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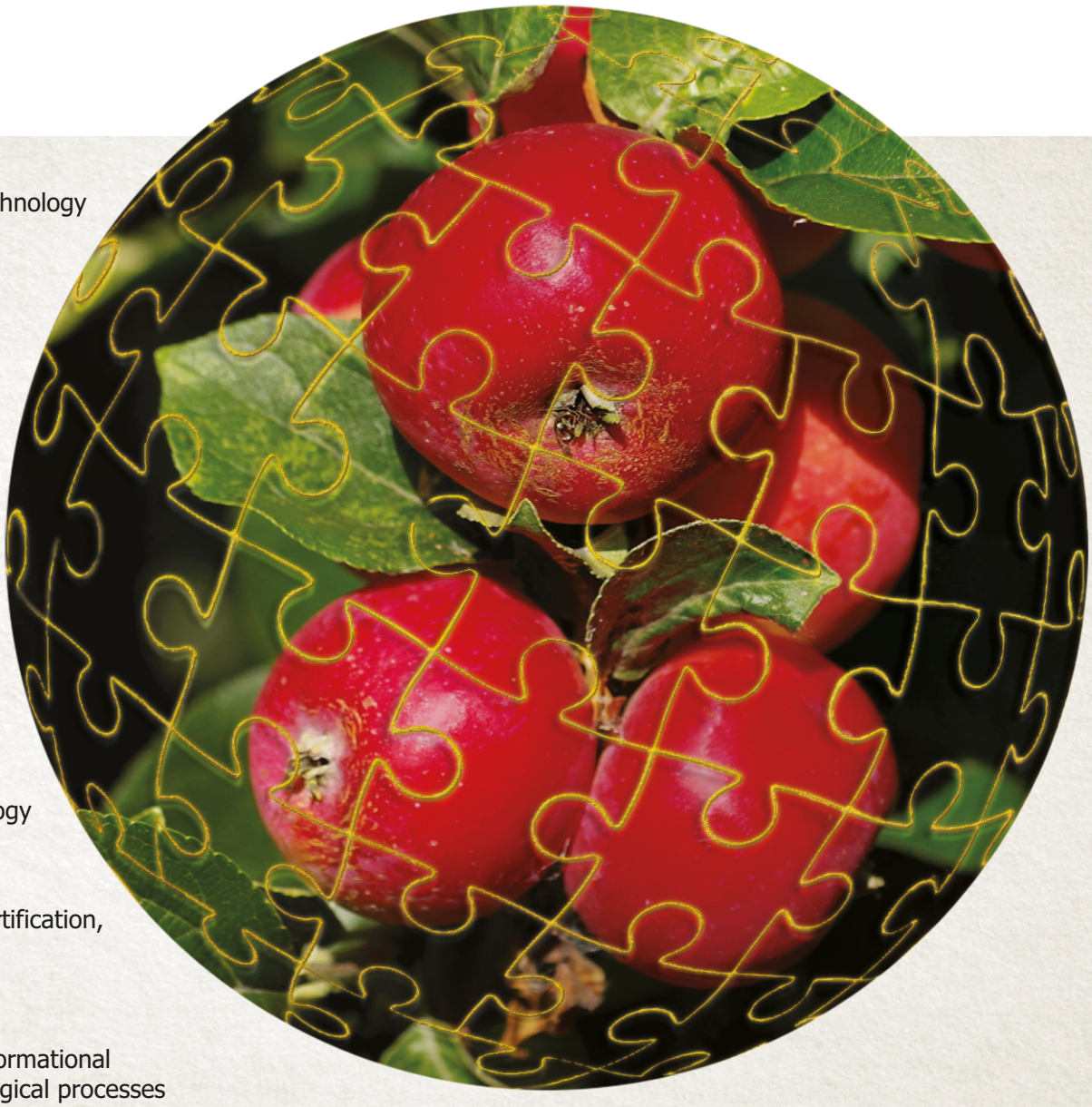

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CONTENTS

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A comparative study of physical properties of selected rice varieties in Nigeria

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Abstract: Rice is now the main food for about 35 million people in Nigeria, and consumption is increasing faster than that of any other food crop in many countries in Africa. This study provided essential engineering data on the physical properties of selected varieties of local rice in Nigeria. Some selected physical properties of Igbemo, Ofada and Abakaliki rice varieties at harvest, market, and storage conditions were evaluated as a function of moisture content. The latter ranged from 12.38 to 25.69% (dry base). We also determined the physical properties of the rice samples, such as moisture content, linear dimensions, geometric mean diameter, arithmetic mean diameter, surface area, aspect ratio, sphericity, bulk density, and hundred kernel weights. A result of the linear dimensions for the major diameter was 8.4–10.3 mm, 6.4–6.55 mm, and 5.9–7.4 mm for harvested, marketed, and stored rice, respectively. The minor diameter ranged from 2.70 to 3.29 mm, 2.49 to 2.63 mm, and 2.56 to 2.74 mm, and the intermediate diameter of the rice varieties at harvest, market, and storage conditions was 1.92–2.29 mm, 1.90–2.02 mm, and 1.87–1.99 mm, respectively. Depending on the conditions and varieties, the bulk density, true density, and porosity, was observed to be between 0.59 to 0.90 g/cm^3, 2.28 to 5.57 g/cm^3 and 70.38 to 85.35% respectively.

Keywords: Rice, moisture content, surface area, aspect ratio, sphericity, bulk density

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INTRODUCTION

Rice is the most widely consumed cereal after maize and sorghum in many parts of Nigeria [1]. Rice grain could be prepared in various forms. Industrially, starch could be made from broken rice which is used in laundry, pharmaceutical, cosmetics, and textile industries. The straw can be used as livestock feed, for thatching of houses, and for making mats and hats. The husk can be used as fuel [2].

The physical properties of rice, which are important in the design and selection of storage structures and processing equipment, depend on grain moisture content. Therefore, the determination and consideration of properties such as bulk density, true density, angle of internal friction, and static coefficient of friction of grain at specific moisture content are essential [3]. Sabbah *et al.* studied the effect of moisture content on the physical properties for three Egyptian paddy rice varieties [4]. They recorded the increase occurring in seed sphericity (S) due to the increase of moisture content. The principal axial dimensions of rye seeds are useful in selecting sieve separators and in calculating power during the rye milling process. Knowing the grain's bulk density, true density and porosity can be useful in sizing grain hoppers and storage facilities: they can affect the rate of heat and mass transfer of moisture during the aeration and drying processes. A grain bed with low porosity will have greater resistance to water-vapour escape during the drying process, which may lead to the need for higher power to drive the aeration fans.

Cereal-grain kernel densities have been of interest in breakage susceptibility and hardness studies [5]. Researchers in [6] reported that various physical properties of green gram (*Phaseulus aureus* L.) were evaluated as

a function of moisture content in the range of 8.39 to 33.40% d.b. The average length, width, thickness and the mass of thousand seeds were 4.21 mm, 3.17 mm, 3.08 mm, and 28.19 g, respectively at moisture content of 8.39% (d.b.). Also, the geometric mean diameter increased from 3.45 mm to 3.77 mm, whereas sphericity decreased from 0.840 to 0.815. They observed that with the increase of moisture content the bulk and true densities decreased from 807 to 708 kg/m³ and from 1,363 to 1,292 kg /m³, respectively. The corresponding bulk porosity increased from 40.77 to 45.16%. The static coefficient of friction is used to determine the angle at which chutes must be positioned to achieve consistent flow of materials through the chute.

The geometric properties such as size and shape are one of the most important physical properties considered during the separation and cleaning of agricultural grains. Theoretically, agricultural seeds are assumed to be spheres or ellipse because of their irregular shapes [3]. [7] determined the physical and mechanical properties of funnel seed as a function of moisture content. They found that there was a parabolic mathematical equation for sphericity, true density, and deformation on both seed length and width sections with changes of moisture content. The shape of the rice was found to be cylindrical with three perpendicular dimensions, namely length (L), width (W), and thickness (T).

Matouk *et al.* developed the mathematical relationships relating the changes of the properties with the seed moisture content. The seed principal dimensions, mass of 1,000 seeds and seed projection area generally increased with the increase of seed moisture content. On the contrary, both shape-index and coefficient of contact surface decreased [8]. Thousand grain mass of rice was utilized in determining the effective diameter which can be used in the theoretical estimation of seed volume [9].

Ghasemi *et al.* designed equipment for processing, sorting, sizing, and other post-harvesting equipment of agricultural products requires information about their physical properties [10]. In their study, various physical properties of rough rice cultivars were determined at a moisture content of 10% (wet basis). In the case of Sorkheh cultivar, the average thousand grain weight, equivalent diameter, surface area, volume, sphericity, aspect ratio, true density, bulk density and porosity were 21.64 g, 3.35 mm, 31.76 mm^2, 20.27 mm^3, 39.71%, 0.28, 1,269.1 kg/m^3, 544.34 kg/m^3, and 56.98%, respectively. For Sazandegi cultivar, the corresponding values were 20.52 g, 3.4 mm, 32.58 mm^2, 21.06 mm^3, 39.88%, 0.29, 1,193.38 kg/m^3, 471.21 kg/m^3, and 60.37%. For Sorkheh cultivar, the average static coefficient of friction varied from 0.2899 on glass to 0.4349 on plywood, while for Sazandegi cultivar those varied from 0.2186 to 0.4279 on the same surfaces. Angle of repose values for Sorkheh and Sazandegi cultivars were 37.66° and 35.83°, respectively. Linear model for describing the mass of rough rice grain was investigated. Mass was estimated with single variable of kernel length with a determination coefficient as 0.862 for Sorkheh cultivar whereas for Sazandegi cultivar was as 0.860. These properties considerably differ from one variety to another.

In Nigeria, there are different local varieties of rice, prominent of these varieties are, 'Ofada', 'Igbemo', and 'Abakaliki' rice. Ofada is a generic name for local rice produced and processed in the rice producing clusters. It originates from Ofada town and its axes including Owode and Wasimi, Ogun State South-West Nigeria. It has recently gained prominence and is fast gaining international attention. Cooked Ofada rice is usually eaten with a special kind of sauce prepared using pepper (Atarodo and Tatase), onion, locust beans, palm oil and assorted meat [11]. The growing of Igbemo rice is a primary activity among the farmers in Igbemo Ekiti, Ekiti State, where 70% are actively engaged in its production, [12]. Igbemo-Ekiti is acquiring national and international reputation for producing rice [13]. Abakaliki is known in agriculture, especially in rice production. It dates back to years before the 1960s.

Despite the popularity of these rice varieties in Nigeria, there is little or no detail of the properties of these varieties. This factor is one of the major issues affecting the development of suitable equipment/structure for the efficient storage and processing of these local rice varieties. This study, therefore, is aimed at investigating some major properties of Ofada, Igbemo, and Abakaliki rice varieties at different conditions. The study also seeks to find the effect of these conditions on the physical properties.

STUDY OBJECTS AND METHODS

1. Sample preparation. One kilogram each of the three rice grain varieties, namely Ofada, Igbemo, and Abakaliki were obtained from farmers from state of their origin, namely from Ogun, Ekiti and Ebonyi State, respectively. Each of the rice variety was divided into 10 equal parts and one part was randomly selected from each of the parts divided. The process was repeated again by mixing all the divided parts together and then separating them into another 10 equal parts. Another sample of grain was taken from each of the divided parts [14]. The process was continued until 500 g of rice were taken from each of the harvested, marketed, and stored rice varieties. The harvested grains were collected directly from the farm after harvest, while the stored and marketed rice varieties were collected from the farmer stores and market outlets respectively.

2. Determination of the physical properties of the rice varieties. We determined the following physical properties: moisture content, shape, size, 1,000 kernel weight, surface area, bulk density, true density, aspect ratio, and sphericity.

2.1 Moisture content. Oven method was adopted for moisture content determination as recommended by the ASAE standards*. The whole grains were placed on dishes for each of the varieties and conditions. After weighing the dishes and grains, dishes were placed in the oven at 105°C for 8 hours. After drying, the dishes were taken out and placed in desiccators. Then the samples were cooled and weighed with a balance. This procedure was performed in three replications for the

* ASAE standards (ASAE [15]).

harvested, marketed, and stored rice samples. Weight differences before and after heating were used to determine moisture content, %:

$$Moisture\ content\ = \frac{W_1 - W_2}{W_1 - W_0} \times 100, \quad (1)$$

where W_0 is weight of moisture dish, kg; W_1 is weight of dish + sample before drying, kg; and W_2 is weight of dish + sample after drying, kg.

2.2 Size. 100 grains selected randomly from 500 g were used, and the basic dimensions of the seed (major, minor, and intermediate) were measured using a digital micrometer (Mitutoyo Digital Micrometer, Series) with accuracy 0.001 mm. The arithmetic and geometric average diameters of rice for each of the varieties and conditions were calculated using equations suggested by K. Shkelqim and M. Joachim [16]:

$$AMD = \frac{L+W+T}{3}, \quad (2)$$

$$GMD = (LWT)^{1/3}, \quad (3)$$

where AMD is arithmetic mean diameter, mm; *GMD* is geometric mean diameter, mm; *L* is length, mm; *W* is width, mm; and *T* is thickness, mm.

2.3 Surface area. Surface area is an important property of grain. It helps the designer in estimating the hopper, processing chamber and the chute. The surface area was found by analogy with a sphere of some geometric mean diameter using the expression cited by Amin *et al*. [17]:

$$S = D_g, \quad (4)$$

where S is surface area (mm^2) and D_g stands for geometric mean diameter (mm) respectively.

2.4 Sphericity. The sphericity was calculated using the equation reported by [18]:

$$Ø = \frac{(LWT)^{1/3}}{L}, \quad (5)$$

where Ø is sphericity; L is the major diameter; W is the minor diameter; and T is the intermediate diameter.

2.5 True density, bulk density and porosity. The bulk density was determined by filling a container of known mass and volume to the brim with each variety of rice and condition for three replicate each. The net mass of rice was obtained by subtracting the mass of the container from the mass of the rice. To achieve uniformity in bulk density, the container was tapped 10 times in the same manner in all measurements to consolidate as reported by Aderinlewo *et al.* [18]. This experiment was carried out in three replicates. The bulk density was then calculated using the equation:

$$Bulk\ density = \frac{M_S}{V_o}, \quad (6)$$

where M_s is the mass of sample in the container, g; V_o is the volume occupied, cm^3.

The true density and porosity were calculated as follow:

$$True\ density = \frac{M_g}{V_g}, \quad (7)$$

$$Porosity = \frac{True\ density - Bulk\ density}{True\ density}. \quad (8)$$

Table 1. Average moisture content of Igbemo, Ofada, and Abakaliki rice varieties under different conditions

Conditions	Moisture content, %		
	Igbemo	Ofada	Abakaliki
Harvest	25.64	20.25	12.85
Storage	12.63	12.44	13.20
Market	12.72	13.41	13.40

2.6 1,000 kernel weight. 1,000 kernel weight was measured by counting 1,000 grains and weighted them on an electronic balance to an accuracy of 0.001 g. This experiment was carried out in three replicates.

2.7 Aspect ratio. Aspect ratio relates the width of a grain to its length which is indicative of its tendency towards being oblong in shape [19]. The aspect ratio R_a was estimated using the equation reported by Aderinlewo *et al.* [18]:

$$R_a = \frac{W}{L}, \quad (9)$$

where W is the minor diameter; L is the major diameter. This experiment was conducted for 100 replicates for the width and length.

RESULTS AND DISCUSSION

1. Moisture content. Table 1 shows the moisture content in the Igbemo, Ofada, and Abakaliki rice at different conditions. The moisture content in the marketed and stored samples was lesser (12.72 and 12.63%) compared with the harvested sample (25.64%). The moisture content of Ofada rice was 20.25%, 12.44%, and 13.41% at harvest, storage, and market conditions, respectively. The Abakaliki rice had the moisture content of 12.85%, 13.20%, and 13.40% for harvested, stored, and marketed samples, respectively.

A one can see in Table 1, the increase in moisture content of the Abakaliki rice occurred after harvest. The rice would have absorbed moisture after harvest unlike the Igbemo and Ofada rice that had a decrease in their moisture content. The Abakaliki rice was usually allowed to dry before harvesting in September with 85% humidity. This led to lower moisture content in the harvested sample when compared to the other rice varieties.

The results are in agreement with [20] that stated that the optimum moisture content of stored grains was 12–13%, while that for harvested grains ideally was 20–25% (wet basis). It also referred to rice as hygroscopic material, which explains why the Abakaliki rice had decreased moisture at harvest condition but increased moisture at market and storage conditions.

2. Geometric properties. The axial dimensions, arithmetic mean, geometric mean diameter, surface area, sphericity, and aspect ratio of the Igbemo, Abakaliki, and Ofada rice at different conditions are presented in Table 2.

2.1 Axial dimension. Table 2 summarizes axial properties of the Igbemo, Ofada and Abakaliki rice at harvest, storage, and market conditions.

Major diameter. We found that the major diameter of the Igbemo, Ofada, and Abakaliki rice was 10.35;

Table 2. Geometric properties of Igbemo, Abakaliki, and Ofada rice varieties under different conditions (n = 100).

Variety	Condition	Major diameter, mm	Minor diameter, mm	Intermediate diameter, mm	Arithmetic mean diameter, mm	Geometric mean diameter, mm	Surface area, mm^2	Sphericity	Aspect ratio
Igbemo	Harvest	$^{e}10.35 \pm 1.09$	$^{f}2.90 \pm 0.23$	$^{f}2.29 \pm 0.12$	$^{d}5.18 \pm 0.38$	$^{f}4.09 \pm 0.25$	$^{h}52.66 \pm 5.74$	$^{a}0.40 \pm 0.07$	$^{b}0.23 \pm 0.08$
	Storage	$^{b}6.55 \pm 0.34$	$^{b}2.57 \pm 0.16$	$^{a}1.87 \pm 0.14$	$^{b}3.66 \pm 0.14$	$^{a}3.15 \pm 0.13$	$^{c}31.28 \pm 2.59$	$^{c}0.48 \pm 0.03$	$^{f}0.29 \pm 0.03$
	Market	$^{b}7.46 \pm 0.54$	$^{c}2.62 \pm 0.15$	$^{b}2.02 \pm 0.12$	$^{e}4.04 \pm 0.23$	$^{c}3.40 \pm 0.17$	$^{f}36.49 \pm 3.58$	$^{c}0.46 \pm 0.02$	$^{d}0.27 \pm 0.02$
Ofada	Harvest	$^{c}8.44 \pm 0.48$	$^{g}3.29 \pm 0.13$	$^{e}2.23 \pm 0.15$	$^{g}4.66 \pm 0.20$	$^{e}3.96 \pm 0.16$	$^{i}49.23 \pm 3.99$	$^{d}0.47 \pm 0.02$	$^{c}0.26 \pm 0.02$
	Storage	$^{a}5.93 \pm 0.39$	$^{d}2.74 \pm 0.14$	$^{d}1.99 \pm 0.11$	$^{a}3.55 \pm 0.14$	$^{b}3.18 \pm 0.11$	$^{d}31.84 \pm 2.20$	$^{g}0.54 \pm 0.03$	$^{h}0.34 \pm 0.03$
	Market	$^{b}6.42 \pm 0.46$	$^{c}2.63 \pm 0.17$	$^{c}1.95 \pm 0.12$	$^{b}3.67 \pm 0.16$	$^{b}3.20 \pm 0.12$	$^{e}32.20 \pm 2.51$	$^{f}0.50 \pm 0.03$	$^{g}0.30 \pm 0.03$
Abakaliki	Harvest	$^{d}8.72 \pm 0.68$	$^{e}2.70 \pm 0.26$	$^{b}1.92 \pm 0.18$	$^{c}4.45 \pm 0.27$	$^{d}3.55 \pm 0.20$	$^{g}39.80 \pm 4.44$	$^{b}0.41 \pm 0.02$	$^{a}0.22 \pm 0.02$
	Storage	$^{b}6.54 \pm 0.41$	$^{b}2.56 \pm 0.42$	$^{a}1.85 \pm 0.06$	$^{b}3.65 \pm 0.21$	$^{a}3.13 \pm 0.16$	$^{b}30.90 \pm 3.57$	$^{c}0.48 \pm 0.03$	$^{e}0.28 \pm 0.02$
	Market	$^{b}6.51 \pm 0.44$	$^{a}2.49 \pm 0.14$	$^{b}1.90 \pm 0.11$	$^{b}3.63 \pm 0.17$	$^{a}3.13 \pm 0.12$	$^{a}30.75 \pm 2.29$	$^{c}0.48 \pm 0.02$	$^{f}0.29 \pm 0.03$

Note: Parameters with the same superscripts in each column have no significant difference at 5% level of significance

8.44; and 8.73 mm respectively at harvest conditions; 6.55; 5.93; and 6.54 mm at storage condition; and 7.46; 6.42; and 6.51 mm at market conditions. Statistical analysis with t-test revealed that there was no significant difference in the major diameter between the three varieties at market condition and Abakaliki rice at storage condition.

Minor diameter. The minor diameter for the Igbemo, Ofada, and Abakaliki rice was 2.90; 3.29; and 2.70 mm respectively at harvest condition; 2.57; 2.74; and 2.56 mm at storage condition; and 2.62; 2.63; and 2.49 at market conditions. The result in Table 2 also showed that there was a significant difference between the minor diameter in all the varieties and conditions, except for the stored Igbemo and Ofada rice that had no significant difference ($p < 0.05$). This is also the case for the market condition of Igbemo and Abakaliki rice.

Intermediate diameter. According to Table 2, the intermediate diameter of the Igbemo, Ofada, and Abakaliki rice was 2.29; 2.23; and 1.92 mm respectively in harvested samples; 1.87; 1.99; and 1.85 mm in stored samples; and 2.02; 1.95; and 1.90 mm were marketed rice. The t-test result revealed the rice varieties and conditions whose intermediate diameters are significant at 5% level of significance. At storage and market conditions, the intermediate diameters of Igbemo and Abakaliki rice were observed not to have any significant difference. Besides, the harvested and marketed Abakaliki rice were also observed not to differ significantly in their intermediate diameter.

2.2 Arithmetic mean diameter. Table 2 also contains the arithmetic mean diameter of the Igbemo, Ofada, and Abakaliki rice under different conditions. The arithmetic mean diameters were 5.18; 4.66; and 4.45 mm, respectively, in the harvested samples; 3.66; 3.55; and 3.65 mm in the stored samples; and 4.04; 3.67 mm; and 3.63 mm in the marketed samples. Further analysis revealed that there was no significant difference in the arithmetic mean diameters among the stored Igbemo rice, the marketed Ofada rice, and the stored and marketed Abakaliki rice.

2.3 Geometric mean diameter. The geometric mean diameters under harvest condition were 4.09; 3.96; and 3.55 mm for the Igbemo, Ofada, and Abakaliki rice, respectively. These values for the stored samples were 3.15; 3.18; and 3.13 mm, and the marketed samples had he geometric mean diameters 3.40; 3.20; and 3.13 mm. Statistical analysis revealed that there was no significant difference between the geometric mean diameters of the stored Igbemo rice and the stored and marketed samples of the Abakaliki rice. In addition, there was no significant difference between the geometric mean diameters of Ofada rice at market and storage conditions. This result implies that a multi-variety screen can be used to clean, sort, or grade Igbemo and Abakaliki rice at storage conditions, Ofada rice at storage and market conditions, and Abakaliki rice at storage and market conditions. This will not be appropriate for the other samples because the discrepancy in their GMD will not allow the use of a screen with a particular diameter. The only possible solution is to have a screen with adjustable size or to replace the screens in the machines when the rice is to be changed.

2.4 Surface area. The surface area of the Igbemo, Ofada, and Abakaliki rice was 52.66; 49.23; and 39.80 mm^2 respectively in the harvested samples; 31.28; 31.84; and 30.90 mm^2 in the stored samples; and, and 36.49; 32.20; and 30.75 mm^2 in the marketed samples. The result also showed that there was no significant difference between the surface area of each condition across the rice varieties ($p > 0.05$). Without varying the air speed or the water pressure in a multi-variety or multi-condition aerodynamic or hydrodynamic applications of these rice varieties, the efficiency will be affected.

2.5 Sphericity. Acording to Table 2, sphericity of the Igbemo, Ofada, and Abakaliki rice was 0.40; 0.47; and 0.48, respectively, at harvest condition; 0.48; 0.54; and 0.48 at storage condition; and 0.46; 0.50; and 0.48 at market condition. In line with the result of GMD, statistical analysis revealed that there was no significant difference in sphericity between the stored Igbemo rice and the samples of the stored and marketed Abakaliki rice. It should be noted that the mean values of sphericity for all the three investigated varieties, which ranged between 0.40 and 0.54, decreased to 0.32–1.00 for most of agricultural products [3].

2.6 Aspect ratio. The aspect ratio of the Igbemo, Ofada, and Abakaliki rice was 0.23; 0.26; and 0.22 for

Table 3. Gravimetric properties of the rice varieties under different conditions (n=3).

Variety	Condition	Bulk density, g/cm^3	True density, g/cm^3	Porosity, %	1,000 kernel weight, g
Igbemo	Harvest	^{a}0.68 ± 0.01	^{a}2.28 ± 0.02	^{a}70.38 ± 0.49	^{a}39.93 ± 0.06
	Market	^{b}0.87 ± 0.01	^{b}5.97 ± 0.31	^{b}85.35 ± 0.77	^{b}28.47 ± 0.64
	Storage	^{b}0.90 ± 0.03	^{c}4.50 ± 0.24	^{c}80.06 ± 0.67	^{c}23.73 ± 0.23
Ofada	Harvest	^{a}0.61 ± 0.01	^{a}2.84 ± 0.07	^{a}78.62 ± 0.68	^{a}31.57 ± 0.25
	Market	^{b}0.89 ± 0.01	^{a}4.54 ± 1.29	^{a}79.30 ± 5.56	^{b}23.50 ± 0.26
	Storage	^{b}0.89 ± 0.01	^{a}4.23 ± 0.24	^{a}78.97 ± 1.26	^{c}24.30 ± 0.20
Abakaliki	Harvest	^{a}0.59 ± 0.01	^{a}2.61 ± 0.25	^{a}77.15 ± 2.20	^{a}22.20 ± 0.10
	Market	^{b}0.89 ± 0.00	^{b}4.13 ± 0.29	^{a}78.40 ± 1.49	^{a}22.30 ± 0.20
	Storage	^{b}0.89 ± 0.00	^{b}4.36 ± 0.44	^{a}79.44 ± 2.16	^{a}22.40 ± 0.30

Note: Parameters with the same superscripts in each column have no significant difference at 5% level of significance

the harvested samples; 0.29; 0.34; and 0.28 for the stored samples; and 0.27; 0.30; and 0.29 for the marketed samples (Table 2). This result did not indicated any significant difference among all the varieties and conditions ($p > 0.05$), except the stored Igbemo rice and the marketed Abakaliki sample.

3. Gravimetric properties. Table 3 presents the bulk density, true density, porosity and 1,000 kernel weight of the three selected local varieties of rice in Nigeria at different conditions.

3.1 Bulk density. As one can see in Table 3, bulk density of the harvested samples was 0.68; 0.61; and 0.58 g/cm^3 for Igbemo, Ofada, and Abakaliki rice, respectively. There was not observed any significant differences at 5% level of significance. The values for the stored and marketed samples were 0.90; 0.89; and 0.89 g/cm^3 and 0.87; 0.89; and 0.89 g/cm^3, respectively. The samples did not show a considerable difference in the bulk density values.

3.2 True density. True density values are also presented in Table 3. In the harvested samples of the Igbemo, Ofada, and Abakaliki rice, the true density was observed to be 2.28; 2.84; and 2.61 g/cm^3, respectively. The values of true density were 5.67; 4.54; and 4.13 g/cm^3 in the marketed rice and 4.50; 4.23; and 4.36 g/cm^3 in the stored rice. The stored samples showed a significant difference unlike the other samples which did not differ considerably.

3.3 Porosity. According to Table 3, porosity of the Igbemo, Ofada, and Abakaliki rice samples was 70.38; 78.62; and 77.15%, respectively, at harvest condition. They did not show a significant difference at 5% level of significance. In the marketed samples, porosity values were 85.35; 79.30; and 78.40% for the Igbemo, Ofada, and Abakaliki rice, respectively. There was no significant difference between the marketed Ofada and Abakaliki rice. In the stored samples, the porosity was 80.06; 78.97; and 79.44%. Similar to market conditions, no significant difference existed between the stored Ofada and Abakaliki rice.

3.4 1,000 kernel weight. This parameter was 39.93; 31.57; and 22.20 g for the stored Igbemo, Ofada, and Abakaliki rice, respectively. The samples did not demonstrate any significant difference at 5% level of significance. For the marketed rice, the 1,000 kernel weight was 28.47; 23.50; and 22.30 g for Igbemo, Ofada, and Abakaliki rice respectively. There was no significant difference existed between the Ofada and Igbemo rice. At storage conditions, the 1,000 kernel weight was 23.73; 24.30; and 22.40 g. Similar to market condition, no significant difference exist between the stored Ofada and Igbemo rice samples.

CONCLUSION

An investigation was carried out to determine and compare the physical properties of Igbemo, Abakaliki, and Ofada rice at harvest, storage, and market conditions. The values of the major diameter, minor diameter, intermediate diameter, arithmetic mean diameter, geometric mean diameter, surface area, sphericity, aspect ratio, bulk density, true density, porosity and 1,000 kernel weights were presented for each variety at the three conditions. Depending on the condition, the moisture content of the Igbemo, Ofada and Abakaliki rice varied from 12.63–25.64, 12.44–20.25 and 12.85–13.40% respectively. The rice condition (or moisture content) influenced some of the axial dimensions, other geometrical properties, and gravimetric properties, likewise the variety. The result presented can be of use in the design of multi-variety or multi-condition separators, pneumatic conveyors, bins, etc. for these three major local rice varieties in Nigeria.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Production of bioethanol from Robusta coffee pulp (*Coffea robusta* L.) in Vietnam

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Abstract: Coffee pulp is the first waste product obtained during the wet processing of coffee beans. Coffee pulp makes up nearly 40% of the total weight of the coffee cherry. Coffee pulp contains 25.88% of cellulose, 3.6% of hemicelluloses, and 20.07% of lignin. Coffee pulp is considered as an ideal substrate of lignocellulose biomass for microbial fermentation to produce such value-added products as ethanol. In this study, we used alkaline pre-treatment of the coffee pulp with NaOH (0.2 g/g biomass) in a microwave system at 120°C during 20 min. This method gave the best results: 71.25% of cellulose remained, and 46.11% of hemicellulose and 76.63% of lignin were removed. After that, the pre-treated biomass was hydrolyzed by Viscozyme Cassava C (enzyme loading was 19.27 FPU/g) at 50°C for 72 hours. The results showed that the highest reducing sugars and glucose concentration after hydrolysis were 38.21 g/l and 30.36 g/l, respectively. Then, the hydrolysis solution was fermented by *S. cerevisiae* (3.10^8 cells/ml) at 30°C for 72 hours. The highest concentration of ethanol obtained was 11.28 g/l. The result illustrated that, available and non-edible as it is, coffee pulp could be a potential feedstock for bioethanol production in Vietnam.

Keywords: Bioethanol, coffee pulp, *Coffea robusta,* lignocellulose biomass, hydrolysis, pre-treatment

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INTRODUCTION

Vietnam is currently the world's largest exporter of Robusta coffee, as well as the world's second-largest exporter of coffee beans after Brazil. In 2016, the total production of coffee beans in Vietnam was about 1,636,500 tons. About 450,000 tons of dried coffee pulp is produced here annually. Coffee pulp is mainly used as a fuel for fruit/coffee beans drying or as a compost and fertilizer on coffee plantations, which causes serious environmental pollution.

All over the world, there have been many researches on the use of coffee pulp. For instance, feeding and digestibility studies were conducted in concrete ponds to evaluate the use of coffee (*Coffea robusta*) pulp as a partial and total replacement for yellow maize in low-cost diets for catfish [1]. The research evaluated the effect of adding coffee husks to animal feed as a substitute for a mixture of corn grain, husks, and cobs. In addition, there have been many studies on how coffee solid wastes can be used. For example, Flammulina velutipes mushroom can be cultivated on coffee spent-ground and coffee husk [2]. Coffee husk can be used as a carbon source for citric acid production in a solid-state fermentation system [3] or for wastewater treatment [4]. However, researchers are more concerned with producing ethanol from coffee pulp using chemical methods [5]. The problem is that these methods remain limited and eco-unfriendly as coffee pulp hydrolysis requires acid and alkali, which means expensive sophisticated equipment. In addition, coffee pulp has a high concentration of carbohydrates and, thus, can be used as a potential raw material for bioethanol production [6]. Besides, recent

studies indicate that residue utilization has an excellent potential for bioethanol production, given that it does not involve costs related to raw material growth. Furthermore, it is estimated that ethanol production from agricultural residues could be sixteen times higher than the current production [7].

Vietnam possesses large quantities of coffee pulp that need utilization. This is also in line with the current global trends to seek alternative renewable energy sources to replace traditional fossil fuels and solve the problem of environmental pollution and climate change. Thus, the present study offers a good solution for these problems.

STUDY OBJECTS AND METHODS

Materials. Robusta coffee pulp was collected at Pong Drang commune, Krong Buk district, Dak Lak province, Vietnam. The berries were of bright-red colour, ripe, neither crushed nor moldy. After harvesting, the pulp was removed and dried at 65°C until the moisture content was 5–8%. After that, the pulp was crushed and sieved; the diameter of the powder was 0.5–1 mm. Finally, the powder was packaged in plastic bags and stored under ambient conditions.

Analytical methods. The moisture content was analyzed according to AOAC method 934.06.

The total ash content was determined by using AOAC method 942.05.

The analysis of total fat was performed by using AOAC method 948.16.

The quantitative analysis of caffeine was performed by using a Genesys UV-Vis Spectrometer (Genesis 10S) [8].

The total polyphenol content in the extracts was determined according to the Folin-Ciocalteu colorimetric method with some modifications [9].

The micro-Lowry method [10] was used to determine the protein content.

The calcicum pectate method was applied to determine the pectin content [11].

Phenol sulphuric acid was used to estimate the total reducing sugars (TRS) using maltose as standard [12].

The reducing sugars (RS) in the hydrolysate were measured by using the DNS method adapted from Miller [13].

A Clever Check blood glucose meter (model TD 4230, Germany) [14] was used to determine the monomeric sugars (glucose).

The cellulose, hemicellulose, and lignin contents were determined by the crude fibre analysis [15].

The ethanol concentration was determined with the help of a Genesis UV-Vis Spectrometer (Genesis 10S) [16].

Pre-treatment method. 50 g of the dried coffee pulp was treated by 500 ml of sodium hydroxide solution (0.2 g NaOH/g biomass). After that, the mixture was pre-treated at 195W and 120°C for 20 min in the microwave system. The pre-treated biomass was recovered by filtration and washed with 1,000 mL of hot water (70°C) to remove the remaining lignin and alkaline substances according to the method offered by Chen *et al.* (2007) [17]. Then the pre-treated residue was pressed to remove excess water and dried at 65°C until moisture content stabilized between 5% and 8%. The concentrations of cellulose, hemicellulose, and lignin remaining in the pre-treated material were calculated by the following equation:

$$R_x = A_p/A_i \times 100, \quad (1)$$

where R_x is the percentage of cellulose (RC), hemicellulose (RH), or lignin (RL) remaining in the pre-treated pulp, %; A_i is the amount of the constituent in the initial dried coffee pulp, g; and A_p is the amount of the constituent after the pre-treatment of the dried coffee pulp, g.

Hydrolysis method (enzyme loading). 5 mL of Viscozyme Cassava C preparation, 150 ml of 0.05 mol/l citrate buffer (pH 4.8), and 15 g (equivalent to 10% of dry material per 100 ml of solution, w/v) of pressed pre-treated dried pulp were mixed in a flask. The containers were incubated in a thermal shaker at 50°C and 150 rpm for 72 hours. After that, the material from each treatment was centrifuged at 2,500 rpm for 10 min [19]. The supernatant was removed to determine RS_s, total reducing sugars (TRS_s), and glucose concentrations. The control samples were not treated by heat and alkaline. The yield from the enzymatic hydrolysis process, %, was calculated using the following equation. Only the cellulose present in the pre-treated coffee pulp was taken into account:

$$YEH = 0.9(G_e - G_w)/C_p \times 100, \quad (2)$$

where G_e is the glucose concentration at the end of the enzymatic hydrolysis, g glucose/l [18]; G_w is the glucose concentration without enzyme treatment, g glucose/l; and C_p is the cellulose concentration in the pre-treated material, g cellulose/l.

Fermentation method. After the hydrolysis, the solution was divided into equal portions of 250 ml each and put in an Erlenmeyer flask. Then $(NH_4)_2SO_4$ (1 g/l), K_2HPO_4 (0.1 g/l) and $MgSO_4.7H_2O$ (0.2 g/l) were added into the solution. The medium was autoclaved at 121°C for 20 min and cooled at room temperature. Fermentation was carried out in an Erlenmeyer flask with 3.10^8 cells/ml of *S. cereviciae* at 30°C, 120 rpm, and pH of 5 [20]. The yeast was collected from the Laboratory of the Food Technology Department at the Industrial University of Ho Chi Minh City. Ethanol concentration was analyzed by using a Genesis UV-Vis Spectrometer at different fermentation times:

$$Y_{p/s} = EC(G_b - G_e), \quad (3)$$

where EC is ethanol concentration at the end of fermentation, g/l; G_b is glucose concentration at the beginning of the fermentation, g/l; G_e is glucose concentration at the end of the fermentation, g/l. The percentage of the theoretical ethanol yield was calculated as follows:

$$Y_{et} = Y_{p/s}/0.51 \times 100, \quad (4)$$

where 0.51 is the maximum theoretical ethanol yield when converting 1g glucose to ethanol.

Table 1. Chemical composition of coffee pulp, g/100g dry basis

Components, %	Present study	a	b	c	d	e
Moisture content	73.85	–	–	77.9	82.0	15.0
Total sugars	9.18	9.70	–		–	28.7
Reducing sugars	8.34	9.63	12.40		–	24.25
Starch	10.20	–	–		–	–
Pectin	4.37	11.37	6.50		–	
Protein	9.52	10.47	10.1		–	7.0
Cellulose	25.88	20.7	17.7	23.0	20.6	16.0
Hemicellulose	3.60	3.60	2.30	20.0	17.2	11.0
Lignin	20.07	14.30	17.5	22.0	15.5	9.0
Lipids	1.22	1.20	–		–	0.3
Ash	6.29	7.33	8.30	15.4	7.9	5.4
Caffeine	0.78	–	1.3		–	1.0
Polyphenols	8.69	–	1.8–8.56		–	5.0

Note: [a][22]; [b][23]; [c][24]; [d][18]; [e][25]

Statistical analysis. All treatments in this study were conducted in triplicate, and 95% of confidence level was applied for the data analysis. ANOVA was used by the one-way analysis of variance, and Statgraphics software (Centurion XV) was used to determine the statistical differences between the treatments.

RESULTS AND DISCUSSION

Characteristics of the solid fraction of coffee pulp. Table 1 shows that the cellulose and lignin content in the coffee pulp (*Robusta coffea*) was 25.88% and 20.07%, respectively. These results were higher than those received by Bonilla-Hermosa *et al*., Elias, and Menezes *et al.* However, the hemicellulose content was similar with the result obtained by Elias (1979) [18, 22, 23]. These differences can be explained by the fact that the previous studies used Arabica, whereas the present research was based on Robusta coffee.

Coffee husks and pulp are comprised of the outer skin and the attached residual pulp, and these solid residues are obtained after de-hulling of the coffee cherries during dry or wet processing, respectively [4]. The coffee pulp only included outer skin and fruit pulp. The sticky coffee husk included skin, fruit pulp, and, perhaps, an insignificant amount of pectin and parchment. Therefore, the total sugars (28.7%) and the reducing sugar content (24.25%) of the sticky coffee husk were higher than those of the coffee pulp (9.7 and 9.63%) [22, 25].

According to Palonen and Hetti [26], lignocellulose biomass is a major structural component of woody plants and other plants, such as grass, rice, and maize. The major constituents of lignocellulose are cellulose, hemicellulose, and lignin. The crude fibre in coffee pulp included: 25.88% of cellulose, 3.6% of hemicelluloses, and 20.07% of lignin. The content of cellulose in the coffee pulp was similar to that in rice husk (24.3%) [27] but lower than in wheat straw (38.2%) [28] and bagasse (38%) [29]. However, there was also a similar proportion between the cellulose content in the coffee pulp (equivalent to 52.23%, g cellulose/100g crude fibre) and the typical proportion of lignocellulose (40–60%) [30]. Therefore, coffee pulp is also considered a source of lignocellulose biomass, which can be used in the production of bioethanol (second generation ethanol production).

Table 2. Percentages of R_x remaining in pre-treated coffee pulp

Lignocellulosic biomass	Before pre-treatment, g/100g dry basis	After pre-treatment, g/100g dry basis	Percentages of R_x
Cellulose, %	25.88[b]	18.44[a]	71.25
Hemicellulose, %	3.60[b]	1.94[a]	53.89
Lignin, %	20.07[b]	4.69[a]	23.37

Note: [a] and [b] in the same row denote a significant difference ($p < 5\%$)

Alkali pretreatment. According to Sun and Cheng [31], the pre-treatment process has a number of advantages: it reduces cellulose crystallinity, removes lignin and hemicellulose, and increases the porosity of the materials. Pre-treatment should meet a number of requirements:

– it cannot produce by-products that are inhibitory to the subsequent hydrolysis and fermentation processes;

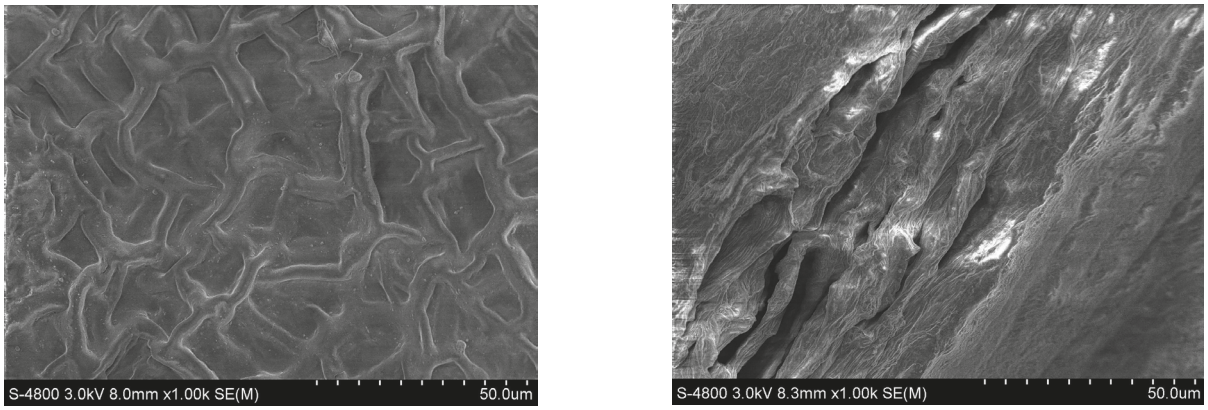
– it cannot result in a loss or degradation of carbohydrate;

– it should improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; and

– it has to be cost-effective. Currently, pre-treatment of lignocellulosic materials can be chemical, physical, physico-chemical, and biological. As for materials that are rich in lignin, alkaline pre-treatment method seems to be the most efficient one.

The efficiency of pre-treatment depends entirely on the type of alkalis, concentration, time, and temperature of the pre-treatment process. To increase the efficiency of lignin removal, the above factors need to be increased. However, the increase in these factors means more cellulose loss. In this study, the coffee pulps were pre-treated with 0.2 g NaOH/g biomass at 120°C for 20 min in a microwave system. The results showed th at 71.25% of cellulose was retained, while 46.11% of hemicellulose and 76.63% of lignin were removed. Although the result was not high, the conversion efficiency could not be regarded as low.

It was necessary to go through the next stages (hydrolysis and fermentation) to evaluate the ethanol conversion efficiency. According to [18], when coffee pulp was pre-treated with 4% NaOH (w/v) at 121°C for 25 min, the commercial efficiency removal of lignin and hemicellulose was 78,41% and 55.85%, respectively, while 69.18% of cellulose was obtained. Wang and Cheng [32] pre-treated coastal Bermuda grass with sodium hydroxide (1% NaOH) and calcium hydroxide (0.1 g $Ca(OH)_2$) (in g/dry biomass) at 121°C during 30 min and obtained about 75% and less than 20% of lignin removal, respectively. In addition, the results of Kim and Holtzapple [33] showed that the optimal conditions of pre-treatment for corn stover were 0.5 g $Ca(OH)_2$/g



(a) (b)

Fig. 1. Scanning electron microphotographs (SEM) of coffee pulp. The coffee pulp (a) was not pre-treated and the coffee pulp (b) was pre-treated with 0.2g NaOH/g biomass at 120°C for 25 min.

raw biomass (55°C). After 4 weeks of pre-treatment, 97.7% of cellulose remained while 32.3% of hemicellulose and 66.9% of lignin were removed. This result showed that the effect of $Ca(OH)_2$ was better than that of NaOH because it was cheaper and produced a larger amount of cellulose. However, hydrolysis and fermentation need to be conducted to evaluate its effectiveness mainly because the final product is ethanol.

Fig. 1 shows that NaOH treatment of lignocellulosic materials caused swelling, which increased the internal surface area, decreased the degree of polymerization and crystallinity, separated the structural linkages between lignin and carbohydrates, and disrupted the lignin structure [34]. The mechanism of alkaline hydrolysis is believed to be in the saponification of intermolecular ester bonds which crosslink xylan hemicelluloses and other components, e.g. lignin and other hemicellulose. The porosity of the lignocellulosic materials increases with the removal of the crosslinks [35]. In addition, Fig. 1 also shows the difference between the raw and the pre-treated samples. The raw samples were rigid, very compact, and non-porous, while the pre-treated sample showed an increase in porosity and a greater surface area, which was attributed to the removal of lignin and hemicellulose.

The alkali pre-treatment can cause redistribution and solubilization of lignin, as well as an increase in the hydrolyzation of cellulose and the porosity of substrates. However, alkali pre-treatment combined with microwave pre-treatment could significantly increase the cellulose hydrolyzation by enzyme because of a larger contact area of cellulose and enzyme [36]. It also causes linkage degradation between cellulose, hemicelluloses, and lignin. In addition, this pre-treatment process has a low temperature and a low alkali concentration, which leads to a lower sugar degradation, and no yeast inhibitor agents are released, so ethanol fermentation yield is higher than when no microwave pre-treatment was conducted. This viewpoint corresponds with the results obtained by Zhao *et al.* [36], who combined alkali pre-treatment and microwave pre-treatment of rice hulls. As a result, the reducing sugar increased by 14% if compared with the samples that underwent no microwave pre-treatment. The results obtained by Xu *et al.* [37] showed that the ethanol yield from fermentation process was nearly 6 times higher than that obtained from the untreated material.

Table 3. Concentration of reducing sugars (RS), total reducing sugars (TRS), and glucose after enzymatic hydrolysis

Test	TRS, g/l	RS, g/l	Glucose, g/l	References
Coffee pulp (Robusta)	48.2[b]	38.21[b]	30.36[b]	Present study
Control	15.8[a]	14.15[a]	12.63[a]	Present study
Coffee pulp (Arabica)	66.15	38.13	27.02	[18]
Wheat straw	–	–	279*	[38]
Bagasse cane	–	–	21	[39]

Note: *mg/g biomass; [a] and [b] in the same column denote a significant difference ($p < 5\%$)

Enzymatic hydrolysis. To assess the effectiveness of alkaline pre-treatment, a certain percentage of hemicellulose and lignin was removed. The ability of enzymatic hydrolysis to produce a particular amount of glucose had to be taken into account. In addition, to evaluate the effectiveness of the pre-treatment process, a control sample was established. The control samples (not pre-treated with alkali) were also hydrolyzed simultaneously with the test sample under the same conditions. However, the hydrolysis effect was completely different (Table 3).

Table 3 shows that YEH (yield of enzymatic hydrolysis) was 76.8% (the result was calculated by formula 2). It means that 76.8% of cellulose was converted to glucose after the hydrolysis process. The productivity was relatively high. According to Menezes *et al.*, hydrolysis of coffee pulp (Arabica) resulted in producing 27.02 g/l of glucose and 60.48% of hydrolysis efficiency [18].

Silverstein *et al.* pre-treated cotton stalks and stems with a NaOH solution (2% NaOH, 60 min, 121°C) and obtained conversion of cellulose equal to 60.8% after the enzymatic hydrolysis. This productivity was lower than the results obtained in the study [40], which can be explained by the following factors. When the alkaline pretreatment of lignocellulose was combined with the

Table 4. Glucose and ethanol contents, ethanol yield ($Y_{p/s}$)

Fermentation time, h	Glucose, g glucose/l	Ethanol, g et/l	$Y_{p/s}$, g et/g glucose	Y_{et} (ethanol yield), %
0	36.58^{*,f}			
24	9.54^{e}	7.11^{b}	0.27^{a}	51.56^{a}
48	7.42^{d}	10.74^{c}	0.36^{c}	72.20^{d}
72	6.10^{c}	11.28^{d}	0.37^{c}	72.55^{d}
72 (control)	4.17^{a}	5.30^{a}	0.31^{b}	60.70^{b}
96	5.55^{b}	11.13^{d}	0.35^{bc}	70.30^{c}

Note: *Including the glucose concentration (g/l) in hydrolysis solution and in yeast culture supplemented during the fermentation. Various lowercase letters (a, b, c, d, e, f) in the same column denote a significant difference ($p < 5\%$)

microwave hydrolysis, the hydrolysis yield was higher. The hydrolyzed sugar content also improved, as compared to the case when alkaline pre-treatment was used on its own. In addition, if combined with microwaves, hydrolysis of the alkaline pre-treatment increases the surface area of cellulose from the breakdown of the hemicellulose and lignin layers. As a result, the sensitivity of hydrolysis enzymes increases [41, 42]. On the other hand, Chen *et al.* [17] pre-treated barley straw with 2% NaOH at 121°C. According to their experiment, 74.03–84.89% of cellulose conversed to glucose during the enzymatic hydrolysis process. The cellulase concentration was 40–60 FPU/g cellulose supplemented with cellobiase (Novozyme 188). Their result was better than that of this study, with an enzyme loading of 19.27 FPU/g of substrate.

The productivity increased considerably when the amount of enzyme was increased (FPU/g), or enzyme cellobiase was supplemented (CBU/g). Chen *et al.* pre-treated corn straw with a 2% NaOH solution for 1 hour at 80°C with an 8% substrate and 20 FPU cellulase/g substrate (which contained 1.64 CBU/g) [43]. The hydrolysis process produced a RS concentration equal to 52 g/l and a high amount of cellobiose. In addition, the RSs content peaked at 64.1 g/l after 60 hour with an increase in the activity of cellobiase to 10 CBU/g substrate. The cellulase activity is inhibited by cellobiose and glucose (to a lesser extent). These problems can be solved by adding cellobiase. In the hydrolysis of lignocellulosic biomass, cellulases attack the cellulose chain to form glucose and cellobiose, then cellobiose decomposes to glucose by cellobiase. Thus, the presence of cellobiase helps reduce the inhibition of cellulase by cellobiose and results in a higher yield of sugars [31].

Fermentation. The glucose concentration in the hydrolysate was consumed by the yeast. The initial concentration was 36.58 g/l and decreased to 6.1 g/l after 72 hour of fermentation. The final concentration of ethanol was 11.28 g/l with a yield of 0.37 g ethanol/g glucose. The control sample (unprotected by hydrolysis and fermentation) showed the initial glucose concentration of 20.85 g/l and 4.17 g/l at the end of fermentation. The ethanol concentration was only 5.3 g/l (Table 4). Therefore, it was necessary to conduct a pre-treatment process to remove lignin and hemicellulose.

According to Menezes *et al.*, coffee pulp (Arabica) was pre-treated by a 4% NaOH solution (w/v) (equivalent to 0.2 g/g biomass) and then was hydrolysed with 13.82 FPU/g of cellulase [18]. The results showed that 27.02 g of glucose/l and 11.99 g of ethanol/l were achieved after hydrolysis and fermentation (Table 5).

Table 5. Comparison of literature data on ethanol production from lignocellulose biomass

Lignocellulose biomass	Ethanol production	$Y_{p/s}$, g et/g glucose	References
Sticky coffee husks	13.6 g/l (8.49 g/100g)	0.38	[25]
Corn stalks	5.0 g/l	0.44	[44]
Barley straw	10.0 g/l	0.44	[44]
Sweet sorghum bagasse	16.2 g/l	0.31	[21]
Wheat straw	18.1 g/l	0.32	[21]
Corn stover	16.8 g/l	0.33	[45]
Coffee pulp (Arabica)	11.99 g/l	0.40	[18]
Coffee husk	7.9 g/l		[46]
Coffee pulp (Robusta)	11.28 (11.36 g/100g)	0.37	Present study

In this study, the pre-treatment process was combined with the microwave system. As a result, the hydrolysis efficiency was high (30.36 g of glucose/l was formed) (Table 3). However, the ethanol yield from fermentation was only 11.28 g/l, which was slightly lower than that of the study by Menezes *et al.* because of the yeast strain or its amount. Besides, they used 3 g/l of dry yeast while the yeast in this study was used as a secondary breed and then added to the hydrolysate with a cell density of 3.10^8 (cells/ml). Chen *et al.* pre-treated barley straw with a 2% NaOH solution at 121°C for 1 hour, and the pre-treated material was hydrolysed by Celluclast 1.5 l at a concentration of 40 FPU/g glucose and Novozyme 188 (cellobiase) [17]. The hydrolysate was inoculated by *S. cerevisiae* (ATCC 24859) and incubated at 30°C for 72 hour. At the end of the fermentation, the ethanol yield ($Y_{p/s}$) was 0.31 g ethanol/g glucose, which is lower than the result of this study (0.37).

The coffee pulp was hydrolysed by using sulphuric acid concentrations of 1%, 2%, and 4% for 1 hour; the achieved ethanol concentrations were 6.097, 4.395, and 3.323 g/l, respectively [47]. The dilute acid hydrolysis resulted in a low ethanol production compared with the deionised water hydrolysis. The maximum ethanol concentration of 6.315 g/l was obtained from the coffee pulp, which was hydrolysed by deionised water [48]. Sugar cane bagasse was pre-treated by steam explosion and hydrolysed by cellulase (26 g RS/l in hydrolysate). The final concentrations of ethanol were 7.4 g/l (0.28 g ethanol/g RS) and 8.2 g/l (0.31 g ethanol/g RS) when two types of yeast were used, i.e. *S. cerevisiae* ATCC96581 and *S. cerevisiae* TMB3001 [39].

150 ml of filtrate yeast (*Saccharomyces cerevisiae*) was added at a concentration of 5.0 g/l and subjected to fermentation for 48 hour at 30°C in a shaker incubator at 120 rpm. The ethanol yield in the fermented broth was found to be 0.50; 0.46; and 0.46 g/g sugar in squeezed CAP, DCP, and WCP. The theoretical ethanol yields

(Y_{max}%) of squeezed cashew apple pulp, dry coffee pulp, and wet coffee pulp were found to be 46.0; 9.35; and 40.0%, respectively [5].

Other studies showed that after fermentation barley straw produced the final ethanol concentration equalled to 10 g/l [44]. In addition, Gouvea *et al.* [25] indicated that when coffee husk was fermented in water (13%, w/v) with *S. cerevisiae* commercial Baker's yeast, the final ethanol concentration equalled 13.6 g/l (Table 5).

The conversion of glucose to high or low ethanol content is attributed either to the low concentration of glucose in the hydrolysis solution or to the poor pre-treatment process, or to the low cellulose content in the raw material. This is consistent with the study conducted by Belkacemi *et al.* [44] that showed an ethanol conversion efficiency of 0.44, while the ethanol content was 5 g/l (Table 5). However, Ballesteros *et al.* obtained quite opposite results [21]: the conversion efficiency reached only 0.32 while the obtained ethanol content was 18.1 g/l (Table 5). Thereby, the conversion efficiency of ethanol indicated that the glucose content in the fermentation broth was high. The high glucose content showed that the hydrolysis process or the fermentation process was very effective.

CONCLUSION

One hundred gram of dry coffee pulp produced 11.36 g of ethanol (the corresponding glucose conversion efficiency of ethanol was 0.37). Comparing with the literature data, it can be seen that ethanol production by fermentation is quite potential. In addition, the present study offers some methods to improve ethanol yields, including the use of a combination of yeast strains for xylose fermentation, hydrolysis coupled with concurrent fermentation, or selection of more potent strains of *S. cerevisiae*.

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Effects of chitosan coating enriched with thyme essential oil and packaging methods on a postharvest quality of Persian walnut under cold storage

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Abstract: This study evaluated the effects of edible coatings and different packaging methods on the shelf-life and quality of walnut kernels. It focused on the coatings with chitosan (1%) and thyme essential oil (TEO) at concentrations of 500 and 1,000 µl L^{-1} (CT_{500}, $CT_{1,000}$) or with chitosan alone (CT). The effects of the coatings was assessed for different packaging methods (LP, loose packaging; PP, packaging in polypropylene bags; and AP, active packaging) as contrasted to control walnuts (C). Walnuts were stored for 120 days in darkness, with relative humidity of 55%, at 4°C. The results showed that the L* index and moisture content of the samples in the chitosan with 500 and 1,000 µl L^{-1} thyme essential oil in active packaging were maximum, whereas peroxide and conjugated diene values were minimum. The lowest rate of mold growth was observed for the chitosan samples with 500 µl L^{-1} thyme essential oil in active packaging. The best overall acceptability score was related to the samples with chitosan alone and the chitosan with 500 µl L^{-1} thyme essential oil in active packaging. The chitosan alone and the chitosan with 500 µl L^{-1} thyme essential oil in active packaging are recommended for storage of kernels at 4°C.

Keywords: Active packaging, chitosan, thyme essential oil, quality, walnut

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INTRODUCTION

Walnuts play an important part in human diet since ancient times. The walnut (*Juglans regia* L.) is quite widespread in Iran. During storage, kernels undergo a series of biochemical, physiological, and structural changes, which make them unacceptable to consumers. Walnut is a nutrient-rich food mainly because of its high biological value proteins (low lysine/arginine ratio), high levels of oil (60 g/100 g in average mainly polyunsaturated fatty acids, or PUFA) [1]. Although fatty acids in walnuts have nutritional value, higher amounts of PUFA (owning unsaturated bands) may cause a poorer quality resistance and a shorter shelf-life [2]. Low oxygen prevent lipid oxidation. The most common oxidation indicators in oils are peroxide value (PV) and conjugated diene value (CDV) [3]. Walnut kernels contain bioactive compounds such as phenols, so polyphenols are subject to oxidation [4, 5]. The walnut kernel can darken due to oxidation of phenolic compounds. L* index shows brightness of products [6]. Moisture is one of the important factors of the quality of nuts [7]. Moisture content (MC) of nuts has a profound effect on their physical, chemical, mechanical, aerodynamic, and thermal properties [8]. Postharvest operations are expected to have a major impact on the microbial contamination of nuts [9]. Among various microbes, fungi are known to play a significant role in the spoilage and loss of stored plant products [10].

Food safety issue requires safe methods with no toxic substances. In recent years, edible coatings have been one of the most innovative ways to improve the

commercial shelf life of fruits. An edible coating, such as chitosan, makes a barrier against moisture, oxygen, and dissolved materials and protects foodstuff from microbial, chemical, and mechanical damages [11, 12]. Chitosan has a higher expansion and elasticity, as well as anti-viral, anti-bacterial, anti-fungi, and antioxidant effects due to different amounts of free amine groups. It can participate in the reactions by forming hydrogen and ionic bonds [13]. Chitosan reduced the growth of *Aspergillus flavus*, the absorption of moisture, and the rate of oxidative reactions [14]. Essential oils (EOs) have been extensively studied as additives in bio-based emulsified coatings. One example is the study by Campos-Requena *et al*. based on carvacrol and thymol, both included in HDPE/modified montmorillonite nanocomposite films [15]. Thyme essential oil (TEO) contains high levels of phenolic compounds, such as thymol and carvacrol. The main component of non-phenolic compounds in TEO is paracymin. Thymol, carvacrol, and paracymin are all antioxidant agents [16]. Although edible coatings create a barrier against oxygen and moisture, they are not perfect replacement of synthetic packaging [17]. A large variety of active packaging systems have been developed and. Today, numerous reviews have emphasized the potential of active packaging technologies to supply safer, 'healthier', and higher-quality foods to consumers [18]. Active packaging is characterized by changing the inside atmosphere of the packed food [19]. Unfavorable flavors, caused by rancidity during storage of the product, did not appear when oxygen adsorbents were applied [2].

This study investigates the effects of chitosan coating enriched with thyme essential oil and types of packaging on the postharvest quality of Persian walnut under cold storage.

STUDY OBJECTS AND METHODS

The study was conducted with walnuts (*Juglans regia* L.) purchased from the local market. Walnuts were shelled manually. The kernels were dried at room temperature till moisture contents of 3.16 ± 0.03%. The chemicals were supplied by Merck and AppliChem Companies. Sachets for active packaging were prepared with ascorbic acid, sodium bicarbonate, and iron powder with the 1:1:1 ratio.

The chitosan solution (1%, w/v) was prepared by dissolving chitosan powder in glacial acetic acid (1%, v/v). The solution was heated, and then glycerol was added as a plasticizer [20]. Tween 80 was used to achieve uniform distribution of essential oil inside the coating solution. TEO (500 and 1,000 µl L^{-1}) was added to the solution; finally the uniform solutions were exposed to UV light for 1 hour for sterilization.

First, the kernels were soaked in the coating solutions for 60 s. Second, the samples were dried at room temperature. The treatments resulted in four samples: control, i.e. uncoated (C); coated with chitosan (CT); coated with chitosan containing 500 µl L^{-1} of thyme essential oil (CT_{500}); and coated with chitosan containing 1,000 µl L^{-1} of thyme essential oil ($CT_{1,000}$). Third, each sample was divided into three equal parts, and then they were packed as follows: loose packaging (LP), packaging in polypropylene bags (PP), and active packaging in polypropylene bags containing sachets (AP). At the end, packets were stored in a dark cold room (55% RH, 4°C for 120 days) and tested every 60 days.

Compositional analysis. The kernels were ground using a home grinder (La Moulinette; Moulinex, Lyon, France). The protein was determined by means of the micro-Kjeldahl procedure, using 5.4 as a conversion factor. The fat contents were evaluated by Soxhlet extraction. The total ash was determined by weighing the dry mineral residue of the samples obtained at 500–550°C. The total amount of carbohydrate was measured by subtracting the amount of ash, protein, and fat from the total dry matter. The moisture contents of the kernels were determined by oven-drying at 103 ± 2°C [21].

Oil extraction and quality analyses. The oil was extracted from kernels using n-hexane solvent without additional heat treatment. About 50 g of ground walnut was mixed with 50 ml of n-hexane (J.T. Baker, Deventer, Holland) and stirred for 30 min. The n-hexane extract was filtered, and the solvent was removed under reduced pressure using a rotavapor (RE 111; Büchi, Flawil, Switzerland) [22].

Peroxide value (PV). First, the acetic acid-chloroform solution and the saturated potassium iodide (KI) solution were added to the oil sample. Second, 30 ml of distilled water was added, then 0.01-N sodium thiosulfate was slowly titrated while shaking the flask vigorously near the end point which was indicated by a faint blue color. Third, the sodium thiosulfate ($Na_2S_2O_3$) was added dropwise until the blue color disappeared. Finally, the peroxide value (meq/kg^{-1}) of oil was calculated according to the following equation:

$$PV = \frac{V \times N \times 1000}{W},$$

where V is the volume of the applied sodium thiosulfate, (ml); N is the normality of the thiosulfate, and W is the oil weight, g [23].

Conjugated diene value (CDV). The CDV was determined at a wavelength of 233 nm, using isooctane as an oil solvent. 0.1–0.3 g of oil was mixed with the isooctane solution. The amount of solution adsorption was determined at a wavelength of 233 nm with a spectrophotometer (Pharmacia, England) [24].

L* index. L* index (black/white) of the kernel was measured using a HunterLab colorimeter (model D65/10) [25].

Mold count. 5 g of each sample was transferred into a sterile stomacher bag under aseptic conditions and diluted 1:10 (w/v) with sterile peptone water (0.1%, w/v, Sigma-Aldrich, Darmstadt, Germany). Then the samples were homogenized for 2 min by means of a stomacher (Seward Laboratory, London, UK). The series of dilutions were prepared by adding 1 ml of each concentration to 9 ml of sterile peptone water (0.1% w/v). In order to count mold, 0.1 ml of each dilution was transferred onto the potato dextrose agar (PDA) medium using the surface culture method and was incubated at 25°C for 5 days [21].

Sensory evaluation. A panel of 10 members evaluated the overall acceptance using the 9-point Hedo-

Table 1. Compositional analysis of walnut kernels

Ash, %	Moisture, %	Fat, %	Protein, %	Carbohydrate, %
1.79 ± 0.11	3.16 ± 0.03	59.14 ± 0.66	15.07 ± 0.65	20.39 ± 0.56

Note: Mean values ± standard deviation over three replicates

Table 2. Effect of coatings and packaging methods on moisture content of kernels

Moisture content, %				
Packaging	Coatings	Storage time, days		
		1	60	120
LP	C	2.25^{Ca}	2.06^{Cab}	1.30^{Db}
	CT	5.56^{Ba}	4.21^{Bb}	2.97^{Cc}
	CT_{500}	5.97^{ABa}	4.35^{Bb}	3.53^{BCc}
	$CT_{1,000}$	6.86^{Aa}	4.81^{Bb}	3.73^{Bc}
PP	C	2.25^{Ca}	2.09^{Ca}	2.02^{CDa}
	CT	5.56^{Ba}	4.95^{Ba}	4.68^{Ba}
	CT_{500}	5.97^{ABa}	5.89^{ABa}	5.46^{Aa}
	$CT_{1,000}$	6.86^{Aa}	6.04^{Aba}	5.96^{Aa}
AP	C	2.25^{Ca}	2.21^{Ca}	2.18^{Ca}
	CT	5.56^{Ba}	5.42^{Ba}	5.20^{ABa}
	CT_{500}	5.97^{ABa}	5.73^{ABa}	5.85^{Aa}
	$CT_{1,000}$	6.86^{Aa}	6.62^{Aa}	6.29^{Aa}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively. Superscript lower letters (a–d) beside mean values in the same row and superscript upper letters (A–D) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.35

nic scale: 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely. The panelists had sensory evaluation experience and were trained in descriptive evaluation of nuts.

Statistical analysis. The research employed a factorial method in the form of a complete randomized design with four replications. Data were subjected to analysis of variance (*ANOVA*) followed by LSD test ($p < 0.05$) to distinguish differences among the treatments. Statistical analyses and Pearson correlation coefficients between traits were analyzed using SPSS software 20.00.

RESULTS AND DISCUSSION

The chemical compositions of the kernels were shown in Table 1.

Moisture. As a result of the analysis of variance, the interaction effect of coating treatments and packaging methods on the moisture content was significant ($p < 0.05$). At the end of storage, the coated samples had the highest moisture. The minimum and maximum moisture content was observed in LP and AP, respectively (Table 2). The hydrophobicity characteristic of chitosan is conditioned by the acetyl groups in its structure, which has not been completely deacetylated. Besides, residual acetyl groups in chitosan play a role in preventing water vapor transmission [26]. Chitosan also decreases weight loss of guava [27].

Moisture absorption in the coating can be effectively decreased due to the hydrophobic characteristics of TEO, which were placed in empty spaces between the polymer chains [28]. In agreement with the previously reported data, the water vapor permeability of coatings was reduced by adding coriander, citronella, tarragon, and TEO [29]. Active packaging ensures a high concentration of carbon dioxide and a high relative humidity inside the package atmosphere [30].

Peroxide value (PV) and conjugated diene value (CDV). The analysis of variance showed the significant interaction effects ($p < 0.05$) for coating treatments, packaging methods, and time of storage. Coating treatments and time of storage, packaging methods and time of storage, coating treatments and packaging methods influenced the peroxide value and conjugated diene value considerably. The trend of CDV was similar to trends obtained for PV. There is a positive correlation between peroxide value and conjugated diene content in walnut [31].

The initial PV in the walnut was about 0.04 meq/kg^{-1}. The fresh walnut kernels in different cultivars had the peroxide values between 0.015–0.29 meq/kg^{-1}; the diversity can be related to the variety of the walnut trees and the weather conditions they grew up in [1]. During storage, the PV and CDV increased in all samples, the maximum amounts were detected in the C and the minimum amounts, in the CT_{500} and $CT_{1,000}$ samples (Table 3). Chitosan prevents the reactive oxygen species (ROS) and the lipid oxidation in food and biological systems because of its antioxidant capacity [32]. Chitosan has antioxidant activity against free radicals [33]. The low PV and CDV of CT_{500} and $CT_{1,000}$ samples can be attributed to antioxidant properties of TEO. Baldwin *et al.* also reported similar results in reducing the PV in oil of pecans [34]. The increasing of PV in the walnut during storage has also been reported [35].

Table 3. Effects of coating treatments and time of storage on PV and CDV of kernels

Test	Coating treatments	Storage time, days		
		1	60	120
Peroxide value, meq/kg oil	C	0.04^{Ac}	1.15^{ABb}	2.82^{Aa}
	CT	0.04^{Ac}	1.45^{Ab}	2.09^{Ba}
	CT_{500}	0.04^{Ac}	1.03^{Bb}	1.62^{Ba}
	$CT_{1,000}$	0.04^{Ac}	0.97^{Bb}	1.25^{Ca}
Conjugated diene value, µmol/g	C	4.88^{Ac}	5.94^{ABb}	7.543^{Aa}
	CT	4.88^{Ac}	6.231^{Ab}	6.844^{Ba}
	CT_{500}	4.88^{Ac}	5.828^{Bb}	6.393^{BCa}
	$CT_{1,000}$	4.88^{Ac}	5.771^{Ba}	6.039^{Ca}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.15

Table 4. Effects of packaging methods and time of storage on PV and CDV of kernels

Test	Packaging	Storage time, days		
		1	60	120
Peroxide value, meq/kg oil	LP	0.04^{Ac}	1.33^{Ab}	2.92^{Aa}
	PP	0.04^{Ac}	0.95^{Bb}	1.99^{Ba}
	AP	0.04^{Ac}	0.58^{Cb}	1.30^{Ca}
Conjugated diene value, μmol/g	LP	4.88^{Ac}	6.11^{Ab}	7.639^{Aa}
	PP	4.88^{Ac}	5.752^{Bb}	6.748^{Ba}
	AP	4.88^{Ac}	5.395^{Bb}	6.08^{Ba}

Note: LP, PP, and AP are loose packaging, polypropylene bags, and active packaging, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.13 for PV and 0.15 for CDV

Tables 4 and 5 showed that the maximum PV and CDV values were with LP and the minimum, with AP. In fact, oxygen is an oxidation resonator; the degree of rancidity was reduced by increasing the amount of carbon dioxide inside the package. The mixture of ascorbic acid and sodium bicarbonate is used in active packaging, so carbon dioxide is produced by combining them. This system is used to increase the shelf-life of fresh meat and fish [36].

L* index. As a result of the analysis of variance, the effect of coating treatments and packaging methods on the L* index was significant ($p < 0.05$). The maximum value was in the $CT_{1,000}$ samples with active packaging and the minimum, in the control sample with loose packaging (Table 6).

The walnut kernel has bioactive compounds such as phenols. The dark color of the walnut kernel in the control sample may be caused by the enzymatic oxidation of carotenoids and phenolic compounds in the kernels [37]. Enzymatic browning of phenols in peanuts correlated with the decrease in the L* index during storage [38]. The reason of high L* in CT_{500} and $CT_{1,000}$ samples can be attributed to the antioxidant properties of chitosan and TEO which inhibited the oxidation of phenolic compounds. The highest L* index in AP resulted from the low oxygen content in the package. The acceptable value of the L* of walnut color was above 40 [39]. The L* indices of all samples were higher than 40, and the coated and actively packed samples had the highest L* index.

Table 5. Effects of coating treatments and packaging methods on PV and CDV of walnut

Test	Coatings	Package		
		LP	PP	AP
Peroxide value, meq/kg oil	C	3.48^{Aa}	2.91^{Ab}	2.48^{Ac}
	CT	2.95^{Ba}	2.25^{ABb}	2.01^{Ab}
	CT_{500}	2.52^{BCa}	2.03^{BCb}	1.16^{Bc}
	$CT_{1,000}$	2.34^{Ca}	2.06^{Cb}	1.06^{Bc}
Conjugated diene value, μmol/g	C	8.175^{Aa}	7.629^{Ab}	7.217^{Ab}
	CT	7.668^{Ba}	6.997^{Bb}	6.767^{Ab}
	CT_{500}	7.256^{Ba}	6.784^{Bb}	5.95^{Bc}
	$CT_{1,000}$	7.08^{Ba}	6.815^{Bb}	5.857^{Bc}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 μl L^{-1} TEO, respectively. Superscript lower letters (a–c) beside mean values in the same row and superscript upper letters (A–C) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 0.15 for PV and 0.17 for CDV

Table 6. Effects of coating treatments and packaging methods on L* index of walnuts

Packaging	Coatings	Storage time, days		
		1	60	120
LP	C	74.87^{Aa}	70.09^{Aab}	59.76^{Cb}
	CT	62.73^{Bb}	68.42^{Ab}	70.03^{Ba}
	CT_{500}	76.34^{Aa}	74.23^{Aa}	72.74^{Ba}
	$CT_{1,000}$	61.61^{Bb}	72.94^{Aa}	75.72^{Ba}
PP	C	74.87^{Aa}	72.95^{Aa}	65.05^{BCb}
	CT	62.73^{Bb}	69.08^{Ab}	74.46^{Ba}
	CT_{500}	76.34^{Aa}	78.18^{Aa}	79.85^{ABa}
	$CT_{1,000}$	61.61^{Bb}	74.86^{Aa}	79.43^{ABa}
AP	C	74.87^{Aa}	67.81^{Bb}	72.58^{Bab}
	CT	62.73^{Bb}	69.80^{ABab}	78.87^{Ba}
	CT_{500}	76.34^{Aa}	77.85^{Aa}	83.17^{Aba}
	$CT_{1,000}$	61.61^{Bb}	65.44^{Cb}	86.25^{Aa}

Note: C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 μl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively. Superscript lower letters (a–d) beside mean values in the same row and superscript upper letters (A–D) beside mean values in the same column show the difference in Duncan's multiple range test ($p < 0.05$). Standard error mean = 2.13

Mold count. As shown in Fig. 1a, the counts of mold in all samples increased during storage, but the control sample count had the highest. In all the treatments, the growth of molds increased within 60 days, but after that in the coated samples, no significant change was observed in the growth of fungi. To the contrary, in the control sample, the number of fungi increased to $\log_{10}$ 3.67 CFU/g^{-1} by the end of storage.

Chitosan extends the product shelf life directly affecting the growth of fungi and other defense operations, such as chitinase accumulation, which reduces the inhibitory effect of fungal cell wall proteinase. Chitosan inhibits microorganisms such as gram-positive and gram-negative bacteria and fungi [40]. According to [41] and [42], chitosan coating in artichoke seeds reduced the activity of various fungi and microorganisms on tomatoes. Antifungal effect of chitosan coating on pears was reported by Xianghong *et al.* [43].

TEO in combination with chitosan coating decreased the fungi count: $CT_{1,000}$ and CT_{500} samples had less microbial load than the others did. The main constituents of TEO are thymol and carvacrol, which have antimicrobial effects [44].

As shown in Fig. 1b, with the LP method, mold growth was significantly higher than with other packaging methods, but there was no significant difference between PP and AP methods.

As shown in Fig. 1c, with the LP method, the highest mold growth was observed in the control sample, but there was no significant difference between the other treatments. The maximum and minimum growth

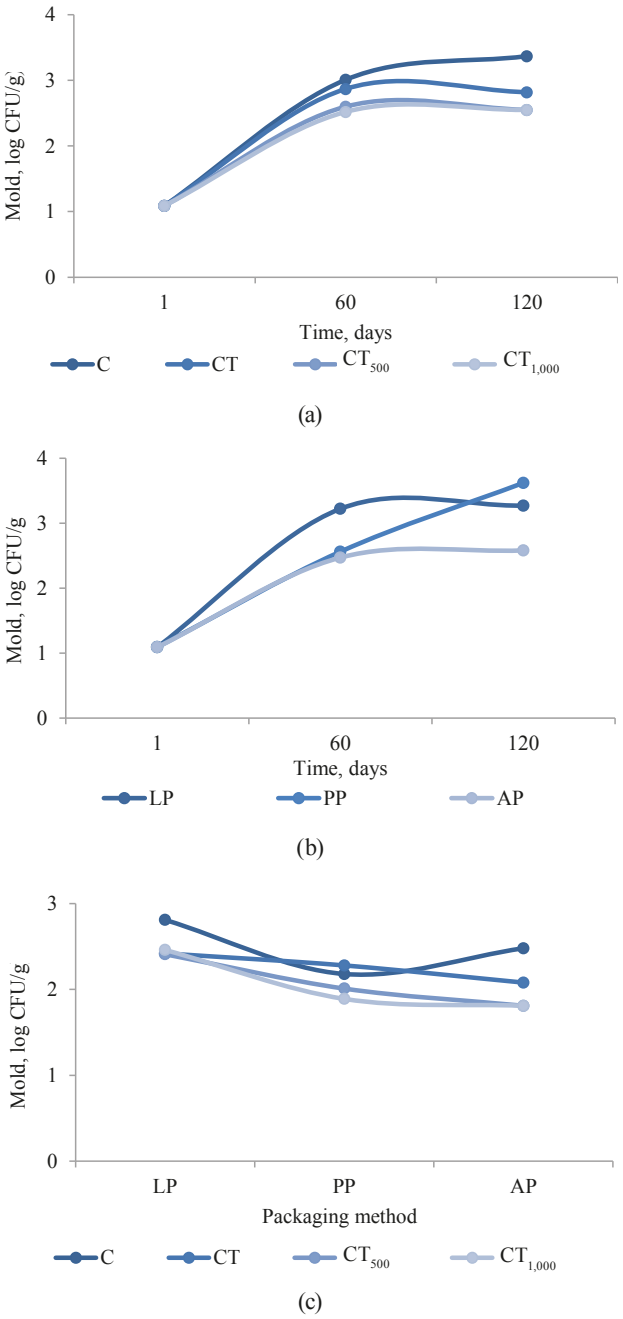

Fig. 1. Effects of coating treatments (a) and packaging methods (b) during storage and the effect of coating treatments and packaging methods (c) on the growth of molds in the kernels. C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively ($p < 0.05$).

was in CT and $CT_{1,000}$ samples, respectively. Active packaging had a better effect on controlling the growth of molds. This could be due to creating an atmosphere with higher CO_2, which inhibits growth of microorganisms. CO_2 is the only gas that has a direct antimicrobial effect and increases the lag phase and growth time during the logarithmic growth stage [45]. Packaging with the modified atmosphere (with a low oxygen and high carbon dioxide content) was effective in controlling fungal rot and protecting the quality in the post-harvest period of fruits [46].

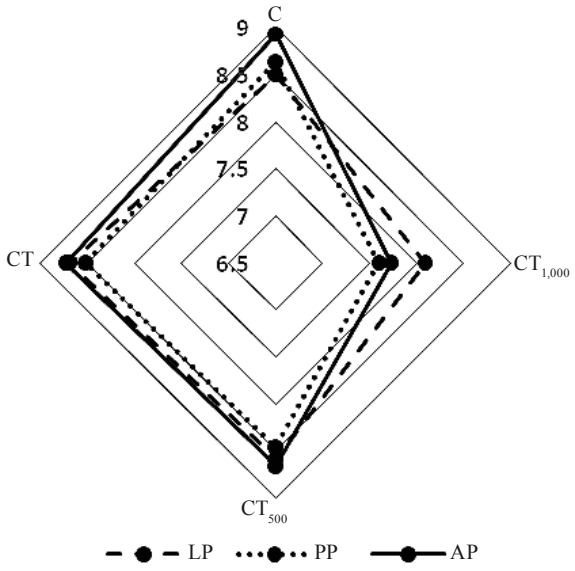

Fig. 2. Effects of treatments and packaging methods on overall acceptability score of walnut kernels. C is the control sample; CT is coated with 1% chitosan; CT_{500} and $CT_{1,000}$ are coated with 1% chitosan containing 500 and 1,000 µl L^{-1} TEO, respectively. LP, PP, and AP are loose packaging, packaging in polypropylene bags, and active packaging, respectively ($p < 0.05$).

Sensory evaluation. As shown in Fig. 2, the AP and LP control samples had the highest and lowest overall acceptability score, respectively. The high overall acceptability score in active packaging (AP) could be attributed to the low peroxide content in the samples. Although peroxides themselves do not directly play a role in off-flavour, but the ingredients of their decomposition produce an undesirable flavour. Hydroperoxides break down to form short-chain compounds, including aldehydes, ketones, alcohols, acids, esters, lactones, ethers and hydrocarbons, which contribute to odor and taste [47].

There was no significant difference in CT and CT_{500} samples in all three packages. The results indicated that chitosan did not adversely affect the general acceptance score of the walnut kernel, which was similar to the previous studies on walnut kernels, strawberries, and peeled lychee [35, 48, 49].

$CT_{1,000}$ treatment resulted in the lowest sensory properties with all three kinds of packaging. Therefore, treatments containing high levels of TEO were evaluated as undesirable. This can be related to a high concentration of the essence oil. The overall acceptance score for this treatment was higher with LP than with other packaging methods. With LP, because of volatile characteristics of TEO, some of the TEO evaporated. It made the flavour score of the sample comparable to those with other packaging methods.

CONCLUSION

The shelf-life and quality of the walnut kernel can be affected by some environment factors during storage. Coating materials and packaging methods can be useful for prolonging the postharvest quality of crops. The study results showed that chitosan

and thyme essential oil coating combined with active packaging had a significant effect on reducing oil oxidation and growth of molds. They also prevented the loss of moisture and a decrease in L* value, improving the sensory properties of the samples during storage. An increase in the essential oil up to 500 µl L^{-1} also improved the functional properties of chitosan coating. Compared with loose packaging, polypropylene packaging was also effective in protecting the qualitative properties of walnut. Active packaging offered the considerable potential and proved the most efficient.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Comprehensive assessment of fruit jelly with an improved carbohydrate profile based on unconventional plant raw materials

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Abstract: One of the negative trends in current nutrition is an increased consumption of refined foods with a low content of useful nutrients and antioxidants and an abundance of oxidation accelerators. Fruit paste and jelly have a relatively low energy value, compared to other confectionery products. Along with this fact, they also have gelling agents and fruit raw materials in their composition, which can classify them as diet food. This paper presents a comprehensive approach to developing a technology for producing fruit jelly with an improved carbohydrate profile. For that, we used viburnum and orange puree, a valuable natural plant material, as a source of carbohydrates, and fructose, as a sugar substitute. The qualimetric model created from the tasting data was used to select the optimal proportions of the main ingredients, viburnum and orange puree, as 25:75 and 75:25. The comparative assessment of antioxidant capacity (AOC) showed that the sample with the 75:25 ratio of viburnum to orange puree had an AOC of 22.33 μmol TEq/g. It was twice as high as AOC of the 25:75 ratio sample. With a glycaemic index of 29.2, this fruit jelly can be recommended as a diet food with preventative properties.

Keywords: Fruit paste, fruit jelly, diet foods, qualimetric modelling, coefficient of concordance, chi-squared test, antioxidant capacity

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INTRODUCTION

Today, most countries witness a profound shift in the structure of major causes of death and a growth in chronic disease, stress, and fatigue, with non-communicable diseases increasingly becoming most common in the 2st century.

One of them is metabolic syndrome (MS), also known as Reaven's syndrome, insulin resistance syndrome, or atherothrombogenic syndrome. It is a complex metabolic disorder often found in patients with arterial hypertension and abdominal obesity. MS is linked to tissue insulin resistance that contributes to the development of hyperinsulinemia, activation of the sympathoadrenal system, increased vascular tone and pathological changes in the lipid spectrum of blood [1].

The main risk factors for MS are hypodynamia, hypercaloric nutrition, easily available pseudo-nutrition with a low content of beneficial nutrients or antioxidants and an abundance of oxidation accelerators, increased activity of the sympathetic nervous system, and frequent stress. It explains why, with the last two factors excluded, A. Regenauer (1998) called MS a 'good life' syndrome, referring to a low-active lifestyle. Indeed, several studies show that a decrease in physical activity and a high carbohydrate diet are the main reasons why the incidence of MS is reaching epidemic proportions. About 25% of the population in Western countries suffer from this disorder [2].

According to the Institute of Nutrition of the Russian Academy of Medical Sciences (www.ion.ru), more than a third of the Russian population are overweight or obese. At the same time, the consumption of confectionery foods is growing due to their variety and affordability, as well as people's stable taste preferences.

Yet, their physiological value is quite low: they are rich in fat (up to 40%) and carbohydrates (up to 70%) but lacking in vitamins and minerals [3].

The RF Government's Order No. 1364-p of June 29, 2016 'The Strategy for Improving the Quality of Foods in the Russian Federation until 2030' (www.government.ru) indicates a need to create conditions for the production of new generation foods with specified quality characteristics. They include specialised, functional, enriched, and organic foods. To do this, we need research aiming to provide a biomedical rationale and develop new formulas and technologies.

Prevention of nutritional diseases is a social task that cannot be fulfilled by doctors alone. Great responsibility in addressing this issue rests with the food industry. It must ensure not only food safety but also optimal quality – nutritional value, sensory properties, and health benefits – and its motto should be 'The 21st century foods are health and taste!' [4–8].

Thus, of scientific and practical interest is the development of technologies that can create confectionery products using non-conventional raw materials rich in essential substances [9, 10].

This study aims to develop a formula and a technology for producing fruit jelly with an improved carbohydrate profile using non-conventional bio-raw material (red viburnum berries). We also intend to measure its antioxidant capacity and physicochemical and organoleptic characteristics.

The improvement of the carbohydrate profile implies a partial or complete replacement of mono- and disaccharides with ingredients having a low glycaemic index [11].

STUDY OBJECTS AND METHODS

The study was conducted by the Department of Grain Processing, Baking, Pasta and Confectionery Technologies at K.G. Razumovsky Moscow State University of Technology and Management (the First Cossack University) in collaboration with the Information Technology Section at V.M. Gorbatov All-Russian Research Institute of Meat Industry and the Core Facility 'Industrial Biotechnology' at the Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences.

We used red viburnum puree and citrus puree (grapefruit, orange, and lemon) were used as the main raw materials to produce test samples and develop the technology. The objects of the study were fruit jelly samples with a different proportion of the main raw materials, namely 25:75, 75:25, 60:40, 40:60, and 50:50.

The control sample was made from apple puree according to the traditional recipe (Table 2) [12].

To prepare the test samples, red viburnum berries and citrus fruits were sieved to make a puree. Then, they were sieved again to separate the puree from any particles of peel or seeds that might be there after the first rough sieve, as well as free it from any foreign matter. For this purpose, it was passed through an MPR-350M-01 (Belarus) pulping machine with a sieve hole diameter of 0.5–1 mm.

Air-dry edible gelatin was soaked in a fourfold volume of water at 20–25°C to swell for 40–60 min. The hydration of macromolecules and the destruction of bonds between them, which occurred during the swelling process, speeded up the dissolution of the gelling agents in water. The swollen gelatin was heated until it was completely dissolved, without bringing it to a boil, and then introduced, in a thin stream and with constant stirring, into a fruit and berry syrup. The syrup was obtained by boiling-out red viburnum puree, citrus puree, and fructose syrup until a dry matter content of 67–69% was reached. The resulting mass was poured into silicone moulds and cooled for 1–2 hours to a temperature of 23–25°C. To form a structure, the jelly mass was cured for 2–3 hours at 23–25°C. Then the samples were removed from the moulds, sprinkled with starch, and dried for 10–12 hours until a crust formed. The crust protects the fruit jelly from getting wet and gives it an attractive appearance.

We tested such sensory characteristics as: taste; colour; aroma; consistency; grittiness; stickiness; and surface condition.

We also examined physicochemical characteristics of the samples, such as acidity, mass fraction of total moisture, glycaemic index, and antioxidant capacity (AOC). For that the following methods were used:

1. Acidity was determined by titration with phenolphthalein.

2. Mass fraction of total moisture was measured by drying on a VChM-A device (Ukraine) at 160–165°C for 3 min.

3. Mass fraction of reducing substances was determined by a method based on the recovery of an alkaline copper solution (hot titration method).

4. The glycaemic index was determined as an amount of glucose accumulated during the product's breakdown *in vitro* [13]. The sample was soaked in 0.1 N hydrochloric acid solution and put into a bath at 37°C. After a 10–15 minute incubation, a preparation of proteolytic enzymes (for example, Panzinorm) was added. Then the reaction mixture was neutralised with sodium bicarbonate to reach a pH of 8.2–8.5. A preparation of the duodenum of laboratory animals (e.g. rats) was added at the same time. Then the sample was incubated for another 1.5 hours at 37°C and filtered or centrifuged. The protein content was determined spectrophotometrically and the glucose content, by an enzymatic method.

5. AOC was measured using an atomic spectrophotometer (Carry 100 Bio, USA). Its operation is based on determining the ratio of two light fluxes that pass through the reference channel and the sample channel in the cuvette compartment. The spectrophotometer also uses the Stop-And-Go scanning principle (stopping the diffraction grating for the chopper rotation cycle). Unlike the traditional non-stop chopper rotation principle, it allows for adequate results without recalibrating the spectrophotometer at any scanning speed, up to 3,000 nm/min in the UV-visible part and up to 8,000 nm/min in the near-IR part of the spectrum. Correct conditions for analysing the spectrum ensure valid analytical results. The

spectrophotometer measured the AOE of the lipophilic and hydrophilic fractions of the samples in relation to the radical cation of the 2-azinobis-3-ethyl benziazolite-6 sulfonic acid diammonium salt (ABTS). Trolox was used as a standard in the AOC analysis and the results were expressed in Trolox equivalents (TEq).

The tasting data were processed by mathematical statistics methods in Microsoft Excel. The experiments were repeated three times. A confidence level of 0.95 was used to test the hypotheses. The consistency of expert opinions was determined by a coefficient of concordance, whose significance was checked with the chi-square test.

RESULTS AND DISCUSSION

The paper describes the main stages of improving the carbohydrate profile of candied fruit jelly, in particular:
– justifying the selection of food ingredients with hypoglycaemic effect;
– developing formulas of fruit jelly samples with an improved carbohydrate profile and testing technological parameters of their production;
– optimising physicochemical and organoleptic characteristics of fruit jelly samples;
– determining the glycaemic index (GI) of fruit jelly samples; and
– studying the antioxidant capacity (AOC) of fruit jelly samples.

The control sample was apple jelly based on the classic recipe and traditional technology.

1. Justifying the use of red viburnum and orange as fruit jelly ingredients. The selection of fruit jelly ingredients (see 'Study Objects and Methods') was based on the comprehensive approach to the development of enriched and specialised foods [14, 15]. This approach contains three groups of criteria, namely:
– biomedical;
– technological; and
– economic.

Medical and biological criteria cover the choice of enriched or specialised products and functional ingredients, setting the amount and restricting the content of specific substances and components, as well as their bioavailability and safety.

Technological criteria are concerned with the simplicity and ease of use of a specific ingredient, its effect on the properties of semi-finished products and the quality of finished ones, its compatibility with the main components, and the method of introducing the ingredient into the formula.

Economic criteria are used to estimate the economic efficiency of an ingredient and its effect on the production cost.

Taking the above criteria into consideration when producing functional and specialised foods will enable us to find an optimal solution based on the technology of effective food functionality.

The plant raw materials used in the study is permitted in the production of sugary confectionery [16], including foods for children aged three and above, and meets the safety requirements specified in the regulatory documents*.

Red viburnum (*Vibúrnum ópulus* L.) is richer in vitamins than many fruits, which makes this beneficial ingredient quite promising in fruit jelly production [17–20].

Due to its pectic substances, viburnum juice can turn into jelly [21], which is essential in fruit jelly production. The percentage of P-active compounds (bioflavonoids) in viburnum berries and juice can reach 300–500 mg/100 g. Viburnum contains vitamins A, D, and E. Interestingly, it has a higher content of vitamin C than citrus fruits. Viburnum is also rich in minerals, such as phosphorus, magnesium, potassium, iron, calcium, copper, manganese, and iodine (Table 1). These berries have plenty of invert sugar (about 30%), tannins, as well as isovaleric and acetic acids [22, 23]. Viburnum seeds contain up to 21% of fatty oil. Isovalerianic acid esters and viburnin glycoside, a natural substance of the cardiovascular and antispasmodic group, give viburnum berries their characteristic smell and bitterness. Despite its beneficial properties, viburnin had a strong negative impact on the sensory indicators of fruit jelly quality. Jelly had an off-odour, so a need arose to eliminate or disguise it in the finished product. The main solutions to this problem were heating, which partially destroys this glycoside, and using strong flavours of natural origin, such as citrus, to neutralise the smell [24].

Citrus fruits have a high biological value due to a variety of biologically active substances. Thus, flavonoids help the body to absorb vitamins, pectic substances protect the cardiovascular system, glycosides (such as naringin) lower blood pressure and cholesterol level, preventing heart attack and acting as a tonic [25].

After testing lemon, orange and grapefruit puree as possible components of viburnum jelly formula that could neutralise the smell of viburnin, orange was selected as the cheapest alternative. Then, we analysed the sensory characteristics (taste, aroma, texture, stickiness, and surface condition) of the test samples made from the above citrus fruits and viburnum puree in a ratio of 75:25 to 25:75. The analysis showed that the fruits only changed the colour and the taste of the finished product and had almost no effect on the other indicators that are essential in the fruit jelly technology.

Orange puree is rich in B vitamins, ascorbic acid, macro- and microelements (Table 1), and fibre. It is a valuable source of amino acids and contains over 170 phytonutrients and 60 flavonoids.

Pectin contained in viburnum puree is not enough to form a strong jelly, therefore gelatin was used as an additional gelling agent.

Gelatin is widely used in the food industry due to its unique structure-forming properties. It gives confectionery a stretchy, jelly-like or foamy texture and has an ability to bind water and stabilise dispersed systems. Proteins make over 85% of gelatin composition, with collagen being the main component. This product contains two essential amino acids, hydroxyproline and proline.

* SanPiN [Sanitary Rules and Norms] 2.3.2.1940-05, Technical Regulations of the Customs Union 029.

Table 1. Chemical composition of red viburnum and orange [26, 27]

Substance	Content per 100 g of finished product	
	Red viburnum	Orange
Chemical composition		
Proteins, g	0.40	0.90
Fats, g	1.50	0.20
Carbohydrates, g	7.00	8.10
Energy value, kcal	26.30	43.00
Vitamins		
Vitamin A, mcg	25.00	8.00
Vitamin C, mg	82.00	60.00
Carotene, mg	1.40	–
Vitamin PP, mg	1.35	0.20
Vitamin B9, mg	0.30	5.00
Vitamin E, mg	2.00	0.20
Minerals		
Potassium, mg	179.50	197.00
Calcium, mg	40.50	34.00
Magnesium, mg	17.50	13.00
Iron, mg	6.10	0.30
Phosphorus, mg	100.00	23.00
Sodium, mg	21.50	13.00
Sulphur, mg	12.00	9.00

Fructose was used as a sweetener. Fructose is slowly absorbed in the intestine and has a glycaemic index of 20. Thus, this contributes to a smooth increase in blood glucose levels, which is especially important for diabetic people. Fructose is 1.75 times as sweet as sucrose and thus can be added to products in smaller quantities to reduce the amount of sucrose by 30–50%. Currently, fructose is used in the production of functional and diet foods, including those for children [28].

2. Developing a formula for diet fruit jelly. A number of experimental studies were conducted to develop products with different ratios of the main raw materials (see 'Study Objects and Methods').

To make fruit jelly dietetic, sugar was replaced with a smaller amount of fructose: it is sweeter than sucrose. This made it possible to significantly increase the amount of puree in the formula. In addition, fructose enhanced the flavour of the fruit jelly.

Table 2 shows the fruit jelly formula developed.

Studies were conducted to measure how the plastic strength of the viburnum-and-orange-based samples made with different amounts of gelatin changed according to the duration of curing, compared to the control sample and the gelatin-free sample.

The gelatin-free jelly had a poor plastic strength of 5.3 kPa (Fig. 1, curve 2), because the amount of pectin contained in viburnum puree is not enough for strong jelly to form.

The percentage of gelatin powder in the test samples was 3%, 5%, and 7% of the formula mass (Fig. 1, curves 3, 4, and 5). Fig. 1 shows how increased amounts of gelatin affected the plastic strength of the jelly mass. With a gelatin amount of 5%, the plastic strength almost reached the value of the control sample (17 kPa), and with a higher amount, it even exceeded it. Thus, we chose a 5% duantity of gelatin powder.

The glycaemic index (GI) of the samples was calculated by measuring the amount of glucose accumulated during the product breakdown *in vitro*.

The analysis (Fig. 2) showed that replacing sugar with fructose reduced the jelly GI almost by half (29.2–32.8 for test samples 1–5 vs. 58.4 for the control sample). This can be explained by a low GI of fructose compared to sucrose (19 vs.75). Some variation of the GI in the test samples seems to be associated with different proportions of viburnum and orange puree in their formulas. The predominance of viburnum puree leads to a slight decrease in the GI.

Table 3 demonstrates the comparison of the nutritional value of five jelly samples with that of the control. As can be seen, all the test samples had a higher content of protein, fats, vitamins, macro- and microelements, compared to the control sample, and almost half of its carbohydrate content and energy value.

The analysis of sensory and physicochemical characteristics of the samples was carried out with expert qualimetry methods by the Department of Grain Processing, Baking, Pasta and Confectionery Technologies at K.G. Razumovsky Moscow State University of Technology and Management.

Table 2. Diet fruit jelly formula based on viburnum and orange puree

Raw material	Dry matter mass fraction, %	Total raw material per 1 ton of finished product, kg					
		Control*	Test 1	Test 2	Test 3	Test 4	Test 5
Granulated sugar	99.85	689.6	–	–	–	–	–
Syrup	78.00	31.0	–	–	–	–	–
Apple puree	10.00	860.0	–	–	–	–	–
Viburnum puree	18.00	–	862.5	690.0	575.0	460.0	287.5
Orange puree	16.00	–	287.5	460.0	575.0	690.0	862.5
Fructose syrup	78.00	–	527.0	527.0	527.0	527.0	527.0
Gelatin powder	84.00	–	92.0	92.0	92.0	92.0	92.0
Lactic acid	40.00	5.3	–	–	–	–	–
Sodium lactate	40.00	9.0	–	–	–	–	–
Total:		1,589.0	1,769.0	1,769.0	1,769.0	1,769.0	1,769.0
Product weight:	69.80	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0

**Note:* the control sample was based on the moulded apple jelly formula. Tests 1–5 refer to the samples with a different proportion of viburnum and orange puree, namely: 75:2; 60:40; 50:50; 40:60; and 25:75.

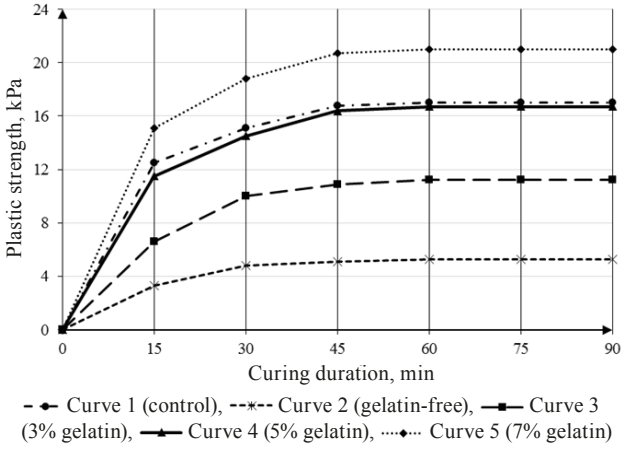

Fig. 1. Changes in the plastic strength of the samples with different amounts of gelatin depending on the curing duration

Twenty respondents aged 20 to 50 took part in the tasting to identify the product relevance among consumers of different age groups.

Of different survey methods used (such as interviewing or questionnaire), interviewing proved most productive: the respondents took the initiative and expressed well-argued opinions.

2.1. Qualimetric modelling as a method of evaluating consumer properties. The key stage of customer satisfaction assessment was data processing based on qualimetric modelling. The method allowed us to combine versatile indicators in one assessment, while taking into account the importance of each of them for the consumer [29]. The qualimetric model was as follows:

$$K = \frac{\sum_{i=1}^{n}\sum_{k=1}^{m}\frac{Y_{ik}}{B_{ik}}}{nm},$$

where m is the number of respondents; n is the number of criteria (indicators); Y_{ik} is the satisfaction of the k-th consumer with the i-th criterion, points; B_{ik} is the importance of the i-th criterion for the k-th consumer, points.

To systematise the data and visualise the results in a compact form, we developed a customer satisfaction matrix that showed individual customer ratings according to selected criteria (taste, colour, aroma, texture, and surface), as well as data processing results.

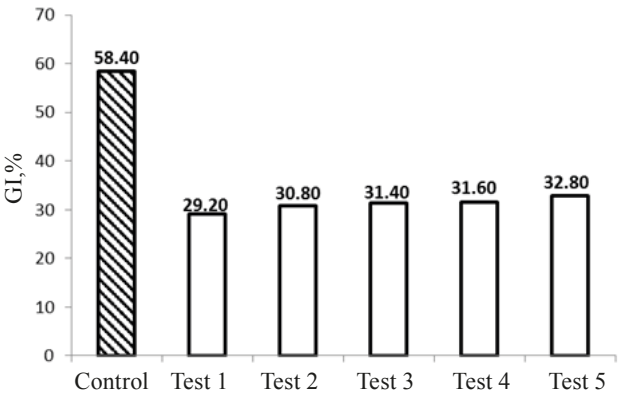

Fig. 2. Effects of the jelly formula on the glycaemic index

The validity of the results was evaluated by analysing the consistency of expert (consumer) opinions using the coefficient of concordance:

$$W = \frac{12\sum_{j=1}^{n} d_j^2}{m^2\left(n^3 - n\right)},$$

where d_j is the deviation of the rank sum for the j-th parameter from the average rank sum.

If there are equal ranks among those given by one expert (consumer), the coefficient of concordance is calculated as follows:

$$W = \frac{12\sum_{j=1}^{n} d_j^2}{m^2\left(n^3 - n\right) - m\sum^{m} T_k},$$

$$T_k = \sum_{k=1}^{m}\left(t_k^3 - t_k\right),$$

where t_k is the number of equal ranks in the k-th group.

The coefficient of concordance W ranges from 0 to 1. $W = 1$ means complete consistency of expert opinions, $W > 0{,}5$ means satisfactory consistency, and $W < 0{,}5$ means poor consistency.

Table 3. Nutritional value of the control and test samples

	Control	Test 1	Test 2	Test 3	Test 4	Test 5
Dry matter	50.44	38.98	38.79	38.66	38.53	38.33
Protein, g	0.32	4.88	4.93	4.96	4.99	5.04
Fat, g	0.11	0.78	0.66	0.57	0.49	0.36
Carbohydrates, g	54.92	27.41	27.51	27.59	27.66	27.76
Moisture, g	49.54	61.02	61.21	61.34	61.47	61.67
Energy value, kcal	222.00	136.19	135.68	135.33	134.99	134.47
Vitamins, mg						
Beta-carotene	–	0.68	0.55	0.46	0.36	0.23
B9	–	0.96	1.42	1.72	2.03	2.49
C	0.86	49.73	47.59	46.16	44.73	42.58
E	0.11	1.01	0.83	0.72	0.60	0.42
PP	0.27	1.44	1.33	1.26	1.18	1.07
Macro- and microelements, mg						
Potassium (K)	68.16	119.59	121.29	122.43	123.57	125.27
Calcium (Ca)	7.77	61.68	61.04	60.62	60.20	59.56
Magnesium (Mg)	3.77	14.81	14.37	14.07	13.78	13.34
Sodium (Na)	0.97	13.17	12.34	11.79	11.23	10.40
Phosphorus (Ph)	9.17	68.10	60.59	55.58	50.58	43.07
Iron (Fe)	0.83	3.13	2.56	2.18	1.81	1.24
Manganese (Mn)	–	0.01	0.01	0.01	0.01	0.01
Copper (Cu)	–	0.08	0.08	0.08	0.08	0.08

Table 4. Qualimetric models of test jelly samples

No. sample	Proportion of ingredients	Qualimetric model
1	Viburnum 75 / Orange 25	$P = 0.20x_1 + 0.18x_2 + 0.16x_3 + 0.17x_4 + 0.15x_5 + 0.13x_6$
2	Viburnum 25 / Orange 75	$P = 0.19x_1 + 0.19x_2 + 0.17x_3 + 0.15x_4 + 0.15x_5 + 0.15x_6$
3	Viburnum 40 / Orange 60	$P = 0.11x_1 + 0.11x_2 + 0.20x_3 + 0.17x_4 + 0.21x_5 + 0.20x_6$
4	Viburnum 60 / Orange 40	$P = 0.14x_1 + 0.16x_2 + 0.17x_3 + 0.16x_4 + 0.18x_5 + 0.18x_6$
5	Viburnum 50 / Orange 50	$P = 0.14x_1 + 0.15x_2 + 0.17x_3 + 0.14x_4 + 0.20x_5 + 0.19x_6$

Table 5. Sensory and physicochemical characteristics of test samples

Indicator	Test sample 1	Test sample 2	Test sample 3	Test sample 4	Test sample 5
			Sensory characteristics		
Colour	Saturated, vivid burgundy	Saturated orange	Unsaturated, pale maroon	Unsaturated, pale orange	Unsaturated, pink
Taste	Sweet, with a pronounced taste of viburnum and a hint of orange	Sweet, with a dominant taste of orange and a hint of viburnum	Sweet taste of orange and viburnum (dominated by orange)	Sweet taste of orange and viburnum (dominated by viburnum)	Sweet, unpronounced taste of fruit and berries
Aroma	Pronounced aroma of viburnum with a hint of orange	Pronounced aroma of orange with a hint of viburnum	Aroma of viburnum with a light hint of orange	Aroma of viburnum with a fruity hint	Aroma of viburnum and orange
Texture	Elastic, gelatinous, tender	Elastic, gelatinous, tender	Elastic, gelatinous, tender	Elastic, gelatinous, tender	Elastic, gelatinous, tender
Shape	Consistent with this type of fruit jelly	Consistent with this type of fruit jelly	Consistent with this type of fruit jelly	Consistent with this type of fruit jelly	Consistent with this type of fruit jelly
Surface	Smooth and shiny	Smooth and shiny	Smooth and shiny	Smooth and shiny	Smooth and shiny
			Physicochemical characteristics		
Acidity, °N	11.1	7.9	6.4	5.9	6.7
Moisture content, %	69.8	67.8	64.3	68.5	66.3
Mass fraction of reducing substances, %	1.5	1.6	1.2	1.3	1.4

Since expert opinions are considered random variables, the criterion χ^2 was used to check the relevance.

Thus, the product quality was assessed at multiple levels, with every consumer opinion taken into account. This once again proves the need for classifying respondents at the first stage of analysis.

A composite quality index is defined as an average weighted index, with individual indicators contributing different weight fractions to its formation.

Thus, the composite quality index for the fruit jelly was determined by six single indicators: x_1 – taste (sweet/bitter), x_2 – taste (like/dislike), x_3 – colour, x_4 – aroma, x_5 – surface condition, and x_6 – texture. Table 4 shows the qualimetric models of the test samples.

Having analysed the above models, we concluded that the least significant indicator for sample 1 was texture; for sample 2 – texture, surface, and aroma; for sample 3 – taste (sweet/bitter) and taste (like/dislike); for sample 4 – taste (sweet/bitter); and for sample 5 – taste (sweet/bitter) and aroma.

We also found that taste was the leading assessment factor for samples 1 and 2, whereas surface condition had the highest value in the rest of the samples.

2.2. Fruit jelly quality indicators. The analysis of the sensory and physicochemical quality indicators enabled us to select the best of the test samples (Table 5).

As can be seen in Table 5, the samples were consistent with the basic requirements for sensory and physicochemical quality indicators, including those stipulated in State Standard**.

The comprehensive organoleptic evaluation and physicochemical studies revealed that samples 1 and 2 manifested the best quality indicators. This could be due to the fact that consumers preferred the samples with pronounced mono taste, aroma, and colour of orange or viburnum, rather than those where they were mixed.

The next stage of research aimed to study the comparative effect that the new formulas of the best jelly samples had on their antioxidant capacity (AOC). This parameter is essential for enhancing the nutritional value and functional significance of the product for the human body.

3. Studying the antioxidant capacity of the jelly samples. It is common knowledge that antioxidants interrupt radical-chain oxidation processes in the human body. These processes are caused by free radicals due to exogenous factors (such as chemical environmental pollutants, ionizing radionuclide emissions) and as a result of biochemical metabolic reactions in body cells [30].

It is also known that using biologically active substances of plant origin can enhance nonspecific immunity

** State Standard 6442-2014

Table 6. Antioxidant capacity of test jelly samples

No. sample	AOC, μmol TEq/g weight *(lipophilic fraction)*	AOC, μmol TEq/g weight *(hydrophilic fraction)*
1	0.04	22.33
2	0.02	9.68

and antioxidant protection of the human body [31]. The latter factor is directly related to the peroxidation of lipids involved in the formation of cell membranes and to the protective functions of the body.

In collaboration with the Bach Institute of Biochemistry, the Russian Academy of Sciences, we measured the antioxidant capacity of the lipophilic and hydrophilic fractions of the test samples. For that, we used a Carry 100 Bio spectrophotometer in relation to the radical cation of the 2-azinobis-3-ethyl benziazolite-6 sulfonic acid diammonium salt (ABTS). Trolox was used as a standard in the AOC analysis and the results were expressed in Trolox equivalents (TEq). The results are presented in Table 6.

As can be seen from Table 4, the main contribution to the jelly antioxidant capacity is made by the hydrophilic fraction. However, the antioxidant capacity of sample 1 is more than twice as high as that of sample 2. Apparently, this is due to a different proportion of orange and viburnum puree in the samples (Table 2). An increased content of viburnum puree in the jelly formula can enhance the antioxidant capacity of the finished product. In addition, sample 1 can be classified as a functional product due to its formula and content of vitamins, as well as macro- and micronutrients.

CONCLUSIONS

The comprehensive analysis of the technology for producing fruit jelly with dietetic and preventative properties and an improved carbohydrate profile was carried out. Here, consumer preferences were taken into account. The analysis opened a prospect for using red viburnum as an unconventional bio raw material with multiple beneficial components.

The study resulted in a fruit jelly formula based on a combination of natural ingredients, viburnum and citrus fruit puree, and fructose used as a sugar substitute. We expect that the new technology will contribute towards the production of specialised preventative confectionery for healthy nutrition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CRITERIA OF AUTHORSHIP

The authors share the responsibility for their work and the information provided in this paper.

The authors were equally involved in conducting the study and writing the manuscript and are equally responsible for plagiarism.

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Effects of natural herbal extracts on hemp *(Cannabis Sativa* L.) oil quality indicators

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Abstract: The present research features such natural herbal extracts as rosemary (*Rosmarinus officinalis* L.), pomegranate (*Punica granatum* L.), and green tea (*Camellia sinensis* L.). Together with vitamin E, they were chosen to investigate hemp oil stability. The experiments revealed the effect of various fatty acids compositions on hemp oil properties. The effect of herbal extracts on the oxidative stability of oils was measured using the Rancimat method. The chemical tests included peroxide value (PV), free fatty acid (FFA) value, and acidity value (AV). Each herbal extract was applied in amounts of 30 mg/l and 50 mg/l. Vitamin E was mixed with the oil in the amount of 2 g/l. The changes in PV, FFA, AV, and fatty acid composition were measured on day 6, 12, 18, and 24. Hemp oil, pure or mixed with the extracts, was analyzed using the Rancimat method at 120°C with an airflow rate of 20 l/h to identify the induction periods (IP). IP values of oils with additional herbal extracts were found to be significantly higher ($p < 0.05$) than those of the control samples. The oil sample with vitamin E demonstrated the longest IP (4.12 hours at 120°C) during the Rancimat test. The herbal extracts and vitamin E had a positive effect on hemp oil PV compared with the control sample. Gas chromatography (GC) showed that hemp oil included a high amount of polyunsaturated Omega-3 fatty acids, and the oil was not resistant to oxidation. In general, the natural herbal extracts and vitamin E in the recommended quantities had a positive effect on the oxidative stability of hemp oil.

Keywords: Hemp oil, natural extracts, quality indicator, Rancimat, peroxide value

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INTRODUCTION

Hemp (*Cannabis sativa* L.) is one of the oldest cultivated plants in history. Even though there seems to be no proved data about it, hemp was cultivated in Asia approximately 20,000 years ago [32]. As a rule, hemp is used as a fibre source. Most hemp seed sorts contain 20-35% of oil. Hemp oil is important for human diet due to its high polyunsaturated fatty acid content. Omega-6/Omega-3 ratio of hemp seed oil is similar with Mediterranean diets, which have great benefits for the cardiovascular system. According to the European Food Safety Agency, Omega-3 and Omega-6 ratio should be 1:3–1:5 [14]. Today, foods with a high amount of polyunsaturated fatty acids (PUFAs) are in greater demand than those with synthetic additives. However, there is a great competition to stabilize such products because they contain a lot of unsaturated fatty acids [22].

Hemp oil is extracted from hemp seeds by the cold pressed extraction method. As a rule, cold pressed oils are extracted from herbs or seeds by using a hydraulic press. The cold press method has several advantages compared with the more conventional solvent extraction method. First, it does not require heat processing or organic solvent. Second, the cold press method preserves such bioactive compounds as phenolics, essential fatty acids, tocopherols, and flavonoids, which improves the quality of oil [36].

Lipid oxidation often makes food unacceptable and decreases its nutritional quality due to the deterioration of essential fatty acids. Therefore, lipid oxidation is one of the important factors which define the quality and

shelf-life of vegetable oils and fats [12]. Under the oxidative degradation, fats and oils produce toxic compounds that are associated with various health issues [13]. Antioxidants are materials that prohibit oxidation of other compounds. Some natural antioxidants to be found in food include ascorbic acid, phenolic compounds, carotenoids, sterols, protein-based components, and sulphur-containing compounds [7]. Synthetic antioxidants are popular in food industry. However, the safety of synthetic materials remains a matter of discussion. It is now obligatory only natural antioxidants should be used in food industry to prevent the oxidative degradation of oils [6]. Herbs and spices contain a high amount of antioxidants due to phytochemicals [35].

In the last decade, many studies have showed that such herbs and spices as pomegranate, rosemary, various tea extracts, oregano, thyme, sage, etc. possess a high antioxidant capacity. It can be explained by the fact that they are rich in phenolic compounds [27]. For instance, pomegranate extract contains flavonoids, hydrolysable tannins, and anthocyanins [18]. Rosemary extract contains such phenolic diterpenes as carnosic acid, carnosol, and rosmanol, which are very effective antioxidants. Carnosic acid is one of the strongest antioxidant components to be found in rosemary extract [37]. Green tea extracts are rich in gallic acid derivatives and flavanols. In addition, green tea extracts contain a wide range of such flavour components as terpenes and organic acids. Green tea extracts are rich in catechins, epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate [38].

The quality indicators of oils, such as oil stability index (OSI), fatty acid composition, PV, and AV, determine the quality of vegetable oils and fats. The Rancimat method evaluates antioxidants by measuring the changes in the conductivity induced by absorption of volatile organic acids. IP is the time needed for the secondary oxidation products to occur. IP defines the resistance properties of oils against oxidation. Thus, IP can be used to compare oil samples. On the other hand, fatty acid composition determines the stability of oils. Unsaturated fatty acids are especially prone to oxidation [15]. A higher rate of unsaturation makes the substance more oxygen-sensitive. Saturation of oils is measured with the help of GC, which also determines the fatty acids composition.

The determination of free fatty acid evaluates that the quantity of hydrolytic efficiency which occurs in the oil and as well as it shows intensity due to the reason of pro-oxidant effects of free fatty acids [16]. The determination of FFA evaluates that the quantity of hydrolytic efficiency which occurs in the oil and as well as it shows intensity due to the reason of pro-oxidant effects of free fatty acids [16]. The PV evaluates hydro peroxides composed throughout the early phase of rancidity of oils and fats. The process is usually settled on a titration with sodium thiosulphate. It evaluates the iodine released from potassium iodide according to peroxides in the oil [33]. In accordance with the Codex Alimentarius Commission [9], PV for cold pressed oils is < 15 meq/kg (milliequivalents of peroxide per kg of oil). On the other hand, peroxide value demonstrates the current status of oxidation of oils and fats, and it does not indicate possible oxidation [27].

Hence, the purpose of the study was to investigate the quality parameters of hemp oil mixed with the different natural extracts and vitamin E. Indicators of oil hemp quality revealed some changes in fatty acids compositions and the quality properties. PV, OSI, AV, FFA, and fatty acid composition were evaluated on days 1, 6, 12, 18, and 24. Omega-3 and Omega-6 ratios were evaluated periodically under storage conditions. The research contributed to a better understanding of various vegetable oils and their oxidative and nutritional properties, as well as their feasibility for human diet.

STUDY OBJECTS AND METHODS

This research was carried out in the laboratory of Food Institute at Kaunas University of Technology. The fresh cold pressed hemp oil was purchased from JSC BORELA (Kaunas). The dried rosemary, green tea leaves, and dried pomegranate were purchased from a local Lithuanian company. Before extraction, the herbs were thoroughly crushed. All the extracts were prepared by pouring boiled deionized water over the dried herbs and stirring for 40 min. The temperature of the water was not maintained during the extraction. After that, the residue was prepared for the freeze drying process. The freeze drying process was applied in a Freeze Drying Plant Sublimator 15 (ZIRBUS Technology, Germany). The materials were placed on the shelf (300 × 400 mm) with the configuration of 5 shelves/80mm distance, 6/65, 7/55, 8/45. The parameters for the process were –45°C and –55°C; the condenser temperature was –18°C; the product temperature at the beginning of the process was –25°C; and the duration of the process was 12–20 hours. After lyophilization (freeze drying) process, the powdered samples were weighed and kept in a sterile plastic bag. Vitamin E was purchased from a local Lithuanian pharmacy. All other reagents for GC and chemical analysis were of analytical grade and obtained from Toronto Research Chemicals (Toronto, Canada). The samples were analyzed for different physicochemical parameters with the help of triple GC and the Rancimat test.

Mixture preparation. The hemp oil was mixed with the extracts of rosemary, green tea, pomegranate, and vitamin E. The extracts were applied in two different amounts: 30 mg/l and 50 mg/l. vitamin E was mixed with the oil at the amount of 2 g/l. During the experiment, all the samples were kept in the dark at room temperature.

Rancimat analysis. The accelerated Rancimat test was used to estimate the hemp oil stability to oxidation. The Rancimat apparatus was used with one evaluation mode. The induction period (IP) was defined as the time corresponding to the inflection point of the conductivity time curve. The Rancimat analysis was conducted using a Rancimat 892 (Metrohm, Herisau, Switzerland) according to the AOCS Official Method Cd 12b-92 [2]. Three grams of samples were put into the test tubes. Pure oil samples were considered as blank control samples. The oil samples were carefully poured into each reaction vessel not to push the substance into the oxy-

Table 1. Induction period of hemp oils with different extracts

Induction period, h							
Control	Vitamin E	Rosemary extract		Pomegranate extract		Green tea extract	
		30 mg/l	50 mg/l	30 mg/l	50 mg/l	30 mg/l	50 mg/l
3.12 ± 0.02	4.12 ± 0.04	3.69 ± 0.03	3.89 ± 0.02	3.67 ± 0.05	3.7 ± 0.01	3.56 ± 0.06	3.61 ± 0.03

gen glass tube. The temperature of 120°C was set; the airflow rate was 20 l/h. IP was automatically determined from the inflection point of the curve by using the software supplied by the company.

Peroxide value (PV) measurement. PV indicates the state of primary oil oxidation. A high PV results from the history of oil, e.g. its exposure to such oxidation factors as high temperatures during processing and storage [19]. PV was determined according to ISO 3960* [20]. The oil samples (3 g each) were accurately weighed and mixed with isooctane-glacial acetic (3:2) solution. After that, potassium iodide mixture, water, and starch solution were applied. Finally, the mixtures were titrated with sodium thiosulfate, and the results were expressed in miliequivalents of active oxygen per kg of oil [23].

Determination of free fatty acid (FFA) and acid value (AV). FFA and AV of the oil samples were determined according to ISO 660:2009** without any modifications. FFA content is the number of milligrams of potassium hydroxide required to neutralize the FFA present in 1g of fat. The acidity content of FFAs was determined in accordance with the procedure specified in the International Standard (ISO 660:2009**). Approximately 5 g of oil samples were carefully weighed and mixed with ethanol (96%) and diethyl (peroxide free) ether in the ratio of 1:1. After that, phenolphthalein was added to the mixture. Finally, the mixtures were titrated with 0.1M sodium hydroxide. The results were expressed in oleic acid (282 g/mol) for the FFA and in milligrams per gram – for the acid value.

Fatty acid composition analysis. The fatty acid composition analysis was based on the AOCS method [1]. The fatty acid composition was analyzed using an Agilent 7890 gas chromatograph coupled with an Agilent 5975 mass spectrometer (GC–MS; Agilent Technology, CA, USA). It was equipped with an HP-88 capillary column (100 mm × 0.25 mm id, 0.2 m film thickness). To prepare fatty acid methyl esters (FAME), oils were saponified with 0.5M KOH and then methylated by using 40% BF3 in methanol. The injection temperature was 250°C. Helium was used as a carrier gas at a pressure of 100 kPa. The split ratio was 1:30. The oven temperature was programmed according to the following sequence: 5 min at 80°C, then an increase up to 150°C at 10°C/min, 2 min at 150°C, followed by another increase up to 230°C at 5°C/min; and 10 min at 230°C. The ionization voltage was 70 eV; the scanning range was 50–550 m/z. Each fatty acid was quantified handling external norm. The fatty acids were expressed as g/100g.

Statistical analysis. All samplings and chemical analyses were tripled. The results of the study were expressed as a mean of the triplicate values with standard deviations.

RESULTS AND DISCUSSION

The experiment revealed that the small quantities of the extracts had no effect on such sensory properties of hemp oil as taste, color, and smell.

Rancimat analysis. Table 1 shows the effect of rosemary, pomegranate, green tea extracts, and vitamin E on the oxidative stability of the fresh hemp oil. The blank hemp oil oxidative stability IP value was 3.12 ± 0.02 h at 120°C; the airflow rate was 20 l/h. The control samples were the most visibly oxidized ones, which was demonstrated with the lowest IP values obtained. The IP values of oils with additional herbal extracts were found to be significantly larger ($p < 0.05$) than that in the control samples.

The Rancimat test assessed IP to detect the formal volatile acids throughout oil oxidation [25]. When vitamin E was added to the hemp oil, it resulted in the highest IP value (4.12 ± 0.04 h). This was because of the strong antioxidant capacity of the tocopherol content in vitamin E. When rosemary was added to the hemp oil, IP values were 3.69 ± 0.03 h (30 mg/l) and 3.89 ± 0.02 h (50 mg/l). IP values for the green tea were 3.67 ± 0.05 h (30 mg/l), 3.7 ± 0.01 h (50 mg/l). IP values for the pomegranate extract were and 3.56 ± 0.06 h (30 mg/l) and 3.6 ± 0.03 h (50 mg/l). According to the results, the oils showed lower IP compared to the control sample. This means that the extracts had a high potential prevention of oxidative stability. According to Parker *et al.* [31], OSI was 8.5 hours for the cold pressed virgin (not refined) hemp seed oil (Wisconsin, USA) at 80°C and 7 l/h air flow rate.

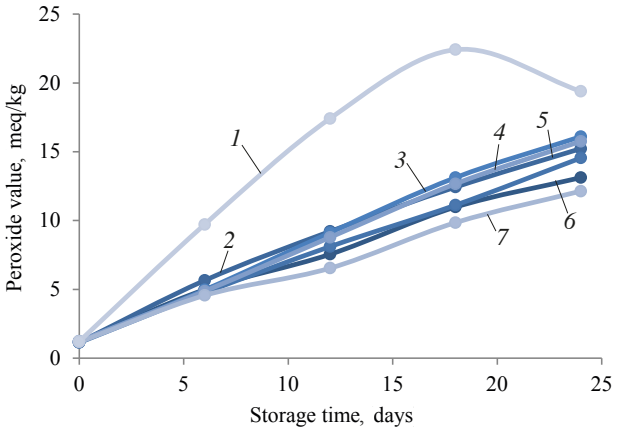

Fig. 1. PVs of hemp oil samples: *1* – control; *2* – green tea extract 30 mg/l; *3* – pomegranate extract 30 mg/l; *4* – pomegranate extract 50 mg/l; *5* – green tea extract 50 mg/l; *6* – rosemary extract 50 mg/l; *7* – vitamine E 2 g/l.

*ISO 3960. Animal and vegetable fats and oils. – Determination of peroxide value. – Iodometric (visual) endpoint determination. 2010.
**ISO 660:2009. Animal and vegetable fats and oils – Determination of acid value and acidity. 2009.

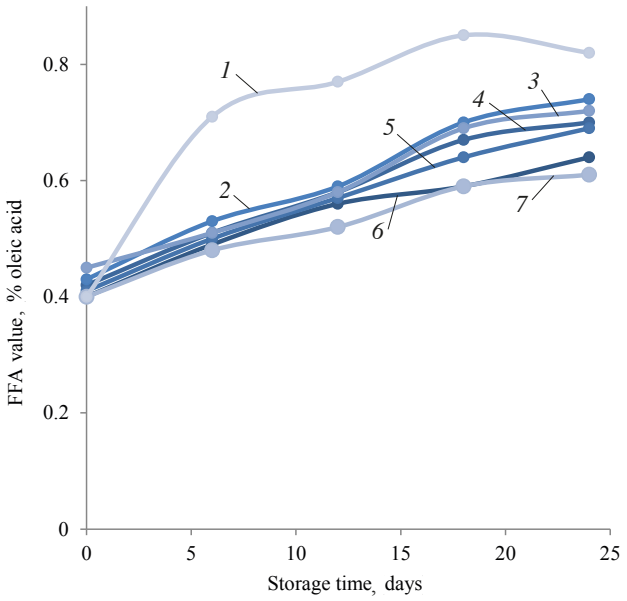

Fig. 2. FFA values of hemp oil samples: *1* – control; *2* – pomegranate extract 30 mg/l; *3* – pomegranate extract 50 mg/l; *4* – green tea extract 30 mg/l; *5* – green tea extract 50 mg/l; *6* – rosemary extract 50 mg/l; *7* – vitamine E 2 g/l.

Peroxide value analysis. Fig. 1 shows the effect the storage at 25°C produced on PV of the seven samples. On day 24, PV of all the oil samples increased. The PVs of the control sample demonstrated a decrease on day 24 after a stable increase from day 0 to day 18. The decrease in the PV rates was caused by the unbalanced initial oxidation products which are responsive to dissociation and carbonyl compounds [34]. On day 24, the blank sample had significantly higher PVs ($p < 0.05$) compared to the samples with rosemary, green tea, pomegranate, and vitamin E. The latter effectively slowed down the formation of hydro peroxide, and therefore postponed lipid oxidation. Plant phenolic compositions decrease lipid oxidation because of their radical-scavenging ability. Phenolic composition could delay the oxidation process owing to the decay of lipid hydro-peroxides. Various phenolic compounds indicate different capacity in delaying lipid oxidation. The difference in their impact is usually attributed to the variation in their structure [26].

PV of the control hemp oil was 19.4 ± 0.12 meq/kg. PVs of experimental samples by day 24 were significantly different ($p < 0.05$) compared to the control sample. Rosemary, green tea extract, and vitamin E revealed a greater ability to delay the PV rates of oils. By day 24, the lowest PV was 12.14 ± 0.17 meq/kg for the vitamin E sample. The effect of rosemary extracts was greater than that of the green tea and pomegranate extracts. PVs of hemp oil with rosemary extract were 14.43 ± 0.06 meq/kg (30 mg/l) and 13.12 ± 0.17 meq/kg (50 mg/l). PVs of hemp oil with pomegranate extract were 16.1 ± 0.09 meq/kg (30 mg/l) and 15.76 ± 0.13 meq/kg (50 mg/l). PVs of hemp oil with green tea extract were 15.23 ± 0.05 (30 mg/l) and 14.55 ± 0.08 meq/kg (50 mg/l). PV of hemp oil with vitamin E was 12.14 ± 0.03 meq/kg.

The peroxide value characterizes the amount of hydro-peroxides created over the primary phase of oxidative rancidity of oils and fats [29]. A higher PV implicates a lower oxidative stability [28]. According to the Codex Alimentarius Commission (1999), PV of refined oils cannot exceed 10 meq/kg, PV of cold pressed virgin oils cannot exceed 15 meq/kg. According to Rossel [33], fresh refined oil must have a PV < 1 meq/kg. However, some oils acquired an off-flavour after refining PVs up to 10 meq/kg at storage condition. PV indicates good relation with flavor scores. However, PV is restricted to the primary stages of oxidation and gives an indication of the available status of oxidation. It does not demonstrate potential oxidation.

Free fatty acids (FFA) and acidity value (AV). Fig. 2 shows FFA values of the hemp oil samples. By day 24, there was a slightly lower rise in FFA in the herbal and vitamin E samples. The FFA of the blank sample demonstrated a more significant increase, if compared to the other samples. The samples with the 2 g/l concentration of vitamin E had the minimum FFA content by the end of the storage period. FFA values of the sample with rosemary extract (30 mg/l, 50 mg/l) were 0.65 ± 0.02 (% oleic acid) and 0.64 ± 0.01 (% oleic acid), respectively. The FFA values of the sample with pomegranate extract (30 mg/l, 50 mg/l) were 0.74 ± 0.03 (% oleic acid) and 0.72 ± 0.02 (% oleic acid), respectively. The FFA values of the sample with green tea extract (30 mg/l, 50 mg/l) were 0.7 ± 0.01 (% oleic acid) and 0.69 ± 0.02 (% oleic acid), respectively. The FFA values of the sample with vitamin E was 0.61 ± 0.03 (% oleic acid) by the end of the storage period. The FFA value of the control sample was 0.82 ± 0.01 (% oleic acid). The results were not significantly different ($p < 0.05$) from the control sample by day 24.

Fig. 3 shows the AVs of the hemp oil samples. At the beginning, AVs of the samples were not significantly different ($p < 0.05$) compared to the control sample. By day 24, AV of the control sample was 1.64 ±

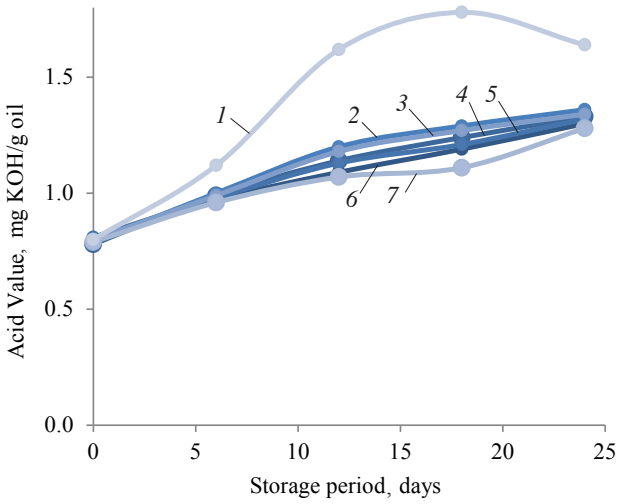

Fig. 3. AVs of hemp oil samples: *1* – control; *2* – pomegranate extract 30 mg/l; *3* – pomegranate extract 50 mg/l; *4* – green tea extract 30 mg/l; pomegranate extract 30 mg/l; *5* – green tea extract 50 mg/l; *6* – rosemary extract 50 mg/l; *7* – vitamine E 2 g/l.

Table 2. Fatty acid composition of various hemp oils on days 0 and 24

Fatty acids	Hemp oil samples								
	Blank		With rosemary extract		With pomegranate extract		With green tea extract		With vitamin E
			30 mg/l	50 mg/l	30 mg/l	50 mg/l	30 mg/l	50 mg/l	2g/l
	Day 0	Day 24	Day 24						
C16:0	9.41	7.12	7.08	7.09	7.21	6.98	7.47	7.42	6.96
C18:0	1.84	2.84	2.80	2.81	2.87	2.81	3.02	3.02	2.80
C16:1	0.12	0.14	0.13	0.12	0.15	0.12	0.18	0.17	0.12
C18:1	9.48	12.21	12.04	11.96	12.26	12.17	12.85	12.62	12.10
C18:2cis	59.32	53.98	54.18	54.17	53.85	54.30	53.11	53.30	53.20
C18:2 trans	4.29	0.89	NA	NA	NA	NA	NA	NA	NA
C18:3	19.15	16.79	16.87	16.87	16.71	16.74	16.47	16.54	16.48
SFAs	10.67	14.43	11.24	11.30	11.46	11.19	11.85	11.80	11.10
MUFAs	10.35	13.43	12.63	12.54	12.88	12.77	13.129	13.28	12.48
PUFAs	78.96	72.10	76.00	75.96	75.45	75.92	74.43	74.67	75.56
n-3 PUFA	19.15	10.51	16.87	16.87	16.71	16.74	16.47	16.54	16.10
n-6 PUFA	59.81	49.87	59.12	59.09	58.73	59.18	57.95	58.13	58.45
Omega 6/3 acids ratio	3.12	3.50	3.50	3.502	3.51	3.534	3.517	3.513	3.631

Note: C16:0 – palmitic acid; C18:0 – stearic acid; C16:1 – palmitoleic acid; C18:1 – oleic acid; C18:2 – linoleic acid; C18:3 – linolenic acid; SFAs – saturated fatty acids; MUFAs – monounsaturated fatty acids; PUFAs – polyunsaturated fatty acids

0.01 mg KOH/g oil. The vitamin E sample demonstrated the lowest AV (1.28 ± 0.04 mg KOH/g oil). Compared to the blank sample, AVs increased slightly during the storage. The rosemary sample AVs were 1.32 ± 0.05 (30 mg/l) and 1.3 ± 0.06 (50 mg/l) mg KOH/g oil. The green tea AVs were 1.33 ± 0.02 (30 mg/l) and 1.32 ± 0.01 (50 mg/l) mg KOH/g oil. The pomegranate AVs were 1.36 ± 0.03 (30 mg/l) and 1.34 ± 0.02 (50 mg/l) mg KOH/g oil by the end of the storage period.

FFA value indicates hydrolysis of fats, more specifically, the existence of FFAs. FFAs appear because of the hydrolysis of triglycerides and could be supported by humidity content [30]. The FFA content of the samples increased during the storage period. FFA of the control sample demonstrated a more significant increase if compared with the other samples.

Fatty acid composition. The changes in the fatty acid compositions reveal the oxidative constancy and nutritional properties of oils. Table 2 shows the fatty acid composition of the hemp oil samples on day 0 and day 24. All the samples were found to contain similar amounts of specific fatty acids, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs). Linoleic acid (C18:2) and α-Linolenic acid (C18:3) predominated. The primary SFA content in the control sample was 10.67%. The levels of the SFA, and MUFA increased in all the samples by day 27. Some changes were considerable, while others were insignificant.

PUFA decreased for all the samples from day 0 to day 24. Vitamin E and natural extracts protected the unity of unsaturated fatty acids, if compared to the blank sample. According to Gargouri *et al*., the unsaturated fatty acids of the oils easily oxidized [17]. An increase in the number of double bonds in unsaturated fatty acids reduced the oxidative constant [8]. The addition of rosemary, green-tea, pomegranate, and vitamin E into oils can be an applicable and effective attempt to preserve the oil from oxidation during storage. Furthermore, natural extracts offer oil industry a novel opportunity to protect unsaturated fatty acids. Thus, vitamin E, rosemary, pomegranate, and green tea extracts provide extra conservation to vegetable oils and fats and improve their nutritional values.

CONCLUSION

While being one of the oldest cultivated plants in history, hemp (*Cannabis sativa L.*) still remains one of the most popular herbs in various branches of industry. Hemp seed oil possesses very important ingredients that have a beneficial effect on human health, e.g. various antioxidants, Omega-3 and Omega-6 fatty acids, etc. However, hemp oil is very unstable against oxidation due to its high content of PUFAs. Rosemary, green tea, pomegranate extracts, and vitamin E can have a positive effect on hemp oil quality. The Rancimat method and other chemical tests proved the positive effect of these extracts on the oxidative stability of hemp oil. The experiment measures the changes in PV, FFA, AV, and fatty acid composition. Thus, natural ingredients raise the quality of oils and can be recommended for oil manufacturers.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this article.

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Effects of various brans on quality and volatile compounds of bread

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Abstract: The present study aimed to evaluate the effect of various bran sources, including wheat, barley, and rice, on the quality and volatile compounds of Egyptian 'balady' bread (Fino). The protein, fat, and total carbohydrates content of the studied brans ranged from 8.49 to 14.16%, 2.16 to 8.12%, and 34.38 to 85.06%, respectively. The mineral composition and colour parameters of the brans were also evaluated. The substitution of wheat flour with 10%, 20%, and 30% of different brans resulted in decreased loaf volume and specific volume, and increased loaf weight. A significant decrease in colour parameters (L, a, and b) of the bread crust and crumb were observed in all the samples. The addition of bran at three concentrations showed a remarkable increase in the total phenolic content of the bread samples, compared to the control. The antioxidant activity of the bread samples fortified with brans showed the following order: RB (rice bran) > BB (barley bran) > WB (wheat bran), as determined by the DPPH and β-carotene assays. Thirty-six volatile compounds identified in the bread samples using GC-MS included 5 alcohols, 6 pyrazines, 2 acids, 9 aldehydes, 5 ketones, 3 esters, and 6 sulphur-containing compounds. Alcohols were the predominant volatile constituents accounting for 58.3; 61.57; 59.08; and 56.15% in the control and in the bread samples prepared with bran from rice, barley, and wheat, respectively.

Keywords: Bran, bread, chemical composition, volatile compounds

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INTRODUCTION

Bread is one of the most important sources of dietary fibres, micronutrients, proteins, and vitamins. Therefore, it is considered effective, when fortified with suitable fibre fractions, in treating various diseases, such as obesity, cardiovascular disease, type 2 diabetes, etc [1]. There is a growing demand for bread in the whole world, especially in developing countries such as Egypt. At the same time, consumers increasingly prefer functional foods that contain ingredients providing health benefits beyond basic nutrition [2, 3].

The whole grain of wheat consists of germ, endosperm, and bran. Milling results in a dramatic loss of healthy biochemical molecules, such as antiradical constituents, fibre, vitamins, and minerals, causing cardiovascular and other types of disease [4]. Only endosperm, which contains a significant amount of carbohydrates, remains after milling. However, cereal products prepared from whole grain are not as popular as those from refined flour due to reduced quality and degraded sensory properties caused by the presence of bran. The detrimental effects of bran can be decreased by various methods such as hydration, fermentation, and size reduction [5–7]. Bran is the main by-product of milling. It is a valuable and inexpensive source of dietary fibre that contains approximately 27% of total carbohydrates, 14% of protein, and 5% of minerals [8, 9]. The chemical composition of wheat bran depends on wheat variety, environmental conditions, etc. Therefore, the source of bran is a critical factor for the quality of wheat grain products [10].

The world production of rice bran reaches 29.3 million tons annually [11]. Introducing defatted rice bran in wheat flour is a useful method of increasing lysine, protein, and fibre contents [12]. A high protein content (11–17%), excellent nutritional value, and a considerable amount of fibre (20–27%) make rice bran a good source of bread fortification [13, 14]. The addition of rice bran at a concentration of 15–30% did not change the physicochemical properties of bread [15].

According to the Food and Agriculture Organisation (FAO), Egypt produces 117,113 tons of barley grains per annum on an area of about 87,752 ha [16]. It was reported by Anderson *et al.* that barley contains a significant amount of β-glucan which helps to reduce low density li-

poprotein (LDL) and total cholesterol in serum of both humans and animals [17–19]. A study into the effect of barley bran on bread quality found that 15% was the best amount of barley bran for bread fortification that ensured high quality and health benefits [20].

Texture, volume, and appearance of bread are important quality criteria for consumers. However, taste and aroma play a dramatic role for both producers and consumers. Approximately 300 volatile compounds are identified in bread that fall into several classes such as alcohols, esters, aldehydes, etc. They result from various interactions between the type and concentration of ingredients during processing, yeast activity during fermentation, and fermentation conditions (time, temperature, etc.) [21, 22].

The current study aims to compare the effects of substituting wheat flour with various brans on the chemical composition, as well as antioxidant and volatile compounds of Egyptian Fino bread.

STUDY OBJECTS AND METHODS

Materials. Wheat flour (WF) (72% extraction) and wheat bran (WB) were obtained from the North Cairo Flour Mills Company (Egypt). Rice bran (RB) was obtained from a local milling factory (Zifta, Egypt). Barley bran (BB) was obtained from a pilot plant at the National Research Centre (Dokki, Egypt). All chemicals used in this study are of analytical grade.

Methods.

Milling. Barley grains (Giza, 136) were manually cleaned, tempered to 14% moisture content, milled using a Quadrumat Junior flour mill (Model MLV-202, Switzerland), and sieved to obtain flour and bran.

Stabilizing rice bran. The bran was immediately stabilized using oven heating (at 110°C for 10 minutes). Immediately subsequent to heating, the sample was removed from the oven and cooled to room temperature (25°C). The stabilized rice bran was milled into flour. The flour was screened through a 30-mesh sieve, supplemented with wheat flour, and stored under freezing conditions.

Making Fino bread. Different Fino bread blends were prepared by using WF (72% extraction) and the studied bran at a concentration of 10%, 20%, and 30%. Active dry yeast (1.5%), NaCl (1.5%), sugar (2%), shortening (1%), bread improver (1%), and water (an amount required to reach 500 Brabender Units of consistency) were added to each sample in the pilot plant at the National Research Centre (NRC) in Dokki, Egypt. Fino bread was made according to Hussein *et al.* in an electric oven (Mondial Formi, 4T 40/60, Italy) [23]. Firstly, yeast was dissolved in warm water (35°C) and added to the dry ingredients and the shortening; then the mixture was kneaded. The dough was fermented at 30°C for 30 min in a fermentation cabinet under 80–85% relative humidity. Then it was divided into 80 g pieces that were placed in the trays and proofed under the same conditions for 45 min. The dough loaves were baked at 325°C for 10–15 min after steaming for 10 sec. To enhance the browning process of protein bread, the dough pieces were brushed with melted margarine prior to baking. The baked loaves were cooled down at room temperature for 60 min. Weight, volume, and specific volume of the bread samples were determined as described by [24].

Analytical methods. Moisture, protein, fat, ash, and fibre of raw materials and Fino bread were determined according to AOAC Official Methods of Analysis International, while carbohydrates were calculated by difference as in Tadrus's study [25, 26]. Individual elements (Ca, P, K, Na, Fe, Mn, and Cu) in all the samples were determined according to Chapman and Pratt [27]. Changes in Hunter colour parameters (L, a & b) of raw materials and Fino bread were followed up using a Tristimulus Colour Analyzer (Hunter, Lab Scan XE, Reston, Virginia) with a standard white tile.

Bread freshness. The freshness of the bread samples was tested at day 0, 3 and 7 of storage at room temperature by alkaline water retention capacity (AWRC) according to the method described by Hussein *et al.* [28].

Sensory properties. The Fino bread samples were evaluated for taste (20), aroma (20), mouth feel (10), crumb texture (15), crumb colour (10), break & shred (10), crust colour (10), and symmetry shape (5) according to the method described in [24].

Total phenolics extraction. Ten grams of powdered bread was extracted with 75 ml 100% methanol at 25°C for 24 hours along with stirring followed by filtration using Whatman no.1 filter paper. The residues were re-extracted twice as described above. The combined methanolic extracts were evaporated at 40°C under vacuum until dry.

Total phenolics determination. The concentration of phenolic compounds in the bread samples was estimated with the Folin–Ciocalteu reagent according to the method described by Singleton and Rossi [29]. One millilitre of a sample (5 mg) was mixed with 1 ml of the Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate (20%) solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 765 nm. Gallic acid was used to construct the standard curve (8–80 μg/ml). The results were expressed as mg of GAEs (gallic acid equivalents/g).

Determination of free radical scavenging activity. The antioxidant activity of the methanol extracts was determined by the DPPH radical scavenging method as described by Woldegiorgis *et al.* [30]. A 0.004% solution of the DPPH radical solution in methanol was prepared and then 2 ml of the DPPH solution was mixed with 1 ml of various concentrations (0.1–0.5 mg/ml) of the extracts in methanol. Finally, the samples were incubated for 30 min in the dark at room temperature. The scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517 nm. The inhibition of free radical DPPH in percent (I %) was then calculated.

The scavenging activity, %, was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{treatment}/=A_{control})] \times 100.$$

β-Carotene-linoleate bleaching assay. The antioxidant activity of the methanol extract with various concentrations (0.1–0.5 mg/ml) was assayed according to the β-Carotene-linoleate bleaching method developed by Velioglu *et al.* [31]. 0.2 mg of β-Carotene (in 1 ml chloroform), 0.02 ml of linoleic acid, and 0.2 ml of Tween were transferred into a round bottom flask. The mixture was then added to 0.2 mg of methanolic extract prepared for the β-carotene-linoleate bleaching assay or 0.2 ml of standard methanol (as a control). Chloroform was removed at room temperature under vacuum at reduced pressure using a rotary evaporator. Following evaporation, 50 ml of distilled water was added to the mixture and then shaken vigorously to form an emulsion. Two millilitres aliquots of the emulsion was taken in test tubes and immediately placed in a water bath at 50°C. The absorbance was measured at 470 nm by a UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. Readings of all the samples were performed immediately (t = 0 min) and after 120 minutes of incubation. The antioxidant activity (%) was evaluated in terms of β-carotene bleaching inhibition using as follows:

$$\% \text{ Inhibition} = [(A_t - C_t)/(C_0 - C_t)] \times 100,$$

where A_t and C_t are the absorbance values measured for the test sample and control, respectively, after 120 min incubation, and C_0 is the absorbance values for the control measured at zero time during incubation. All the experiments were carried out in triplicate.

Ascorbic acid was used as a standard, and the extract-free mixture was used as a control.

Volatile compounds analysis. Flavour compounds were identified by GC/MS analyses. The instruments included a static headspace (Agilent 7890 GC coupled to a 5977 MS detector) with a column (DB-5; J&W Scientific Inc.) of 60 m in length, 0.25 mm in inside diameter, and 0.25 μm in membrane thickness. We also used a mass spectrometer (Automass SUN-200S; JEOL Ltd.) with a mass spectral search programme (Version 2.0; National Institute of Standards & Technology) for library search and identification, as well as software (EZChrom Elite; GL Sciences Inc.) for the quantification of identified total ion peak areas. For the static headspace, 3 grams of the whole bread samples were encapsulated in a glass vial container (22 ml). The analytical conditions of the headspace included a sample weight of 3 g, a sample temperature of 80°C, an injection temperature of 160°C, an injection duration of 36 sec, a needle temperature of 80°C, and a transferring temperature of 160°C. High-purity helium carrier gas (1.2 ml/min) was employed for gas chromatography. The column was held at 50°C for 3 min, with the temperature programmed to 220°C at a heating rate of 4°C/min, and then held at 220°C for 15 min. The analytical conditions of the mass spectrometer included an interface temperature of 220°C, a transferring temperature of 160°C, and an ion source temperature of 230°C. The ionization energy of the mass spectrometer was 70 eV and a scan cycle time was 0.5 ms (33–40 m/z).

Compounds identification. The linear retention index (RI) values for unknowns were determined based on retention time data obtained by analyzing a series of normal alkanes (C_6–C_{22}). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory (Adams, 2007).

Statistical analysis. The obtained results were evaluated statistically using analysis of variance as reported by Mc-Clave and Benson [32].

RESULTS AND DISCUSSION

Chemical composition of wheat flour and bran. The proximate composition of the brans under study is presented in Table 1. The protein content of different brans ranged from 8.49 to 14.16%. The fat content varied from 2.16 to 8.12% in BB and RB, respectively. Rice bran exhibited the most concentrated source of dietary fibre (36.18%) among all cereal brans. Barley

Table 1. Chemical composition of brans, %

Sample	Moisture	Protein	Fat	Fibre	Ash		Carbohydrates
WF (72%)	11.50 ± 0.12	12.65 ± 0.36	1.16 ± 0.06	0.48 ± 0.06	0.65 ± 0.03		85.06 ± 0.72
WB	9.12 ± 0.11	8.49 ± 0.10	3.82 ± 0.09	15.16 ± 0.11	14.59 ± 0.15		57.94 ± 1.22
RB	10.55 ± 0.15	14.16 ± 0.19	8.12 ± 0.05	36.18 ± 0.15	7.16 ± 0.19		34.38 ± 1.03
BB	13.85 ± 0.22	12.52 ± 0.17	2.16 ± 0.03	12.35 ± 0.19	5.62 ± 0.17		67.35 ± 0.86
			Mineral composition, mg/100 g				
	Na	K	P	Ca	Fe	Zn	Cu
WF (72%)	130.12 ± 0.96	45.12 ± 0.33	182 ± 0.99	26 ± 0.12	2. 65 ± 0.09	0.96 ± 0.01	0.18 ± 0.001
WB	1.2 ± 0.03	686 ± 3.19	588 ± 2.15	42.35 ± 0.15	6.1 ± 0.10	4.2 ± 0.10	0.6 ± 0.003
RB	5.9 ± 0.06	1650 ± 4.12	1780 ± 3.19	67.5 ± 0.22	12.9 ± 0.13	7.6 ± 0.17	0.9 ± 0.005
BB	6.5 ± 0.09	860 ± 3.18	790 ± 2.66	72.5 ± 0.39	8.32 ± 0.15	5.9 ± 0.23	0.72 ± 0.002
			Hunter colour parameters				
	L		a		b		
WF (72%)	92.20 ± 1.15		0.62 ± 0.03		11.36 ± 0.13		
WB	70.64 ± 1.29		6.13 ± 0.22		20.12 ± 0.19		
RB	73.92 ± 1.35		4.76 ± 0.19		17.45 ± 0.22		
BB	70.70 ± 1.62		6.13 ± 0.12		20.12 ± 0.14		

Note: WF (72%) is wheat flour extract 72%; WB is wheat bran; and RB is rice bran; BB: barley bran

bran had the maximum value for total carbohydrates (67.35%). Maximum ash content (14.59%) was observed in wheat bran. Wheat flour (72%) contained 85.06% of carbohydrates. Similar results were reported by O. Ozdestan *et al.* [33, 34].

Mineral content. According to Table 1, the brans under study were superior in sodium, potassium, phosphorus, calcium, iron, zinc, and copper, compared to wheat flour. These data agree with those found by Faria *et al.* who reported that Ca and P contents were 63.3 and 979 mg/100 g in rice bran and 65 and 979 mg/100 g in stabilized rice bran, respectively [35]. Also, the results in Table 1 showed an increased amount of K and P in rice bran, compared to the other raw materials.

Colour attributes. Colour plays an important role in the consumer's choice of foods, especially bakery products. The colour parameters of the brans, as well as wheat flour 72%, were evaluated using a Hunter laboratory colourimeter (Table 1). The bran samples were darker than WF. The same trend was observed with yellowness (a*): it was higher for different brans, compared to wheat flour. The obtained results are in good agreement with Kim *et al.* And Ramy *et al.*: the presence of bran produces darker bakery products [36, 37]. Therefore, we should control its concentration or use suitable additives to reduce this browning.

Bran effects on physical properties. The physical characteristics of the Fino bread produced with different concentrations of brans are presented in Table 2. The results revealed an increase in the loaf weight and a decrease in the loaf volume as the bran level increased, which was true for all types of bran. A significant volume decrease was observed in the bread supplemented with rice bran, compared to the other brans. We believe that this effect was caused by a high fibre content in rice bran (Table 1) and its higher water holding capacity. Another explanation of the reduced loaf volume could be the dilution effect of bran on gluten and a lower retention of CO_2. The specific loaf volume of bread containing 20 or 30% of bran had lower values compared to that of the control sample (Table 2). The volume parameters are critical for consumer acceptance [38]. The loaf volume gradually decreased in all the bread samples, compared to the control, with its values ranging from 220 (for 30% of BB) to 280 cm^3 (for 10% of WB).

Table 2. Physical properties of bread with various amounts of brans

Sample	Weight, g	Volume, cm	Specific volume, cm^3/g
Control	70.5 ± 0.12	290 ± 1.65	4.11 ± 0.19
WB concentration,%:			
10	73.2 ± 0.15	280 ± 1.20	3.83 ± 0.19
20	77.5 ± 0.17	265 ± 1.35	3.42 ± 0.32
30	82.0 ± 0.21	250 ± 1.42	3.05 ± 0.39
RB concentration, %:			
10	74.2 ± 0.22	270 ± 2.6	3.64 ± 0.19
20	78.5 ± 0.15	250 ± 1.35	3.18 ± 0.32
30	84.0 ± 0.11	230 ± 2.15	2.74 ± 0.39
BB concentration, %:			
10	76.2 ± 0.10	260 ± 1.75	3.41 ± 0.42
20	80.5 ± 0.20	240 ± 2.0	2.98 ± 0.49
30	86.0 ± 0.17	220 ± 1.65	2.56 ± 0.51

These results were in agreement with [39] that substituted wheat flour with high concentrations of rice bran (20 and 30%), which decreased the loaf volume.

Bran effects on colour attributes. The colour measurements of different bread samples are shown in Table 3. The bread samples containing different proportions of bran had lower values of L, b, and a; moreover, the values decreased as the concentration of bran increased. All the fortified samples had slightly lower L values for crust than the control and therefore a slightly darker crumb colour was noticed.

Bran effects on sensory evaluation. The sensory characteristics of the Fino bread samples with different amounts of WB, RB, and BB are shown in Table 4. The results indicated that the addition of bran did not have a clear effect on the crust and crumb colour, whereas its effect on taste and smoothness was quite remarkable. All the changes, however, were in the acceptable range. The colour changes may be due a higher content of reducing sugars in bran, compared to wheat flour, and the Maillard reaction during the baking process. We can also

Table 3. Hunter colour parameters of Fino bread with various amounts of brans

Sample	Crust			Crumb		
	L	a	b	L	a	b
Control	$60.18^{a} \pm 0.11$	$12.90^{a} \pm 0.09$	$33.50^{a} \pm 0.15$	$73.15^{a} \pm 0.22$	$2.18^{d} \pm 0.19$	$24.55^{a} \pm 0.33$
WB amount,%:						
10	$52.39^{b} \pm 0.13$	$10.90^{c} \pm 0.11$	$30.41^{b} \pm 0.30$	$57.15^{b} \pm 0.22$	$6.20^{c} \pm 0.08$	$23.18^{a} \pm 0.10$
20	$48.60^{c} \pm 0.22$	$11.65^{b} \pm 0.19$	$28.15^{c} \pm 0.26$	$46.63^{c} \pm 0.26$	$7.26^{b} \pm 0.09$	$22.15^{c} \pm 0.18$
30	$44.19^{d} \pm 0.17$	$11.95^{b} \pm 0.17$	$26.11^{d} \pm 0.21$	$42.60^{d} \pm 0.15$	$8.13^{a} \pm 0.08$	$21.22^{d} \pm 0.21$
RB amount,%:						
10	$55.13^{b} \pm 0.69$	$12.03^{ab} \pm 0.19$	$30.15^{b} \pm 0.63$	$60.19^{a} \pm 1.11$	$7.12^{b} \pm 0.09$	$24.50^{a} \pm 0.11$
20	$52.11^{b} \pm 0.55$	$11.75^{b} \pm 0.25$	$27.19^{c} \pm 0.35$	$52.15^{c} \pm 1.25$	$7.95^{ab} \pm 0.11$	$22.19^{b} \pm 0.19$
30	$46.50^{d} \pm 0.45$	$11.33^{c} \pm 0.39$	$25.18^{d} \pm 0.29$	$44.20^{d} \pm 1.19$	$8.20^{a} \pm 0.13$	$19.65^{e} \pm 0.26$
BB amount,%:						
10	$53.50^{b} \pm 0.62$	$12.00^{ab} \pm 0.10$	$29.17^{b} \pm 0.25$	$58.65^{b} \pm 0.15$	$6.70^{b} \pm 0.09$	$23.20^{b} \pm 0.44$
20	$48.35^{c} \pm 052$	$11.65^{b} \pm 0.08$	$26.15^{c} \pm 0.31$	$49.70^{c} \pm 0.19$	$7.55^{ab} \pm 0.07$	$21.15^{c} \pm 0.65$
30	$44.61^{d} \pm 0.39$	$11.21^{c} \pm 0.06$	$24.20^{d} \pm 0.44$	$42.50^{d} \pm 0.32$	$8.80^{a} \pm 0.03$	$18.19^{d} \pm 0.33$
LSD at 0.05	3.65	0.26	1.22	3.39	2.52	0.35

Table 4. Sensory evaluation of bread with various amounts of brans

Sample	Taste (20)	Aroma (20)	Mouth feel (10)	Crumb texture (15)	Crumb colour (10)	Break &shred (10)	Crust colour (10)	Symmetry shape (5)
Control	$18.5^{a} \pm 0.52$	$18.7^{a} \pm 0.55$	$9.3^{a} \pm 0.39$	$12.6^{a} \pm 0.32$	$9.0^{a} \pm 0.39$	9.12 ± 0.19	$8.6^{a} \pm 0.22$	4.5 ± 0.42
WB amount, %:								
10	$16.9^{b} \pm 0.53$	$18.2^{b} \pm 0.72$	$8.9^{b} \pm 0.35$	$11.9^{b} \pm 0.45$	$8.6^{b} \pm 0.52$	8.82 ± 0.35	$7.6^{ab} \pm 0.18$	4.2 ± 0.35
20	$15.8^{c} \pm 0.35$	$17.5^{c} \pm 0.69$	$7.9^{c} \pm 0.27$	$11.2^{c} \pm 0.60$	$7.6^{c} \pm 0.49$	8.65 ± 0.42	$6.7^{b} \pm 0.26$	4.2 ± 0.33
30	$14.6^{d} \pm 0.22$	$16.6^{d} \pm 0.61$	$7.5^{d} \pm 0.33$	$10.6^{d} \pm 0.38$	$6.8^{d} \pm 0.43$	8.45 ± 0.39	$5.9^{b} \pm 0.19$	4.0 ± 0.31
RB amount, %:								
10	$17.5^{ab} \pm 1.13$	$17.33^{a} \pm 1.12$	$8.3^{b} \pm 0.45$	$12.1^{ab} \pm 0.45$	$8.4^{b} \pm 0.33$	8.25 ± 0.49	$8.2^{a} \pm 0.15$	3.9 ± 0.18
20	$16.3^{bc} \pm 1.22$	$16.3^{c} \pm 1.25$	$7.8^{c} \pm 0.42$	$11.9^{b} \pm 0.50$	$7.5^{c} \pm 0.71$	8.19 ± 0.52	$7.6^{ab} \pm 0.36$	3.5 ± 0.25
30	$16.0^{c} \pm 2.11$	$16.0^{d} \pm 1.15$	$7.3^{d} \pm 0.35$	$11.2^{c} \pm 0.62$	$6.9^{d} \pm 0.61$	8.11 ± 0.29	$6.2^{b} \pm 0.42$	3.2 ± 0.30
BB amount, %:								
10	$17.3^{b} \pm 1.45$	$17.3^{a} \pm 1.31$	7.4 ± 0.27	$12.10^{ab} \pm 0.65$	$8.5^{b} \pm 0.61$	8.08 ± 0.35	$8.6^{a} \pm 0.19$	4.0 ± 0.17
20	$17.0^{b} \pm 1.62$	$16.5^{b} \pm 1.43$	7.5 ± 0.23	$11.7^{b} \pm 0.52$	$7.3^{c} \pm 0.56$	7.88 ± 0.36	$7.2^{ab} \pm 0.35$	3.90 ± 0.15
30	$16.2^{bc} \pm 0.96$	$16.2^{c} \pm 1.10$	7.7 ± 0.17	$11.0^{c} \pm 0.13$	$6.9^{d} \pm 0.35$	7.70 ± 0.62	$6.7^{b} \pm 0.61$	3.8 ± 0.11
LSD at 0.05	1.22	0.34	0.45	0.62	0.65	NS	0.95	NS

notice that increased concentrations of bran lead to a gradual decrease in hardness and smoothness, especially in the bread samples containing 30% of WB, RB, and BB. The results also showed a significant effect of WB, RB, and BB on the aroma of bread, primarily due to the flavour compounds.

The bread samples with 30% of WB, RB, and BB were significantly harder than the others. This may be due to the dilution of gluten and the thickening of the walls surrounding air bubbles in the crumb [40, 41]. All the Fino bread samples containing WB, RB, and BB showed an observed acceptability. Also, the addition of the brans changed the bread colour slightly and reduced the size of the holes, as confirmed by Sharma and Chauhan [39]. As can be noticed, there were no significant differences in break and shred and symmetry shape between the Fino bread from WF (control) and the samples with 10% of WB. However, the samples fortified with bran manifested significant differences in taste, aroma, mouth feel, crumb texture and colour, and crust colour. As the bran level increased, the crust colour score decreased.

Table 5. Changes in freshness of bread with various amounts of brans during storage

Sample	Storage time, days		
	0	3	7
Control	$300^{a} \pm 0.11$	$295.3^{a} \pm 0.13$	$290.4^{a} \pm 0.09$
WB amount, %:			
10	$290^{b} \pm 0.19$	$285.6^{b} \pm 0.15$	$280.1^{b} \pm 0.13$
20	$280^{c} \pm 0.22$	$275.3^{c} \pm 0.22$	$270.3^{c} \pm 0.19$
30	$275^{cd} \pm 0.17$	$270.5^{d} \pm 0.19$	$263.5^{d} \pm 0.25$
RB amount, %:			
10	$295^{ab} \pm 0.15$	$286.2^{b} \pm 0.20$	$278.5^{b} \pm 0.30$
20	$287^{bc} \pm 0.12$	$276.3^{c} \pm 0.16$	$269.8^{c} \pm 0.26$
30	$280^{c} \pm 0.10$	$271.5^{d} \pm 0.14$	$262.7^{d} \pm 0.17$
BB amount, %:			
10	$292^{b} \pm 0.25$	$287.3^{b} \pm 0.23$	$278.5^{b} \pm 0.15$
20	$285^{c} \pm 0.17$	$279.5^{c} \pm 0.19$	$269.4^{c} \pm 0.13$
30	$278^{d} \pm 0.23$	$269.7^{d} \pm 0.15$	$263.3^{d} \pm 0.32$
LSD at 0.05	5.9	9.4	8.9

Bran effects on staling. The changes that occur after baking can be defined as staling. They can be measured by the alkaline water retention capacity (AWRC) test. Increases in the AWRC showed the freshness of baked products [42]. Our results revealed a gradual increase in the staling rate for all the Fino bread samples during a prolonged storage time of about 7 days (Table 5). No differences were observed in the first 3 days, while 7 days of storage caused an increase in the staling rate for all the bread samples. It is clear that the Fino bread with WB, RB, and BB at concentrations of 10, 20, and 30% was fresher than the control under the same conditions due to its higher water retention capacity and a consequent improvement of its staling rate. This might be due to a higher content of fibres in bran-fortified bread compared to the control. The Fino bread samples with 10 or 20% of bran had a higher water retention capacity, compared to the control. Such an increase can be related to a higher hydrophilic nature of proteins. It was noticed that the bread fortified with 10 or 20% of bran showed a better consistency or high texture characteristics.

Bran effects on total phenolic content and antioxidant activity. The total phenolic content (TFC) of the brans under study, as well as the bread samples fortified with different concentrations of bran, was determined by the Folin-Ciocalteu method (Fig. 1). It was clear that the addition of all the brans showed a remarkable increase in the total phenolic content of the bread samples, com-

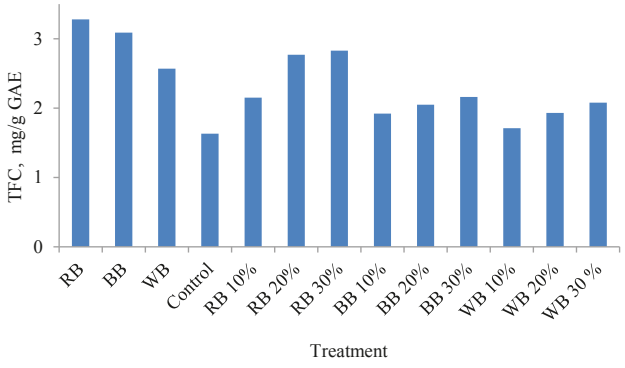

Fig. 1. Total phenolic content of bran and bread prepared with different amounts of brans.

pared to the control. The highest increase was found in the rice bran treatment, followed by barley bran and wheat bran, respectively.

The data obtained agree well with M. Irakli *et al.* who found an increase in both free and bound phenolic content in bread prepared with rice bran [43]. Also, a significant increase in the total phenolic content was reported by Laokuldilok *et al.* for the bread baked with red and black rice bran, compared to the control samples [44]. The surveys show no clear trend regarding the effect of thermal treatment on the total phenolic content of bread after baking. The degradation or damage of antioxidant components in flour during heating or baking may increase the total phenolic content, as reported by Holtekjolen *et al.* [45]. Another explanation, offered by Dordevic *et al.*, is that fermentation increases the concentration of various bioactive components in cereals, as well as the Maillard reaction, which may increase the total phenolic content during evaluation [46].

The antioxidant activity of the control and the bread samples with different concentrations of brans were determined by the DPPH and β-carotene assays. Fig. 2 shows a significant increase in the antioxidant activity of the bread samples, compared to the control, especially in the 30% amount of the brains. According to Fig. 2, the order of the antioxidant activity for the bread samples fortified with bran was as follows: RB > BB > WB. The correlation between the total phenolic content of bran and the antioxidant activity is in good agreement with Stratil *et al.* [47]. It was found that increased amounts of bran led to an increased antioxidant activity (Fig. 2).

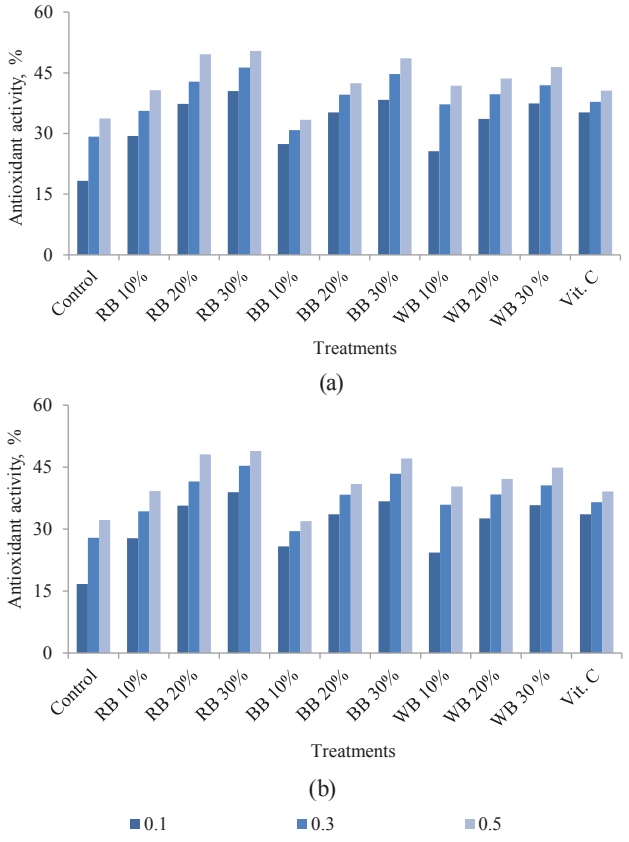

Fig. 2. Antioxidant activity of bread prepared with different amounts of bran as determined by DPPH (a) and β-carotene (b) assays.

Table 6. Volatile composition of whole Fino bread prepared with various brans

Volatile compounds	RI [a]	Control	RB	BB	WB
Alcohols					
2-Methyl-1-propanol	631 [b]	19.46	25.61	21.47	18.93
2-Methyl-1-butanol	746	5.21	4.93	5.91	6.18
3-Methyl-1-butanol	749	21.32	19.04	21.54	22.39
1-Hexanol	882	8.45	7.92	6.32	5.71
1-Octen-3-ol	993	3.94	4.07	3.84	2.94
Sub total		58.38	61.57	59.08	56.15
Pyrazines					
2-Methylpyrazine	836	2.43	3.16	4.82	2.18
2,5-Dimethylpyrazine	924	0.46	0.91	1.25	3.53
2,6-Dimethylpyrazine	927	1.03	0.03	3.49	1.24
2-Ethyl-3-methylpyrazine	1,008	0.78	0.54	1.02	0.62
2-Propylpyrazine	1,021	1.12	1.13	0.84	0.97
2,3-Diethylpyrazine	1,091	0.08	0.76	0.02	1.03
Sub total		5.9	6.53	11.44	9.57
Acids					
Octanoic acid	1,187	1.17	2.63	2.74	1.78
Nonanoic acid	1,279	0.95	1.89	1.96	1.02
Sub total		2.12	4.52	4.7	2.8
Aldehydes					
2-Methylbutanal	672	2.13	1.27	1.19	1.64
3-Methylbutanal	679	5.52	2.31	2.67	4.95
Pentanal	695	0.16	0.43	0.58	0.13
Hexanal	813	0.05	1.73	1.45	0.62
Heptanal	912	1.18	2.03	0.78	0.45
Octanal	1,015	2.31	1.48	1.33	1.89
Nonanal	1,114	1.78	1.29	0.69	2.34
Decanal	1,218	1.25	1.05	1.17	1.17
(Z)-2-decenal	1,261	0.04	0.57	1.03	0.02
Sub total		14.42	12.16	10.89	13.21
Ketones					
2,3-Pentanedione	693	0.07	0.61	1.34	0.08
2-Heptanone	904	1.18	0.95	0.51	1.23
3-Octen-2-one	1,047	1.29	1.73	1.32	1.16
(E,E)-3,5-octadien-2-one	1,103	0.25	0.46	0.49	0.18
Geranyl acetone	1,459	2.16	0.73	0.82	1.79
Sub total		4.95	4.48	4.48	4.44
Esters					
Isoamyl acetate	891	1.53	0.78	1.06	1.42
Ethyl hexanoate	1,013	0.97	0.56	0.83	0.85
Ethyl octanoate	1,211	2.04	1.94	2.05	1.67
Sub total		4.54	3.28	3.94	3.94
Sulphur-containing compounds					
2-Methylthiophene	783	1.18	0.83	1.29	1.03
2-Ethylthiophene	878	0.96	1.79	0.45	0.64
2-Furanmethanethiol	927	1.04	0.56	0.72	0.93
2-Propylthiophene	967	3.27	1.65	1.69	2.86
Dimethyl trisulfide	993	1.03	1.24	0.83	0.92
3-Formylthiophene	1,026	1.45	0.56	0.19	1.53
Sub total		8.93	6.63	5.17	7.91
Total		99.24	99.17	99.7	98.02

Note: a is RI retention indices determined on DB-5 capillary column; b is values expressed as relative area percentage to the total volatile compounds identified

Bran effects on volatile compounds. The sensory evaluation of the control and the bread samples made with various brans showed that in increased amounts of substitution had a negative effect on the sensory properties, especially on taste and aroma. Therefore, we selected the samples with 10% of the brans for volatile analysis using HS-GC/MS (Table 6).

The volatile compounds identified in bread (thirty-six) belonged to major chemical compounds: 5 alcohols, 6 pyrazines, 2 acids, 9 aldehydes, 5 ketones, 3 esters, and 6 sulphur-containing compounds [48, 49]. The volatile compounds identified as relative peak areas are listed in Table 6.

The analysis of volatile compounds using GC-MS showed that alcohols were the predominant volatile constituents accounting for 58.38; 61.57; 59.08; and 56.15% in the control and in the breads with bran from rice, barley, and wheat, respectively (Table 6). The main alcohols were 3-methyl-1-butanol and 2-methyl-1-propanol in the control sample at concentrations of 21.32 % and 19.46%, respectively. The high concentration of alcohols (61.57%) in the bread with rice bran may explain the low scores of sensory evaluation in this treatment. Some alcohols, such as 1-Octen-3-ol and hexanol, have a negative effect on the aroma of bread [50]. Our results revealed that these alcohols had higher concentrations in RB-containing bread, compared to the control and the other bread samples (Table 6). The second major volatile compound was aldehydes: 14.42% in the control sample and 13.21; 12.16; and 10.89% in the breads with brans from wheat, rice, and barley, respectively (Table 6). Among the most important aldehydes are octanal and nonanal, which have a positive effect on the flavour of bread [51]. These aldehydes were higher in the bread with wheat bran, compared to the other samples (Table 6), which can explain its higher acceptability according to the sensory evaluation (Table 4). Generally, the most common compounds of alcohols and aldehydes produce a positive effect on the bread flavour together with low levels of acids, and they could be used to explain the sensory analysis, as reported by Quilez *et al*. [52]. The contribution of an aroma compound to the flavour of food depends on its odour threshold, concentration, and food matrix, as well as the release of this volatile during mastication [53].

CONCLUSION

The present study emphasizes the importance of substituting wheat flour with bran to improve the nutritional value and fibre content of bread. While no significant changes in sensory evaluation were observed at a substitution amount of 10%, higher concentrations of bran significantly increased the total phenolic content and the antioxidant activity, and led to a negative sensory evaluation, compared to the control sample. However, all the changes were in the range of acceptability. Therefore, the study will extend to evaluate the changes during storage under various conditions to valorise the use of these cheap and nutraceutical ingredients in bread-making to produce highly acceptable functional foods with health benefits.

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Effects of Vietnamese tamarind fish sauce enriched with iron and zinc on green mussel quality

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Abstract: Marinating is a traditional technology to improve the shelf - life and quality of products. Enriched Vietnamese tamarind fish sauce was prepared and used to marinate green mussels. Subjects of this research were: green mussels (C1), marinated green mussels (C2), and marinated green mussels packaged in modified atmosphere (M1, M2, and M3). A percentage of O_2:CO_2:N_2 was 5:50:45 for M1, 5:70:25 for M2, and 5:90:5 for M3. Microbiological, chemical, and sensory qualities of the samples were analyzed during storage at 4°C for 30 days. The results indicate that glycogen, iron, and zinc contents as well as pH values decreased in all the cases during storage. In addition, TVB-N and TBARS values as well as mesophilic and lactic acid bacteria increased with time in all the samples. The number of psychrophilic bacteria in marinated samples was small or not detected at all. *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Vibrio cholera* were below standard values. Based on sensory acceptability, the shelf-life of C1, C2, M1, M2, and M3 samples was 12, 18, 24, 24, and 27 days, respectively.

Keywords: Vietnamese tamarind fish sauce, iron, zinc, marinated green mussel, modified atmosphere packaging (MAP)

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INTRODUCTION

Green mussel (*Perna viridis* L.) is a common marine animal in the Asia-Pacific region [1]. This is a large bivalve with smooth, elongated shell typical of several mytilids. Flesh of the green mussel is considered an excellent source of selenium, calcium, iron, magnesium, phosphorous and vitamins (A, B_1, B_2, B_6, B_{12} and C) [2, 3]. In addition, mussel fat is rich in polyunsaturated fatty acids (PUFA, 37–48% of total fatty acids) mainly ω–3 PUFA [4], which is biologically important and can decrease the risk of cardiovascular disease [5]. However, this filter feeder animal has a high risk of microbiological contamination that lead to a short shelf-life, poor quality, and economic losses. In addition, only well–cooked green mussels are microbiologically safe.

Marinating is a seasoning process mainly using organic acid containing liquids [6]. Liquid marinades generally include sugar, spices, oil, and acid such as vinegar, fruit juice or wine. Marinating improves tenderness, juiciness, taste and aroma, as well as improves the shelf-life of red meat, poultry, seafood, and vegetables [7–10].

Vietnamese tamarind fish sauce is popular in its country of origin. The main ingredients are tamarind pulp, fish sauce and sugar, while some fresh garlic and chili are used to enhance taste. The Vietnamese consume this sauce with various foods, such as seafood, fried food, dried food. It is even used as a condiment in various kinds of food products. Ready-to-cook products are expected to gain popularity in South-East Asia as well. Mineral deficiencies, including iron and zinc, remain problematic in developing countries. Thus, the enrichment of food products with minerals can be an effective way to solve this problem. However, according to a market survey, green mussels marinated with enriched tamarind fish sauce are not commercially avai-

lable, especially when packed in a modified atmosphere. Modified atmosphere packaging (MAP) is a technology that prolongs the shelf-life of food products by retarding microbial growth and biochemical reactions, often with an elevated carbon dioxide level [11]. Moreover, in MAP oxygen may inhibit the growth of anaerobic bacteria and accumulate toxins from *Clostridium botulinum*, type E [12]. However, botulinum toxins can form at below 2% oxygen [13, 14].

Therefore, the aim of this study was to determine the shelf-life of green mussels marinated with tamarind fish sauce enriched with iron and zinc. The mussels were packed in modified atmosphere and stored at 4°C.

STUDY OBJECTS AND METHODS

Materials. Tamarind pulp without seeds, fish sauce, white sugar, finger chili, garlic, and the fresh green mussels (40–50 individuals/kg) were purchased from a supermarket located in Songkhla province. Experiments were carried out at the Food Technology Department, the Agro-Industry Faculty of the Prince of Songkla University, Thailand. Iron sodium ethylenediaminotetraacetate ($FeNaEDTA{\cdot}3(H_2O)$) and zinc sulfate ($ZnSO_4{\cdot}7(H_2O)$) were taken from Sigma–Aldrich Company (Steinheim, Germany).

Marinated green mussel preparation. The green mussels were washed during 2 hours with tap water, then blanched in boiling water at 100°C for 30 s to facilitate meat removal. A ratio of sample to water was 1:2 w/w. Afterwards, the meat of green mussels was separated from the shells. We prepared tamarind fish sauce contained 42% of tamarind slurry, 37% of sugar, and 21% of fish sauce. 236 mg of $FeNaEDTA{\cdot}3(H_2O)$ and 88.5 mg of $ZnSO_4{\cdot}7(H_2O)$ were added into 30 g of the sauce, which was 36 mg of Fe and 20 mg of Zn, respectively. The green mussel meat was soaked in the enriched sauce at the ratio of 1:1. The marinated meat was packaged in 15 x 30 cm Havel Vacuum bags, b.v. (Europac Co., Ltd) with O_2 transmission rate 46.6 cm^3m^2 day^{-1} at 38°C, 1 atm. We tested five samples of green mussels: meat without marinade solution packed under normal air (C1), marinated meat without gases (C2), and meat packed in the modified atmosphere (M1, M2, and M3). A percentage of O_2:CO_2:N_2 was 5:50:45 for M1, 5:70:25 for M2, and 5:90:5 for M3. All samples were stored at 4°C for 30 days. Microbiological, chemical and sensory analyses were performed every 3 days.

pH. The marinated green mussels were blended with sterilized distilled water at the ratio of 1:5 and allowed to stand for 2 min. Then pH was measured by using a pH meter (Mettler 350, Singapore).

Thiobarbituric acid reactive substances (TBARS). The thiobarbituric acid reactive substances (TBARS) were determined by the method described in [15]. 10 g of chopped samples were homogenized with 50 ml of distilled water for 2 min and then transferred to distillation tubes. 47.5 ml of distilled water was additionally infused into the distillation tube to avoid losing the sample. 2.5 ml of 4 N HCl and a few drops of an anti-foam agent were added. 5 ml from 50 ml of the distillate were mixed with 5 ml of 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid (TBA reagent). The mixture was placed in a vial that was capped and heated in a water bath for 35 min. Afterwards, the vial was cooled down and the sample was measured at 532 nm using a spectrophotometer (UV/VIS T180, PG Instrument Ltd., UK). The results are expressed in mg malondialdehyde/kg sample.

Glycogen content. Glycogen content was determined based on the method of [16]. 50 mg of chopped marinated green mussels were boiled for 20 min with 400 µl of 33% KOH, cooled down, and 700 µl of 96% ethanol was added. The mixture was placed in an ice bath for 2 hours to reach complete precipitation. Thereafter, the mixture was centrifuged at 7,500 g for 20 min using a microlitre centrifuge (Z-233-M2, Hermle–Germany) and the supernatant was collected. The supernatant was mixed with 1 ml of distilled water and sonicated for 5 min using Crest Ultrasonic (575 DAE, Malaysia). Then 100 µl of the solution and 2 ml of anthrone reagent were kept at 90°C for 20 min in darkness. The reagent consisted of 38 ml of concentrated sulphuric acid with 15 ml of distilled water and 0.075 g of anthrone. The solution was measured at 620 nm using a spectrophotometer. Glycogen concentration was calculated from a standard curve for glycogen from Sigma–Aldrich Company (Steinheim, Germany).

Total volatile base nitrogen (TVB – N). Total volatile base (TVB – N) contents in the green mussels were determined using Conway micro-diffusion method described in [17]. 2 g of a sample were mixed with 8 ml of 4% tricloroacetic acid (TCA), then homogenized with a high-speed homogenizer for 1 min. The supernatant was filtered by Whatman No. 41 filter paper (Whatman International, Ltd., Maidstone, UK). 1 ml of the sample was placed in the outer ring, while 1% boric acid containing the Conway indicator was pipetted into the inner ring. 1 ml of saturated K_2CO_3 was added to the sample to initiate the reaction after mixing. Thereafter, the mixture was incubated at room temperature for 3 hours. Afterwards, the inner ring solution was titrated using 0.02N HCl until the green color turned to pink similar to a blank sample. TVB-N content was calculated as follows, mg/100 g:

$$\text{TVB} - \text{N}\,(\text{mg}/100\ \text{g}) = \frac{\text{N} \times 14 \times (\text{A} - \text{B}) \times \text{V} \times 100}{\text{Weight of sample}},$$

where: N is normality of HCl in titrant; A is titration volume for actual sample; B is titration volume for blank; V is total initial volume of sample with TCA.

Iron and zinc content. Samples of 0.5 g in mass were digested in 5 ml of concentrated nitric acid with 2 ml of 30% hydrogen peroxide under pressure in a closed vessel before heating in a microwave oven until the samples were digested. Afterwards, the samples were cooled down and filtered by Whatman filter paper No. 1 with a pore size of 110 nm (Whatman International, Ltd., Maidstone, UK)). Each sample solu-

tion was then transferred into a 25 ml volumetric flask and the volume was made up with distilled water [18]. The extract samples were used to determine iron and zinc by Inductively Couple Plasma Mass Spectrometry (ICP-MS) (Perkin-Elmer SCIEX, Shelton, CT, USA). The RF power was 1,350 W, the plasma gas flow rate was 15 l/min, the carrier gas flow was 0.94 l/min, and make-up gas flow was 0.15 l/min. The sample take rate was 100 µl/min and the sample depth was 6.0 mm.

Microbiological quality. 25 g of the marinated green mussels were blended with 225 ml of 0.1% sterilized peptone water. From the 10^{-1} dilution, dilutions to 10^{-2} and 10^{-3} were prepared with 0.1% sterilized peptone water. Mesophilic and psychrophilic bacteria, *Escherichia coli*, *Salmonella* spp, *Vibrio cholera*, and *Staphylococcus aureus* were determined using the method in [19]. Lactic acid bacteria were determined using de Man Rogosa and Shape agar by pour plate method, with incubation in anaerobic conditions at 35°C for 48 hours before colony count [20].

Sensory evaluation. Marinated samples were baked in a casserole pan (House worth HW-1707S, China) at 180°C for 10 min until the core temperature of the meat sample reached 80 ± 2°C. Then the samples were served to fifty untrained panelists of post-graduate students and technicians from the Department of Food Technology, Prince of Songkla University. The panelists were asked to evaluate the likability scores for appearance, aroma, texture, taste and overall on a nine-point hedonic scale.

Statistical analyses. All experiments were run in triplicates. The data were subjected to Analysis of Variance (ANOVA) and the differences between means were assessed for significance by Duncan's Multiple Range Test [21]. Data analysis was performed with the SPSS package (SPSS 6.0 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

We found that the sauce had pH 3.31, total titratable acidity 32.13%, and water activity 0.87. The viscosity of this enriched sauce was 840 cP, with non-Newtonian behavior. The colour coordinates *L**, *a**, and *b** were 26.44, 15.8 and 33.7, respectively.

pH. pH levels of un-treated and treated green mussels are shown in Fig. 1. In general, pH with all treatments decreased with storage time. The initial pH of un-treated green mussels (C1) was 6.77 and decreased to 6.21 at 15 days of storage ($p < 0.05$). The marinated samples had significantly lower pH than the control group due to acidity of the sauce (pH 3.31) ($p < 0.05$). The pH of marinated green mussels without gases (C2) was 4.48 on day 1 and declined to 4.07 by day 21 of storage ($p < 0.05$). The initial pH of marinated green mussel (M1, M2, and M3) was about 4.51–4.64 and dropped to 4.24, 4.2 and 3.95, respectively, at 30 days of storage ($p < 0.05$). The decrease in pH of meat during chilled storage is normally caused by conversion of glycogen to lactic acid and other volatile acids, as a result of muscle degradation [22]. Cao *et al.* also reported that the spoilage of mollusk shellfish was partly by fermentation indicated by decreasing pH [23]. Moreover, pH was lower with MAP using gases than with other treatments,

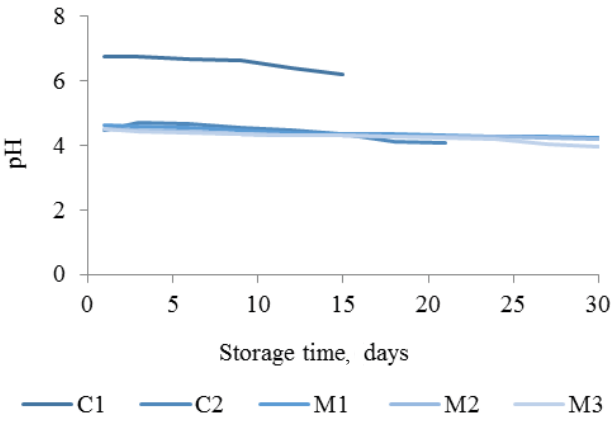

Fig. 1. Dependence of pH values of green mussels on storage time. C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively.

due to the dissolution of CO_2 that produced carbonic acid [24] and reduced production of alkaline compounds in the marinated samples [24, 25]. Higher concentrations of CO_2 (M3 > M2 > M1) gave lower pH levels in our experiments, although without significant differences. The pH value in our experiments was mainly due to the acidic marinade sauce with tamarind pulp.

TBARS. Fig. 2 demonstrates the TBARS levels in green mussels during storage for the various treatments. TBARS is indicator of the malondialdehyde content, which is a secondary lipid oxidation product from polyunsaturated fatty acids [26] causing off-flavors in meat products [27]. The initial TBARS of the control (C1) was 2.62 mg MDA/kg, while the marinated samples (C2, M1, M2, M3) had 0.99, 0.81, 0.92, 0.88 mg MDA/kg, respectively. It should be noted that the marinated green mussels had lower TBARS values than the control samples, apparently due to osmosis during marinating conveying TBARS out from the mussels. Also the bacterial count was higher for C1 than for the other samples, which could also play a role in producing TBARS [28]. The lower TBARS values of the marina-

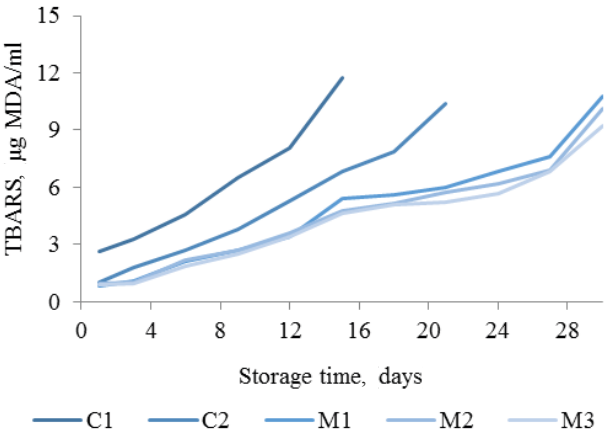

Fig. 2. Dependence of TBARS values of green mussel on storage time. C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively.

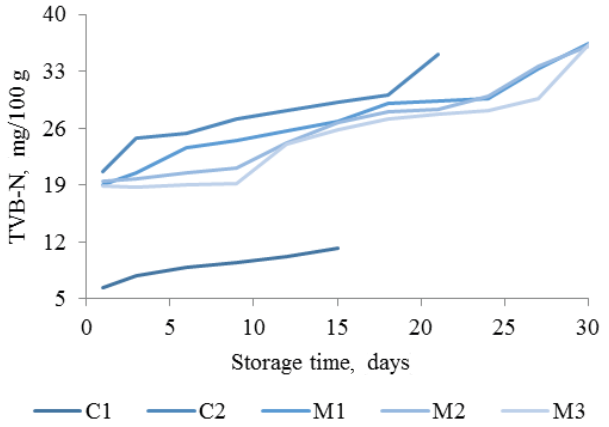

Fig. 3. Dependence of TVB – N values of green mussels on storage time. C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively.

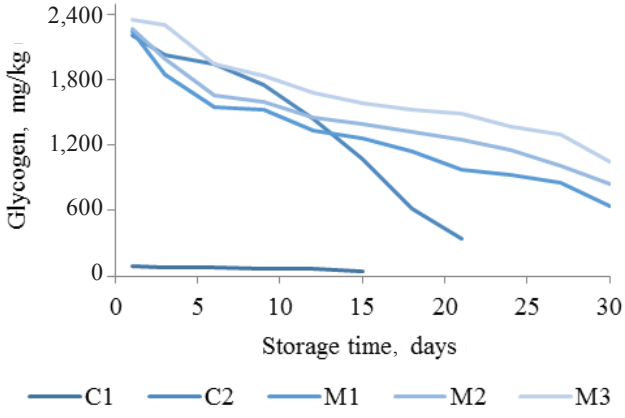

Fig. 4. Dependence of glycogen content of green mussels on storage time. C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively.

ted green mussels were consistent throughout the storage, and sulfur compounds from garlic in the marinade may have played a role as antioxidants [29]. However, TBARS in all cases increased during storage because of high content of polyunsaturated fatty acids in mussel fat, as mentioned in the introduction. Moreover, MAP packaging reduced TBARS may be due to O_2-elimination by enriched CO_2 proportion [22]. Masniyom *et al.* reported that seabass flesh in MAP did not suffer from increased lipid oxidation [30]. The TBARS level of 5–8 mg/kg malonaldehyde is a standard criterion of the poor quality of oily food [31, 32]. TBARS in the control (C1) reached 8.03 mg MDA/kg on day 12 of storage, while C2 had 7.85 mg MDA/kg on day 18. M1, M2, and M3 had TBARS levels below the standard on day 27, namely 7.59, 6.89, and 6.82 mg MDA/kg, respectively. The marinated green mussels packed at the percentage of O_2:CO_2:N_2 equaled 5:90:5 gave the least TBARS level at the end of storage ($p > 0.05$).

Total volatile base nitrogen (TVB-N). Changes in the TVB-N value of the treated and un-treated green mussels during storage are shown in Fig. 3. The TVB-N increased with storage time. The initial TVB-N of green mussels (C1) was 6.44 mg/100 g and increased gradually to 10.22 mg/100 g during storage. The treated samples C2, M1, M2, and M3 had initial TVB-N values 20.6, 18.97, 19.53 and 18.94 mg/100 g, respectively. By the end of the storage they reached 30.1, 33.33, 33.67, and 29.69 mg/100 g, respectively ($p < 0.05$). TVB-N is composed of volatile amines, typically dimethylamine, trimethylamine and ammonia [22], that commonly determine the spoilage of seafood [33]. The marinated samples had higher TVB-N values than the control even on day 1, due to volatile amines in the fish sauce [34]. In addition, the marinade contained garlic with sulfur compounds that could also react with boric acid, contributing to TVB-N content [29]. The increasing trend of TVB-N with time in this study agrees with the findings of [35]. Fishery products are acceptable for human consumption when TVB-N does not exceed 30 mg/100 g of sample [34]. Based on this, the shelf-lives of M3, M2, and M1 samples were 27, 24, and 25 days, respectively. In addition, [36] noticed that for seafood TVB-N indicated the freshness of raw materials and acceptability for human consumption. They pointed out that TVB-N increased with storage time as a result of quality deterioration. However, the initial TVB-N of the marinated products may not indicate the quality, considering that fish sauce and garlic used as ingredients cause erroneous results.

Glycogen content. During storage glycogen content decreased gradually in all samples, but declined rapidly in C2 samples ($p < 0.05$), as shown in Fig. 4. The initial glycogen content in the control sample (C1) was significantly lower than that in the marinated samples (C2, M1, M2, and M3). Glycogen is a polysaccharide including glucose units in a branched structure. Extraction and hydrolysis were used to obtain simple sugars in the glycogen content determination. As mentioned earlier, the marinade solution had 37% of sugar, so it positively contributed to the glycogen content. Jermyn [37] mentioned

Table 1. Iron and zinc content in green mussel samples during storage

Minerals	Samples	Initially	End of storage
Iron content, mg/kg	C1	71.44 ± 0.77^{a}	67.61 ± 0.67^{b}
	C2	599.62 ± 12.60^{a}	371.47 ± 2.33^{b}
	M1	389.67 ± 1.14^{a}	381.34 ± 1.99^{b}
	M2	399.2 ± 1.98^{a}	390.00 ± 1.41^{b}
	M3	481.93 ± 1.36^{a}	392.36 ± 0.44^{b}
Zinc content, mg/kg	C1	11.09 ± 0.40^{a}	8.95 ± 0.13^{b}
	C2	382.19 ± 8.22^{a}	249.00 ± 1.41^{b}
	M1	267.20 ± 1.56^{a}	257.74 ± 2.51^{b}
	M2	280.35 ± 0.64^{a}	266.17 ± 1.17^{b}
	M3	331.00 ± 1.98^{a}	271.11 ± 2.40^{b}

Note: $^{a–b}$ Means within rows with different superscripts are significantly different ($p < 0.05$). C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively

Table 2. Microbiological levels in marinated and non-marinated green mussel kept under different conditions during the storage at 4°C for 30 days.

Bacterial type	Treatment	Storage time, days										
		1	3	6	9	12	15	18	21	24	27	30
Mesophile, CFU/g	C1	9.3×10^3	7.0×10^4	2.5×10^5	6.3×10^5	8.6×10^5	1.2×10^6					
	C2	2.8×10^3	3.7×10^3	8.9×10^4	9.8×10^4	1.04×10^5	5.3×10^5	7.1×10^5	1.13×10^6			
	M1	< 100	3.6×10^3	6.5×10^3	7.9×10^3	8.6×10^3	1.30×10^4	1.63×10^4	1.90×10^4	2.45×10^4	9.0×10^4	1.42×10^6
	M2	< 100	2.5×10^3	5.2×10^3	5.7×10^3	6.3×10^3	9.2×10^2	1.05×10^4	1.47×10^4	2.10×10^4	2.6×10^4	1.12×10^6
	M3	< 100	< 100	< 100	3.0×10^3	5.2×10^3	7.4×10^3	9.6×10^3	1.10×10^4	1.80×10^4	2.48×10^4	9.8×10^5
Psychrophile, CFU/g	C1	8.5×10^3	1.56×10^4	2.0×10^4	2.13×10^4	2.32×10^4	5.0×10^4					
	C2	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10			
	M1	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	M2	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
	M3	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
E.coli, MPN/g	C1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0					
	C2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0			
	M1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M3	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Salmonella spp., CFU/g	C1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	C2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	M1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	M2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	M3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S.aureus, MPN/g	C1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0					
	C2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0			
	M1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M3	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
V.cholerae, MPN/g	C1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0					
	C2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0			
	M1	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M2	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
	M3	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0	< 3.0
Lactic acid bacteria , CFU/g	C1	9.9×10^3	1.5×10^4	1.76×10^4	2.1×10^4	2.6×10^4	3.5×10^4					
	C2	6.0×10^3	1.02×10^4	1.66×10^4	1.81×10^4	2.41×10^4	4.8×10^4	7.2×10^4	1.14×10^5			
	M1	8.0×10^3	9.5×10^3	1.43×10^4	1.72×10^4	2.10×10^4	2.5×10^4	4.6×10^4	6.2×10^4	8.5×10^4	1.12×10^5	2.12×10^5
	M2	6.0×10^3	7.6×10^3	9.4×10^3	1.02×10^4	1.71×10^4	2.38×10^4	2.5×10^4	3.5×10^4	4.1×10^4	5.6×10^4	1.36×10^5
	M3	2.5×10^3	3.4×10^3	5.4×10^3	8.3×10^3	1.34×10^4	1.87×10^4	2.25×10^4	3.2×10^4	3.5×10^4	4.2×10^4	6.2×10^4

Note: C1 = control, C2 = normal marinated, M1 = marinated at $5\%O_2$:$50\%CO_2$:$45\%N_2$, M2 = $5\%O_2$:$70\%CO_2$:$25\%N_2$, M3 = $5\%O_2$:$90\%CO_2$:$5\%N_2$

that in the determination of carbohydrates by anthrone method, other simple sugars in the system interfered. He pointed out that using glycogen content for glycolysis determination, which should decrease with storage time, might not give good results for high sugar products. The glycogen content decreased with storage time due to metabolism by glycolysis pathway in some bacteria [22]. The glycogen content of the control sample (C1) dropped to 61.42 mg/kg by day 12 of storage, while the marinated without MAP sample (C2) had 614.01 mg/kg after 18 days ($p < 0.05$). In the marinated samples packed with gases (M1, M2, and M3) the glycogen contents were 857.33; 1,005.68; and 1,293.17 mg/kg. The marinade with sugar strongly increased glycogen levels in marinated products. However, in C1the glycogen content decreased from 94.75 to 61.42 mg/kg during 12 days. The rapid decrease in glycogen levels of C2 may due to faster bacterial growth than with the treatments that included MAP, and the garlic from marinade solution seems to accelerate glycogen loss relative to the control (C1) perhaps by sulfur compounds and prebiotics in garlic that support lactic acid bacterial growth [22].

Iron and zinc content. Iron and zinc contents of green mussels with various treatments are presented in Table 1. On day 1, the iron content in C1 was 71.44 mg/kg, while that in the marinated samples C2, M1, M2, and M3 was 599.62; 389.67; 399.2; and 481.93 mg/kg, respectively. The initial zinc contents in C1, C2, M1, M2, and M3 were 11.09; 382.19; 267.2; 280.35; and 331 mg/kg, respectively. The iron and zinc contents may have fluctuated due to uncontrolled factors, such as uneven marinade absorption, the amount of marinade drip from the surface of green mussels during sample preparation for testing, or the small weight (1.0 g) of samples tested. If the samples are not uniform, then consistent results may not be obtained. The iron and zinc content in the marinated samples was higher than that in the control sample due to the absorption of iron and zinc from the marinade. Generally, the iron and zinc contents decreased by the end of the shelf-life to 67.61; 371.47; 381.34; 390.00;

Table 3. Sensory acceptability score of green mussel kept under different condition during storage at 4°C for 30 days

Storage time, day	Treatments	Attributes					
		Appearance	Flavor	Colour	Texture	Taste	Overall
1	C1	6.63 ± 0.72^{a}	6.40 ± 0.62^{b}	6.90 ± 0.99^{a}	7.07 ± 0.74^{ab}	6.57 ± 0.68^{a}	6.63 ± 0.73^{b}
	C2	6.90 ± 0.76^{a}	7.10 ± 0.84^{a}	6.90 ± 0.80^{a}	7.30 ± 0.75^{a}	7.03 ± 0.61^{a}	7.10 ± 0.84^{a}
	M1	6.93 ± 0.98^{a}	6.57 ± 0.63^{b}	6.73 ± 0.69^{a}	6.73 ± 0.52^{bc}	6.77 ± 0.68^{a}	6.60 ± 0.56^{b}
	M2	6.83 ± 0.65^{a}	6.80 ± 0.71^{ab}	6.53 ± 0.57^{a}	6.33 ± 0.48^{c}	6.77 ± 0.73^{a}	6.60 ± 0.67^{b}
	M3	6.90 ± 0.61^{a}	6.80 ± 0.81^{ab}	6.90 ± 0.96^{a}	6.83 ± 0.70$^{a\ bc}$	6.83 ± 0.70^{a}	6.80 ± 0.48^{ab}
3	C1	6.50 ± 0.73^{a}	6.27 ± 0.98^{b}	6.47 ± 0.78^{a}	6.97 ± 0.85^{a}	6.40 ± 0.93^{b}	6.23 ± 0.94^{c}
	C2	6.83 ± 0.91^{a}	6.87 ± 0.94^{a}	6.73 ± 0.83^{a}	6.80 ± 0.71^{a}	6.83 ± 0.83^{ab}	6.80 ± 0.76^{ab}
	M1	6.60 ± 0.81^{a}	6.40 ± 0.67^{b}	6.57 ± 0.50^{a}	6.63 ± 0.93^{ab}	6.50 ± 0.86^{ab}	6.53 ± 0.78^{bc}
	M2	6.70 ± 0.79^{a}	6.90 ± 0.84^{a}	6.60 ± 0.67^{a}	6.27 ± 0.52^{b}	6.40 ± 0.56^{b}	6.43 ± 0.50^{bc}
	M3	6.83 ± 0.79^{a}	6.67 ± 0.76^{ab}	6.83 ± 0.75^{a}	6.77 ± 0.63^{a}	6.93 ± 0.78^{a}	6.97 ± 0.96^{a}
6	C1	6.23 ± 0.63^{b}	6.13 ± 0.43^{c}	6.43 ± 0.63^{ab}	6.77 ± 0.82^{a}	6.70 ± 0.53^{abc}	6.13 ± 0.68^{c}
	C2	6.67 ± 0.71^{a}	6.80 ± 0.92^{a}	6.70 ± 0.88^{a}	6.57 ± 0.50^{abc}	6.80 ± 0.76^{a}	6.77 ± 0.90^{a}
	M1	6.60 ± 0.72^{a}	6.30 ± 0.53^{bc}	6.27 ± 0.52^{b}	6.33 ± 0.61^{bc}	6.43 ± 0.63^{bc}	6.57 ± 0.57^{ab}
	M2	6.60 ± 0.56^{a}	6.53 ± 0.63^{ab}	6.40 ± 0.50^{b}	6.20 ± 0.76^{c}	6.40 ± 0.62^{c}	6.27 ± 0.52^{bc}
	M3	6.83 ± 0.70^{a}	6.63 ± 0.67^{ab}	6.60 ± 0.67^{ab}	6.70 ± 0.70^{ab}	6.77 ± 0.68^{ab}	6.80 ± 0.71^{a}
9	C1	6.17 ± 0.53^{b}	6.03 ± 0.41^{c}	6.13 ± 0.68^{b}	6.50 ± 0.51^{ab}	6.03 ± 0.61^{b}	6.07 ± 0.69^{b}
	C2	6.57 ± 0.68^{a}	6.70 ± 0.79^{a}	7.00 ± 0.79^{a}	6.20 ± 0.61^{bc}	6.57 ± 0.90^{a}	6.63 ± 0.76^{a}
	M1	6.47 ± 0.51^{ab}	6.27 ± 0.69^{bc}	6.13 ± 0.63^{b}	6.23 ± 0.73^{bc}	6.30 ± 0.65^{ab}	6.37 ± 0.56^{bc}
	M2	6.53 ± 0.63^{a}	6.43 ± 0.50^{ab}	6.30 ± 0.47^{b}	6.13 ± 0.57^{c}	6.30 ± 0.60^{ab}	6.17 ± 0.59^{b}
	M3	6.47 ± 0.73^{ab}	6.53 ± 0.73^{ab}	6.50 ± 0.78^{b}	6.60 ± 0.67^{a}	6.63 ± 0.61^{a}	6.70 ± 0.60^{a}
12	C1	6.10 ± 0.55^{ab}	5.73 ± 0.58^{b}	6.03 ± 0.41^{c}	6.17 ± 0.53^{b}	5.87 ± 0.57^{c}	6.00 ± 0.45^{b}
	C2	6.03 ± 0.76^{b}	6.27 ± 0.45^{a}	6.57 ± 0.68^{a}	6.13 ± 0.63^{b}	6.20 ± 0.48^{bc}	6.13 ± 0.43^{b}
	M1	6.37 ± 0.49^{ab}	6.17 ± 0.38^{a}	6.07 ± 0.52^{c}	6.17 ± 0.59^{b}	6.17 ± 0.53^{b}	6.27 ± 0.58^{b}
	M2	6.47 ± 0.57^{a}	6.40 ± 0.72^{a}	6.23 ± 0.50^{c}	6.07 ± 0.58^{b}	6.23 ± 0.63^{ab}	6.10 ± 0.66^{b}
	M3	6.30 ± 0.53^{ab}	6.37 ± 0.56^{a}	6.43 ± 0.82^{ab}	6.50 ± 0.63^{a}	6.50 ± 0.63^{a}	6.57 ± 0.63^{a}
15	C2	5.90 ± 0.66^{a}	6.17 ± 0.91^{a}	6.40 ± 0.77^{a}	6.10 ± 0.76^{a}	6.13 ± 0.76^{a}	6.10 ± 0.61^{b}
	M1	6.23 ± 0.57^{a}	6.07 ± 0.78^{a}	6.00 ± 0.91^{b}	6.10 ± 0.40^{a}	6.07 ± 0.37^{a}	6.13 ± 0.63^{ab}
	M2	6.20 ± 0.61^{a}	6.27 ± 0.45^{a}	6.17 ± 0.38^{ab}	6.03 ± 0.49^{a}	6.20 ± 0.66^{a}	6.03 ± 0.67^{a}
	M3	6.27 ± 0.52^{a}	6.23 ± 0.68^{a}	6.33 ± 0.61^{a}	6.43 ± 0.68^{a}	6.27 ± 0.45^{a}	6.37 ± 0.76^{a}
18	C2	5.83 ± 0.79^{a}	5.93 ± 0.78^{a}	6.27 ± 0.78^{a}	6.03 ± 0.67^{a}	5.97 ± 0.76^{a}	5.90 ± 0.66^{b}
	M1	6.13 ± 0.57^{a}	6.03 ± 0.56^{a}	5.87 ± 0.455^{b}	6.07±0.45^{a}	6.00 ± 0.79^{a}	6.07 ± 0.64^{ab}
	M2	6.17 ± 0.75^{a}	6.13 ± 0.63^{a}	6.10 ± 0.66^{ab}	6.00 ± 0.69^{a}	6.13 ± 0.43^{a}	6.00 ± 0.74a^{b}
	M3	6.17 ± 0.38^{a}	6.17 ± 0.53^{a}	6.27 ± 0.58^{a}	6.30 ± 0.47^{a}	6.18 ± 0.46^{a}	6.30 ± 0.47^{a}
21	M1	6.07 ± 0.52^{a}	5.90 ± 0.61^{a}	5.70 ± 0.75^{b}	6.00 ± 0.53^{a}	5.93 ± 0.52^{a}	6.03 ± 0.72^{a}
	M2	6.10 ± 0.76^{a}	6.07 ± 1.11^{a}	6.07 ± 0.64^{a}	5.90 ± 0.55^{b}	6.07 ± 0.58^{a}	5.97 ± 0.61^{a}
	M3	6.10 ± 0.55^{a}	6.13 ± 0.63^{a}	6.17 ± 0.59^{a}	6.23 ± 0.43^{a}	6.10 ± 0.31^{a}	6.23 ± 0.50^{a}
24	M1	6.03 ± 0.72^{a}	5.90 ± 0.80^{a}	5.70 ± 0.84^{a}	5.97 ± 0.67^{a}	5.80 ± 0.71^{a}	5.97 ± 0.76^{a}
	M2	6.00 ± 0.53^{a}	6.03 ± 0.76^{a}	5.93 ± 0.64^{a}	5.90 ± 0.61^{a}	6.00 ± 0.37^{a}	5.87 ± 0.63^{a}
	M3	6.03 ± 0.72^{a}	6.03 ± 0.56^{a}	6.10 ± 1.09^{a}	6.13 ± 0.51^{a}	6.00 ± 0.45^{a}	6.13 ± 0.82^{a}
27	M3	5.93 ± 0.37	5.97 ± 0.72	5.93 ± 0.58	5.90 ± 0.55	6.00 ± 0.26	6.07 ± 0.37

Note: Mean ± SD from 50 panalists; $^{a–f}$Means within a column with different superscripts are significantly different ($p < 0.05$). C1 = control; C2 = marinated with no gases; M1, M2, and M3 = marinated at the O_2:CO_2:N_2 percentage of 5:50:45; 5:70:25; and 5:90:5, respectively

and 392.36 mg/kg for C1, C2, M1, M2, and M3, respectively. The zinc contents similarly were 8.95; 249.00; 257.74; 266.17; and 271.11 mg/kg for the same samples. The decrease in iron and zinc contents during storage may be due to the degradation of proteins by browning reaction, which may easily interact with iron and zinc.

El-Din *et al.* [38] mentioned that when iron and zinc minerals were added into dairy products, such as milk, the iron was bound with amino acids into casein micelles. Zinc associated with colloidal calcium phosphate in the casein micelles. Therefore, the contents of available iron and zinc may be reduced. In addition, it was noted that the drip increased with storage time due to high salt content (~ 5.5% NaCl) and acidity of the marinade, leaching iron and zinc out from the mussels. Baygar *et al.* [39] observed that water content decreased during marinating.

Microbiological quality. Mesophilic and lactic acid bacteria were found in all treated samples throughout the experiment. However, psychrophilic bacteria were found only in the control sample (C1), while *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Vibrio cholera* were very low or not detected, as shown in Table 2. Psychrophilic bacteria in the marinated sam-

ples (C2, M1, M2, and M3) were present in the amount of no more than 10 CFU/g. This indicates that the hurdle effect of the sauce (a_w 0.87, pH 3.31, TA 32.13%, and salt 5.26%) strongly controlled psychrophilic bacterial growth. During storage, mesophilic and lactic acid bacteria increased with time ($p < 0.05$). Not surprisingly that the bacterial count in the control sample (C1) was the highest, followed by the marinated samples (C2), (M1), (M2) and (M3) in this order. ICMSF [40] recommended 10^6CFU/g as the standard limit of total viable count in freshwater fish and seafood. By this threshold the shelf-life of the control was 12 days, while the marinated green mussel (C2) had 18 days. With higher CO_2 contents lower bacterial counts were found. This suggests that MAP can prolong the shelf-life when CO_2 inhibits microbial growth. Farber [25] also stated that CO_2 becomed more antibacterial as its concentration increased. CO_2 can decarboxylate enzymes and inhibit the metabolic activity of microflora, thereby extending the lag phase and reducing logarithimic phase growth of spoilage bacteria [25, 41, 12]. Goulas *et al.* [42] reported that when mussels (*Mytilus galloprovincialis*) were kept in 80%CO_2:20%N_2 atmosphere, the total viable count of bacteria was retarded compared to other tested conditions. Masniyom *et al.* [43] also reported that MAP with 80–100% of CO_2 effectively extended the shelf-life of green mussels (*Perna viridis*). Our current study indicates that 90% CO_2 atmosphere is the most effective to prolong the shelf-life of marinated green mussels. Therefore, combined acidity, salt and atmospheric CO_2 were effective in retarding microbial growth.

Sensory evaluation. Generally, sensory evaluation is applied in the estimation of seafood quality and correlates well with the microbiological and chemical analyses [44]. The sensorial scores from fifty non-trained panelists for green mussels with the various treatments during storage are summarized in Table 3. The scores from panelists became worse with storage time ($p < 0.05$). The threshold score for rejection was chosen to be 6, and only aroma showed significant differences between un-treated and treated green mussels during storage (Table 3). While all the sensory attributes with any treatment degraded towards the end of shelf-life, the scores remained on an acceptable level. Unexpectedly, the marinated mussels did not score better than the control group. This may be due to too soft texture, and unusual aroma and taste from the sauce that was highly acidic and contained iron and zinc. This altered the good natural aroma and texture of the mussels. This type of sauces is served as dips in Vietnam, not as marinades.

CONCLUSION

TVB-N, TBARS, TVC, and lactic acid bacteria increased in all samples during storage, while pH, glycogen, iron, and zinc contents decreased. The shelf-life of marinated green mussel was extended from 12 to 18, 24, or 27 days based on microbiological quality, TBARS and TVB-N value, by use of CO_2 rich MAP packaging. For marinated green mussels, high concentration of CO_2 (90%) appears to be both feasible and beneficial to shelf-life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Effects of protein-containing additives on pasta quality and biological value

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Abstract: The present work includes results on enrichment of pasta, a mass-consumption product, with protein. Meat products, legume (*Leguminosae* L.) flour and plant protein isolates were used as protein-containing additives. The content of protein and essential amino acids in the additives makes them promising for improving the biological value of pasta. We studied effects of the additives on raw gluten and wheat flour starch properties, pasta dough rheological characteristics, and finished product quality. As a result, the following optimal amounts of the additives were established: 15% by weight of flour for meat, 10% by weight of the mixture for pea and lentil flour as well as plant isolates, and 7.5% by weight of the mixture for soy flour. The enriching components added in pasta dough were found to have a positive effect on pasta quality. These increased protein content by 1.59–8.19%, biological value by 6–16%, utility coefficient of amino acid composition by 0.2–0.26, protein digestibility by pepsin by 11–24%, and daily protein intake level by 31.4–12.5%.

Keywords: Pasta, protein enrichment, biological value

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INTRODUCTION

Pasta is a mass-consumption product in Russia: more than 94% of the population uses it in their diets.

According to the current State Standard for pasta, high-grade and first-grade wheat flour can be used as basic raw material in the bread making industry. Protein content in pasta made from such flour is insignificant [1] and able to meet, on average, only 10.2–16.9% of daily protein requirement for adults [2]. However, the required protein level in a human body is a necessary condition for other nutrients to exert their biological functions. Moreover, it is not protein itself that is of significance but amino acids released in the gastrointestinal tract as a result of hydrolysis; it is amino acids that characterise the biological value of proteins.

In this research, we studied basic nutrients contents in pasta produced from bread flour that was obtained by milling common wheat grains grown in the central region of Russia. Thus, a maximum amount of protein did not exceed 11.1%, and scores of five out of eight essential amino acids were less than 90% [3].

Alternative raw materials as well as additives with higher content of protein and amino acids than that in wheat flour can be an effective way to increase the biological value of pasta. Those can be buckwheat processing products [4], egg products, including dry egg white [5, 6, 7], rye protein concentrate [8], mushroom powder [9], faba beans (*Vicia faba* L.) [10], soy protein concentrate [11], corn gluten [12], and even beef emulsion [13]. In some cases wheat flour is replaced with lupin (*Lupinus* L.) [12], defatted soy [9], kidney beans (*Phasedus vulgaris* L.) [12], whole grain amaranth, banana, and rice flours [12, 14, 15], etc.

As known, the human body assimilates proteins and carbohydrates effectively if their ratio is 1.4:1.45. In the case of pasta, the ratio of proteins to carbohydrates is 1:7. We took this fact into consideration when developing a new type of pasta with increased biological value and

Table 1. Characteristics of high-grade wheat flour samples for bread making

Parameters	Samples		
	no.1	no.2	no.3
Moisture content, %	10.60	10.80	13.00
Ash content, %	0.55	0.54	0.54
Acidity, °	2.00	2.00	2.20
Raw gluten content, %	28.80	29.50	29.50
GDI (gluten deformation index), units	100.00	77.50	80.00
Cohesion strength, N	4.10	3.10	5.50
Water-absorbing ability, %	200.70	183.00	198.00

when determining optimal amounts of protein-containing additives.

Since effectiveness of the latter directly depends on specific concentration of proteins, it is this parameter that was a key factor that we considered in choosing the additives. The additives would tend to reduce the content of gluten proteins which take part in product structure formation. Therefore, the more protein the additives contain, the lower amount of those is needed to provide pasta with this critical nutrient and ensure a high quality of the finished product.

The aim of this project was scientific and practical justification for the using of protein-containing additives of plant and animal origin to increase the biological value of pasta while maintaining/improving quality characteristics of the additives.

In this research we set the following tasks: to study effects of protein-containing additives on properties of raw gluten and wheat flour starch as texturizing agents, as well as their effects on rheological properties of pasta dough and quality characteristics of pasta, primarily on their organoleptic and cooking properties; to study effects of the enriching components added into pasta dough on protein content in pasta, biological value and amino acid balance of protein, utility coefficient of amino acid composition, protein digestibility by the proteolytic enzyme pepsin, and on daily protein intake level.

STUDY OBJECTS AND METHODS

Subjects of the research were wheat flour for bread making State Standard 52189-2003* (Table 1) as well as samples of pasta dough and finished pasta. We used the following enriching ingredients: meat products, namely chicken breasts and cooled B-grade veal meat (in amounts of 10% and 15% by weight of flour); legume flours, namely deodorized, low-fat soy flour (5%, 7.5% and 10% by weight of the mixture), pea and lentil flours (5% and 10% by weight of the mixture) in accordance with regulatory documents; and isolates of soy, pea, and corn proteins (in amounts of 5%, 10%, 15% and 20% by weight of the mixture). The isolates were produced by Dezhou Ruikang Food Co. (China), Cosucra Group (Belgium), and OOO Zvyaginki Krakhmalnyy Zavod (Russia), respectively. The corn isolate met the requirements of TU 9189-008-27291178-2005**. All the components contained protein with balanced amino acid composition (Table 2).

A traditional technology was used to produce high-protein pasta. The technology included the following steps: raw materials preparation, basic raw and enriching additives weighing-out, pasta dough kneading and pressing, pasta formation, cutting, pre-drying, putting on drying surfaces, final drying, stabilizing, and cooling. The additives were mixed with legume flours and plant protein isolates. In the case of meat additives, minced meat of at most 325 μm in size with formulation water quantity was introduced into pasta dough. To determine optimal amounts of the additives, we studied their effects on wheat gluten and starch characteristics, pasta dough rheological properties, and finished product quality.

To analyse the properties of raw wheat gluten, starch, and pasta dough, we applied ordinary techniques. The following devices were also used: a drying cabinet (Mogilev-Podolskiy Priborostroitel, Ukraine), a luxmeter 'Kvarts-21M' (Russia), a ST-1 texture analyser (mode 2 and 3) (OOO NPP Radius, Russia), and 'Amilotest' (mode 2) (OOO NPP Radius, Russia).

The rheological properties of pasta dough were determined by means of a device similar to a capillary viscometer, with a capillary length of 30 mm and a diameter of 3 mm.

Protein mass fraction in the additives and the finished pasta were determined by Nessler method [16] and Lowry method [17] (calibration curves were constructed using serum albumin). The amino acid composition of protein we determined by a liquid chromatography method with some modifications (Spackman method) using an amino acid analyser Chromaspek (USA). Essential amino acid scores (AAS), %, and coefficients of distinction amino acid scores (CDAAS), i.e. average values of essential amino acid scores redundancy in comparison to the lowest score value of an essential amino acid, were calculated as follows:

$$AAS = \frac{\text{content of amino acid in 1 g of test protein}}{\text{content of the same amino acid in 1 g of reference protein}} \cdot 100, \quad (1)$$

$$CDAAS = \frac{\sum_{i=1}^{N}(AAS_i - \text{the lowest AAS})}{N}, \quad (2)$$

where N is essential amino acids content, and AAS_i is amino acid score of the i-th amino acid, %.

To calculate biological value (BV), %, which is an indicator of protein quality, the following equation was used:

$$BV = 100 - CDAAS. \quad (3)$$

Utility coefficient (U), which characterises essential amino acid balance to the reference value, was found as follows:

$$U = C_{min} \frac{\sum M_{ri}}{\sum M_i}, \quad (4)$$

where C_{min} is the lowest amino acid score; M_{ri} is the content of the i-th essential amino acid that corresponds with the reference value, g/100 g protein; and M_i is the content of the i-th essential amino acid in the test pro-

* State Standard 52189-2003. 'Wheat flour. General technical specifications'.

Table 2. Amino acid composition and content of protein in additives

Parameter	Protein-containing additives							
	chicken	veal	legume flours			plant protein isolates		
			soy	pea	lentil	pea	corn	soy
Protein content, %	23.0 ± 0.1	22.3 ± 0.1	44.2 ± 0.1	24.25 ± 0.1	31.4 ± 0.1	90.9 ± 0.1	91.3 ± 0.1	92.5 ± 0.1
Amino acid content, mg/100 g product / Score, %:								
Isoleucine	916 / 100	1,148 / 129	1,807 / 102	1,407 / 145	1,291 / 103	4,190 / 115	4,510 / 123	4,440 / 120
Leucine	2,017 / 125	1,712 / 110	2,678 / 87	1,964 / 116	2,393 / 109	7,910 / 124	12,760 / 199	7,678 / 119
Valine	994 / 86	1,287 / 115	2,517 / 114	1,285 / 106	1,608 / 102	4,730 / 104	4,510 / 99	4,348 / 94
Lysine	1,879 / 149	1,918 / 156	2,195 / 90	1,619 / 121	2,178 / 126	7,000 / 140	2,770 / 55	5,920 / 116
Threonine	1,052 / 114	975 / 109	1,887 / 107	1,062 / 109	1,215 / 97	3,545 / 97	3,650 / 99	3,608 / 98
Methionine + Cysteine	918 / 133	805 / 103	1,062 / 69	648 / 76	652 / 59	2,090 / 67	2,050 / 64	2,312 / 71
Tryptophan	365 / 159	284 / 127	581 / 131	239 / 99	279 / 89	910 / 100	980 / 107	1,018 / 110
Phenylalanine + Tyrosine	1,274 / 92	1,680 / 125	3,891 / 147	2,196 / 151	2,603 / 138	8,640 / 158	7,260 / 133	8,500 / 153

Table 3. Amino acid scoring system recommended by FAO/WHO

Amino acid	Content in 1 g of ideal protein, mg
Isoleucine (Ileu)	40
Leucine (Leu)	70
Valine (Val)	50
Lysine (Lys)	55
Threonine (Thr)	40
Methionine (Met) + Cysteine (Cys)	35
Tryptophan (Trp)	10
Phenylalanine (Phe) + Tyrosine (Tyr)	60

duct, g/100 g protein.

We used the amino acid scoring system recommended by FAO/WHO Committee to compare content of each individual amino acid with that of the same amino acid in the ideal protein (Table 3). Unsettleable proteolytic products were an indicator of digestibility of the pasta proteins, with use of trichloroacetic acid (TCA) and Folin's reagent. The statistical analysis of the results was carried out by means of MS Excel and Statistica 6.0 programmes.

RESULTS AND DISCUSSION

We studied effects of the protein-containing additives on the quality characteristics of the pasta developed, such as sensory and cooking properties, properties of raw gluten and starch as basic texturizing agents, as well as rheological characteristics of the pasta dough (Tables 4 and 5, Fig. 1) [3]. Three control samples of wheat flour (Table 1) were used for the experiments.

According to the results obtained, the following optimal amounts of the additives were established: 15% by weight of flour for the meat additives, 7.5% by weight of the mixture for soy flour, 10% by weight of the mixture for pea and lentil flours, as well as for the protein isolates of corn, pea, and soy. It was found that these amounts of the enriching components:

– improved consumption characteristics;

– improved wheat gluten elasticity (N_{el}^{GDI}) by 35; 2–10; and 12.5–22.5 units for meat additives, legume flours, and plant protein isolates respectively;

Table 4. Effects of protein-containing additives on properties of raw wheat gluten and starch

Pasta samples	Values						
	Raw gluten content, %	GDI, units	Cohesion strength of gluten, N	Water-absorbing ability of gluten, %	Gelatinization temperature of starch, °C	Temperature of maximum viscosity of starch gel, °C	Viscosity of starch gel at gelatinization temperature, N
Control sample no. 1	28.83 ± 0.1	100.0	4.1	200.7 ± 0.75	65.0	93.5	10.5 ± 0.1
Experimental samples with meat additives , % by weight of flour:							
Veal (15 %)	28.5 ± 0.1	65.0	4.0	212.5 ± 0.75	61.0	90.5	8.95 ± 0.1
CHicken (15 %)	28.6 ± 0.1	65.0	3.9	207.0 ± 0.75	62.0	91.0	9.23 ± 0.1
Control Sample no. 2	29.5 ± 0.1	77.5	3.1	183.0 ± 0.75	65.0	94.0	8.34 ± 0.1
Experimental samples with legume flours , % by weight of mixture:							
Soy flour (7,5 %) Lentil flour (10 %) Pea flour (10 %)							
Control sample no. 3	29.5 ± 0.1	80.0	5.5	198.0 ± 0.75	75.0	95.0	5.55 ± 0.1
Experimental samples with isolates of plant proteins, % by weight of mixture:							
Corn (10 %)	26.78 ± 0.1	72.5	7.7	184.0 ± 0.75	75.0	95.5	5.37 ± 0.1
Pea (10 %)	29.21 ± 0.1	57.5	5.9	165.3 ± 0.75	75.0	95.5	4.94 ± 0.1
Soy (10 %)	28.42 ± 0.1	67.5	6.9	166.7 ± 0.75	74.5	95.5	4.83 ± 0.1

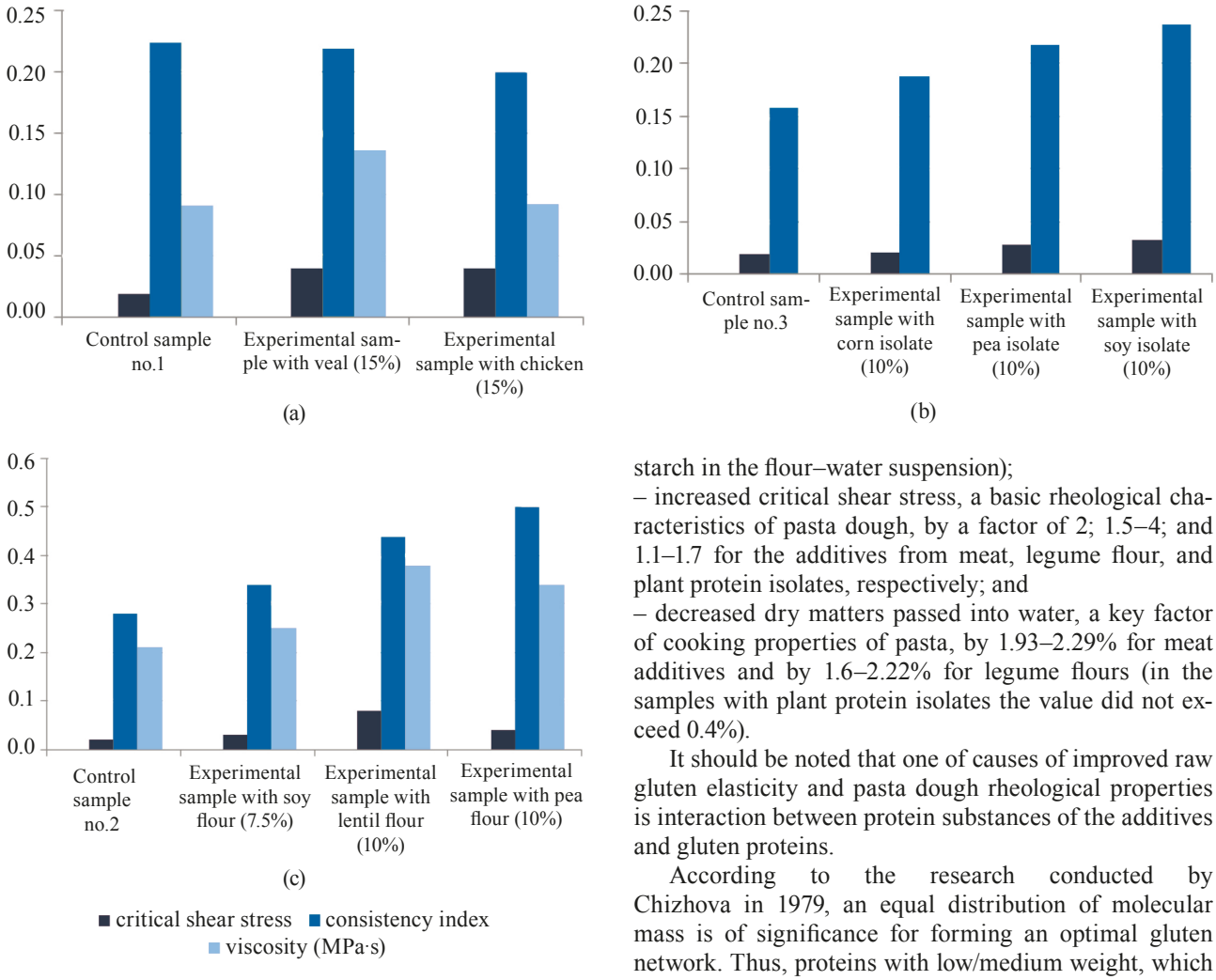

Fig. 1. Rheological properties of pasta with and without enriching additives: (a) meat; (b) plant protein isolates; (c) legume flours.

– increased the temperature of starch gel maximum viscosity by 0.5–1.5°C for legume flours and plant protein isolates (for meat additives, this parameter remained unaffected apparently due to a lower mass fraction of starch in the flour–water suspension);

– increased critical shear stress, a basic rheological characteristics of pasta dough, by a factor of 2; 1.5–4; and 1.1–1.7 for the additives from meat, legume flour, and plant protein isolates, respectively; and

– decreased dry matters passed into water, a key factor of cooking properties of pasta, by 1.93–2.29% for meat additives and by 1.6–2.22% for legume flours (in the samples with plant protein isolates the value did not exceed 0.4%).

It should be noted that one of causes of improved raw gluten elasticity and pasta dough rheological properties is interaction between protein substances of the additives and gluten proteins.

According to the research conducted by Chizhova in 1979, an equal distribution of molecular mass is of significance for forming an optimal gluten network. Thus, proteins with low/medium weight, which are soluble in water and salt solutions, can become an intermediate building material. Medvedev has proved that protein substances having lower molecular weight than wheat flour proteins interact with gluten proteins in dough and contribute to coagulation structure formation.

As known, the strength of intermolecular bonds, which determine gluten gel structure, directly depends on solubility of gluten proteins. To analyse the interaction of protein substances of the additives with gluten, we

Table 5. Effects of protein-containing additives on pasta quality

Pasta samples	Values			
	Titratable acidity, °	Shear strength, N	Weight ratio of dry pasta to cooked pasta	Dry matters passed into cooking water, %
Control sample no.1	2.0	4.30	2.10	9.66 ± 0.1
Experimental samples with meat additives:				
Veal (15%)	2.5	5.20	1.88	7.37 ± 0.1
Chicken (15%)	2.8	5.00	1.90	7.73 ± 0.1
Control sample no.2		2.96	1.83	6.20 ± 0.1
Experimental samples with legume flours:				
Soy (7,5%)	–	3.09	2.30	4.76 ± 0.1
Lentil (10%)	–	3.85	2.46	3.98 ± 0.1
Pea (10%)	–	3.61	2.46	4.21 ± 0.1
Control sample no.3	2.2	2.80	2.89	8.30 ± 0.1
Experimental samples with isolates of plant proteins:				
Corn (10%)	2.2	3.40	2.93	8.50 ± 0.1
Pea (10%)	2.2	3.70	2.94	8.60 ± 0.1
Soy (10%)	2.2	3.80	2.95	8.70 ± 0.1

studied their influence on the solubility of gluten in a polar solvent. In other words, we determined an amount of gluten protein which passed into the solvent from gluten, as the less proteins interact with each other, the more proteins pass into the solvent. 6M urea solution was used as a solvent since, as Medvedev found in 2004, in this case the type and amount of a protein-containing additive, rather than properties of gluten itself, had an effect on its solubility. An amount of protein passed into the polar solvent was determined by Lowry method. A graduation curve was constructed on serum albumin.

Optimal amounts of protein-containing additives in the pasta dough were found to reduce the passage of proteins from gluten into 6M urea solution. This fact proved the presence of strong bonds between molecules or aggregates of gluten proteins and the change in their structures. Moreover, the moving of dough inside an extruder barrel causes plastic deformation of particles and their sticking. As a result, interaction forces between proteins of gluten and those of the additives undergo increase (Fig. 2), which reduces the protein solubility.

As one can see from Table 5, the addition of plant isolates into dough impair the cooking properties of pasta, namely contribute to passage of dry substances into cooking water. Therefore, we studied qualitative characteristics of initial raw materials, above all mass fraction of raw wheat gluten, and their effects on the passage of dry substances into water during cooking. For that, three samples of wheat flour with different raw gluten content were used. According to the results of the experiment, the lower the raw gluten content is in flour, the greater amounts of dry matters pass into the cooking water.

The statistical analysis of the results showed a dependence of raw wheat gluten content and plant isolates amounts on the content of dry substances passing into water during the cooking process:

$$P_{d.m.} = y = 8{,}3(0.83 + 0.0133x_1)(1.53 - 0.02x_2), \quad (5)$$

where y is an amount of dry substances passed into the cooking water, %; x_1 is an amount of a plant isolate, % by weight of the mixture; x_2 is gluten content,%.

Table 6 gives the main quality characteristics of the protein in the enriched pasta. Analysis of the obtained data revealed:

The protein-containing additives, namely chicken and veal meats, pea, soy, and lentil flours, as well as corn, pea, and soy protein isolates, significantly increased protein content in the pasta – by 2.81, 3.08, 2.48, 1.59, 1.93, 7.49, 7.10, and 8.19% respectively.

Essential amino acids content in total amino acids was 29% for the samples with chicken and veal; 30, 29.2, and 31.3% for the samples with legume flours; and 35%, on average, for pasta with plant proteins isolates. It is a known fact that the amount of essential amino acids, including cysteine and tyrosine, should be 36% of total amino acid content for adults. The content of essential amino acids in the experimental samples was 4.1, 5.1, 4.3, 6.4, and 10.1% higher than in the control samples. Moreover, the content and scores of each essential amino acids, with some exceptions, had higher values than the control samples.

The biological value of protein in the samples with the additives was 6–16% higher than that in the control samples (except for corn protein isolate).

The utility coefficient of amino acid composition (whose theoretical value was 1) was 0.2–0.26 units higher in the experimental samples than that in the control samples.

100 g of the enriched pasta was able to meet 31.4–12.5% of daily protein requirement. That value for the control samples was 18–11%.

The protein digestibility of the enriched cooked pasta by pepsin was, on average, 11–24% higher than that of the control samples. One of the causes is balanced amino acid composition: proteins with a high biological value are digested and assimilated well. Another cause is an increased mass fractions of water-soluble and salt-soluble proteins in the pasta with protein-containing additives: albumins and globulins of both animal and plant origin are readily broken down by pepsin.

Since combined protein mixtures are considered to be quite effective in the development of a biologically valuable product, we calculated (by means of Microsoft Excel) a formulation of the pasta with mixtures of plant protein isolates in the amount of 10%. Thus, the following ratios of wheat flour to soy, pea, and corn isolates ensuring a high biological value of the finished pasta were established: 90:0:5:5 (BV = 73%), 90:3:3:4 (BV = 72%), 90:4:2:4 (BV = 72%), and 90:7:0:3 (BV = 72.5%). Furthermore, protein content in the pasta was 19.1–19.4%, essential amino acids content was, on average, 33.5% by total amino acids, and the utility coefficient was 0.69–0.70 units.

The experiments established that the combined protein mixtures enhanced the mechanical strength of dry pasta by 35.7–57.1% and the weight ratio of dry pasta to cooked pasta by 1.7–3.1%. Moreover, the value of dry matters passed into cooking water was virtually equal to that of the control sample. Thus, the combined protein mixtures allowed us to make qualitative pasta with higher biological value.

It should be noted that the duration of the drying process reduced for all samples with the protein-containing additives, except for pasta with soy flour. This might be due to an increased content of protein fractions whose molecular mass is lower than those of gluten proteins. They bind water mainly osmotically, and the binding is rather weak, so removing absorbent-bound water greatly reduces. The drying time of the pasta with soy flour, however, increased by 10 min compared to the control sample. This is due to the fact that soy contains lipids and a greater amount of dietary fibre (2.9 g per 100 g) forming strong bonds with water, the removal of which took longer than in the control sample [3].

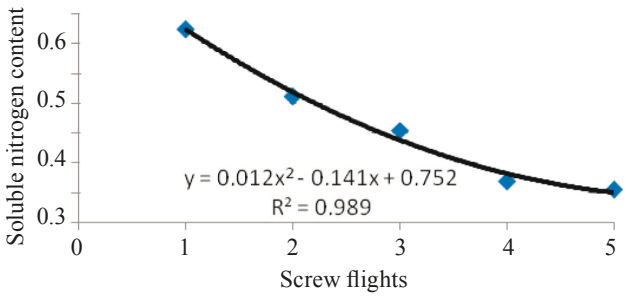

Fig. 2. Content of ammonium nitrogen passed into solution (in dough samples from different flights of the screw).

Table 6. Quality characteristics and composition of protein in pasta with protein-containing additives

Parameter	Pasta samples							
	control	chicken (15%)	veal (15%)	soy flour (7,5%)	pea flour (10%)	lentil flour (10%)	pea isolate (10%)	corn isolate (10%)
Protein, %	11.10	13.91	14.18	13.58	12.69	13.03	18.59	18.68
Daily value, %	18–11	22.6–13.6	23–14	22.1–13.3	20.6–12.5	21.2–12.8	30.2–18.2	30.4–18.3
Essential amino acids, %	2.69	3.53	3.82	3.58	3.30	3.52	5.52	6.21
Amino acid score, %							78	
isoleucine	65	66	71	74	75	75	85	91
leucine	71	72	77	75	83	80	75	135
valine	56	59	61	71	63	68	77	69
lysine	36	56	60	50	54	59	153	47
threonine	146	134	145	136	120	136	60	126
methionine + cysteine	54	64	66	61	56	66	97	60
thryptophan	97	109	103	110	102	100	108	105
phenylalanine + tyrosine	92	91	98	103	95	93		142
Essential amino acids, % by all amino acids	24.9	25.6	27.3	27.0	26.7	28.6	30.5	33.8
Biological value, %	59	75	75	65	73	74	68	51
Utility coefficient of amino acid composition, units	0.53	0.79	0.79	0.74	0.75	0.79	0.73	0.53
Coefficient of distinction amino acid score, %	41	25	25	35	27	26	32	49

As an example, Fig. 3 gives drying curves for semi-finished pasta (the control sample and the sample with pea protein isolate).

Statistical analysis of the experimental data resulted in a third-order equation describing the dependence of the drying time of the semi-finished pasta with the protein-containing additives on the protein content in it:
for the first stage of drying (at 55°C, until the moisture of 20%)

$$y = 0.012x^3 – 0.5587x^2 + 8.4295x – 38,452, \text{ and}$$

for the second stage (at 45°C, to the final moisture)

$$y = 0.0041x^3 – 0.1961x^2 + 3.0197x – 10.797,$$

where y is drying duration, min; and x is protein content in pasta, %.

The last step of the research included the evaluation of the novel pasta competitiveness. The integral index characterisng a product competitivenes, was higher than 1 for all the experimantal samples. The summery index reflecting the quality and nutritional value of the pasta with the protein additives, was 70% higher than that of the control sample (1.7 vs. 1.0).

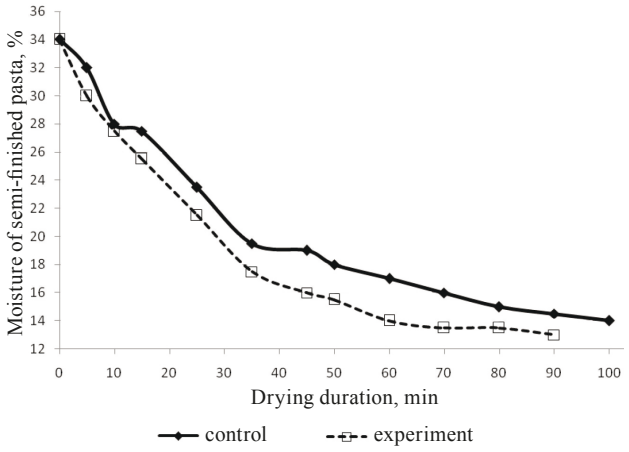

Fig. 3. Drying curves for semi-finished pasta.

CONCLUSION

In summary, the protein-containing components added into the pasta dough contribute to high quality formation of the finished product as well as the quality and quantity of protein substances in pasta made from bread flour. It should be noted that meat additives, pea and lentil flours, as well as soy isolate were the most effective to improve the biological value of the pasta. This characteristic in the developed pasta increased by 6–16% compared to the control samples. Additionally, the amino acid balance also improved. Consequently, the use of protein-rich pasta in the human diet will improve its nutritional value.

All new types of pasta enriched with protein-containing additives are supported by technical specifications and technological instructions. Pasta dough formulations are protected by the Patent of the Russian Federation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Effects of *Granucol* activated carbons on sensory properties of sea-buckthorn (*Hippophae rhamnoides* L.) wines

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Abstract: The paper introduces some experimental data on activated carbons of Granucol series that can improve the colour of sea-buckthorn wines and stabilize them during storage. Such treatment is necessary because sea buckthorn contains reactive phenolic compounds that trigger non-enzymatic oxidative browning in sea-buckthorn wine. A direct regulation of the amount of phenolic compounds can improve sensory characteristics of sea-buckthorn wines, as well as increase their shelf-life. The research featured table dry wine made of 10 varieties of sea buckthorn grown in the Altai region. The chromatic characteristics were studied according to the existing guidelines of the International Organization of Vine and Wine (OIV, France). The index of yellowness served as an additional indicator for the colour assessment of the sea-buckthorn wines. Another objective indicator of colour assessment was the index of the displacement of the colour of *x* and *y* coordinates that corresponded with the green-red and yellow-blue chromatic axes. When 20–60 mg/100 ml of Granucol activated carbon was used during the winemaking process, it significantly improved the harmony of the sea-buckthorn wines. In particular, it had a positive effect on the colour characteristics. Granucol carbon reduced such unfavourable taste characteristics as excessive roughness (the total amount of polyphenolic compounds fell by 1.5–2 times) and significantly improved the aroma by erasing the yeasty and fusel odours.

Key words: Sea-buckthorn wines, activated carbon, colour stability, chromatic characteristics, browning

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INTRODUCTION

In the Altai region, researches on the industrial use of sea-buckthorn berries began almost simultaneously with the cultivation of the plant. Nowadays, sea buckthorn covers enough acreage to allow for its industrial processing. There have been *in vitro* and *in vivo* studies of sea-buckthorn products (juices, jam, oil, etc.) on humans and animals. These nutrition and pharmaceutical products proved to have an anti-inflammatory, antitumoural, and antisclerotic effect on a living organism [1, 2]. As a rule, such preventive and therapeutic effect is attributed to phenol, vitamins, mineral substances, amino acids, fatty acids, and phitosterols. Sea buckthorn contains up to 11 satureted as well as mono- and polyunsarurated fatty acids. In addition, the berries contain α- and γ-tocopherols and α-tocotrienol, as well as some phitosterols, including campesterol, β-sitosterol, Δ^5-avenasterol, cycloartenol, and gramisterol, which have a strong antioxidant effect [3, 4]. Sea-buckthorn berries are known to contain a large amount of cartienoids and their ethers, such as astaxanthin, zeaxanthin, zeaxanthin-palmitate, α-, β-, and γ-carotenes, cis-β-carotene, β-cryptoxanthin, lycopene, lutein-palmitate-myristate, and other biologically active compounds [5–9].

Nevertheless, the huge potential of sea-buckthorn is hardly used for fruit wine production because of a high oil content in sea-buckthorn berries. Thus, the berries are difficult to process, and the resulting drinks are sensory unstable [10].

According to the previous research [11], the low stability of sea buckthorn wine is probably connected with high-reactive substances of the phenol origin in its composition. The substances are prone to copolymerization and condensation reactions; as a result, the drinks tend to be of dark colour. A high concentration of phenol substances proved to be an essential feature of sea-buckthorn berries. Sea-buckthorn flavonoids are represented by catechins, leucoanthocyanins, prosyanidins, flavan-3-ol, and, to a lesser extent, by flavones. Also, the berries con-

tain coumarins and tanning substances [12–16]. Nevertheless, the polyphenols are able to inhibit the formation of Maillard reaction products. Presumably, the mechanism can be explained by the fact that some polyphenols might interact with the intermediate products of the Maillard reaction: polyphenolic amides obstruct the reaction and result in sugar and amino acid degradation products of dark colour.

The phenol compounds of grape and fruit wines affect such sensory properties as colour and taste [18–20]. Excessive phenol compounds make white wines rough and harsh. Usually, the rough taste is attributed to the tanning substances [21–23], e.g. prosyanidins. The effect of polyphenols on the colour of white wines is determined by both enzymic and nonenzymic oxidation when exposed to oxygen. As a result, the wine acquires amber colour, which may turn dark-brown if exposed to oxygen for too long. Such changes of colour are inappropriate for table wines [20].

Activated carbon can improve the sensory characteristics of sea-buckthorn wines. In fact, activated carbon of Granucol series is often used to improve the taste and colour of grape wine [32, 33]. This brand of carbon can be used for different technological purposes. For instance, Granucol GE adsorbs unwanted taste and smell; Granucol FA is used to remove the reddish tint in young wine; Granucol BI can lower the amount of phenol and monomer substances. Fruit wine industry has developed a lot of ways to improve such indices of must as sugariness or acidity. However, there are little experimental data on how to lower and stabilize polyphenols, in spite of the fact that it is polyphenols that are responsible for the harsh and rough taste, as well as browning during storage.

Eye appeal is an important aspect that determines the reaction of customers when they choose wines and winy beverages of an unfamiliar trademark [34]. Thus, competitiveness requires that local wines should be attractive without losing their shelf stability. Appearance can be objectively assessed by analysing chromatic characteristics, e.g. colour intensity, tint, and coordinates in the CIE Lab system [35–40]. By determining the chromatic properties of wine and winy beverages, it is also possible to measure its yellowness, since yellowness has recently been introduced into control practice for many nutrition products. It characterizes the change in colour of a test sample from clear or white toward yellow [41–43].

The research objective was to analyse the effect various amounts of Granucol activated carbon produce on the polyphenols content and the chromatic and sensory characteristics of dry sea-buckthorn wine.

STUDY OBJECTS AND METHODS

The research featured dry wine materials of sea buckthorn (Novost Altaya variety) harvested in 2014 in Barnaul at M.A. Lisavenko Research Institute of Siberian Gardening. The initial amount of polyphenols was 480 ± 4.5 mg/dm^3. The wine materials were produced by submerged cap fermentation with the help of Oenoferm yeast, race LW 317-28 (Erbslöh Geisenheim AG, Germany). Clarification of the wine material was performed using 2.0–2.5 g/dm^3 of bentonite. The final filtration of the wine materials was made with the help of a SEITS-KS80 filter-paperboard. The ageing time of the wine material was 42 weeks at 5 ± 1°C. The general amount of SO_2 was 80 mg/dm^3. Granucol carbon (Erbslöh Geisenheim AG, Germany) was applied in rising concentrations from 10 to 150 mg/100 ml at 10 mg/100 ml intervals.

The mass concentration of general phenolic substance was determined according to the colourimetric method with the Folin-Ciocalteu reagent [44–46] on the spectrophotometer PE-5300VI (Ecros, Russia). The samples were preliminary diluted by 100.

The optic and chromatic characteristics of the samples before and after activated carbon treatment were determined in accordance with the methodic recommendations compiled by the OIV [47, 48] with the help of a UV-1800 spectrophotometer (Shimadzu, Japan).

Based on the spectral characteristics of the wine materials, we calculated:

– the value of colour intensity (I) represented by the sum of absorption values of the wine materials at the wave lengths of 420, 520, and 620 nm:

$$I = A_{420} + A_{520} + A_{620}; \quad (1)$$

– the value of wine material colour tint (N) represented by the ratio of absorption value at the wave lengths of 420 and 520 nm:

$$N = A_{420} / A_{520}; \quad (2)$$

– the value of yellowness (G, %) according to the formula introduced in [49]:

$$G = \frac{(1.28X - 1.06Z)100}{Y}, \quad (3)$$

where X, Y, and Z are coordinates of colour in the CIE system:

$$X = 0.42 \cdot T_{625} + 0.35 \cdot T_{550} + 0.21 \cdot T_{445}, \quad (4)$$

$$Y = 0.20 \cdot T_{625} + 0.63 \cdot T_{550} + 0.17 \cdot T_{495}, \quad (5)$$

$$Z = 0.24 \cdot T_{495} + 0.94 \cdot T_{445}, \quad (6)$$

where T_{625}, T_{550}, T_{445}, and T_{495} are coefficients of transmittance relative to distilled water at the corresponding wave lengths, %.

To analyse the effect of Granucol carbons on the sensory characteristics of sea-buckthorn wines, different amounts of the activated carbon were added into the processed and aged wine materials and stirred for two hours. Finally, the wine was filtered from carbon. After that, the samples were tested for mass concentration of polyphenols and the optic characteristics of wine materials.

RESULTS AND DISCUSSION

Fig. 1 shows the dynamic changes in the amount of the phenolic compounds in the wine material according to the concentration and type of Granucol carbon.

Fig. 2 shows that the usage of Granucol carbon reduced the polyphenol concentration in the sea-buck-

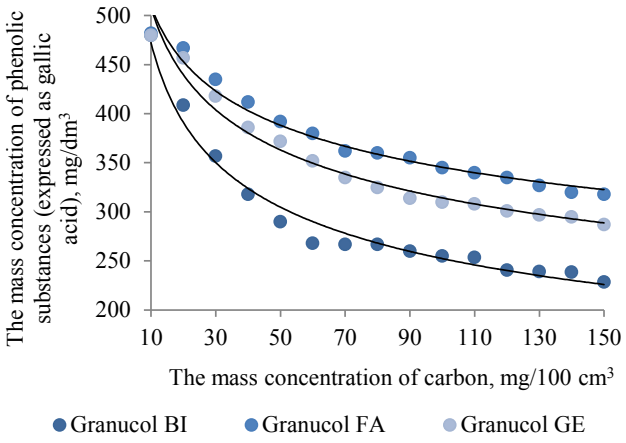

Fig. 1. Effects of the mass concentration of the phenolic substances in the sea-buckthorn wine material on the concentration and type of Granucol carbon.

thorn wine material. Granucol BI demonstrated the best results. In general, this type of carbon helped lower the amount of phenolic substances in the sea-buckthorn wine by 2.1 times when the maximum carbon amount was 150 mg/100 ml. Granucol FA and Granucol GE also lowered the amount of polyphenols. However, they were less effective and reduced the amount of polyphenols only by 1.52 and 1.56 times, respectively. Fig. 2 shows the empiric isotherms of phenolic substances adsorption by different activated Granucol carbons.

We calculated the specific adsorption by the following formula:

$$A = \frac{C_0 - C}{m} \cdot V, \quad (7)$$

where C_0 is the mass concentration of phenolic substances in the starting wine material, mg/dm^3;

C is the mass concentration of phenolic substances in the processed wine material, mg/dm^3;

m is the mass of the used sorbent, mg; and

V is the volume of the processed solution, dm^3.

Here we can see that the most effective concentration of Granucol BI was 20–60 mg/dm^3. Probably, this type of

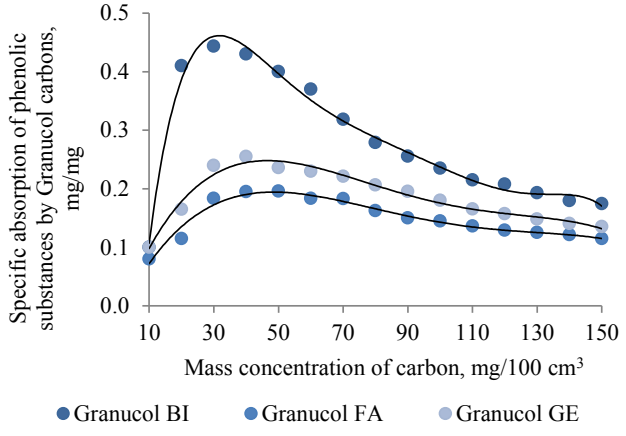

Fig. 2. Isotherms of adsorption of phenolic substances in sea-buckthorn wine material by different types of Granucol carbons.

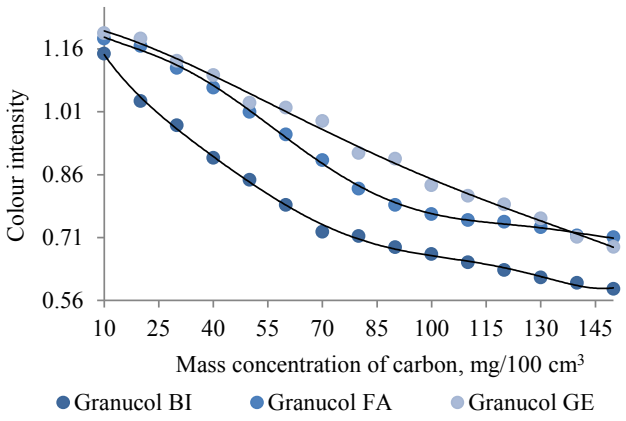

Fig. 3. Effects of the concentration and type of Granucol carbons on the colour intensity.

carbon absorbs the phenolic substances that exhaust the media due to their monomer nature.

The optical properties of wine material help determine its quality, age, and technological peculiarities. For instance, one can define the age and composition by the colour of wine. Any deviations from the colour norm mean that the wine in question is defective.

A Shimadzu UV-1800 spectrophotometer was used

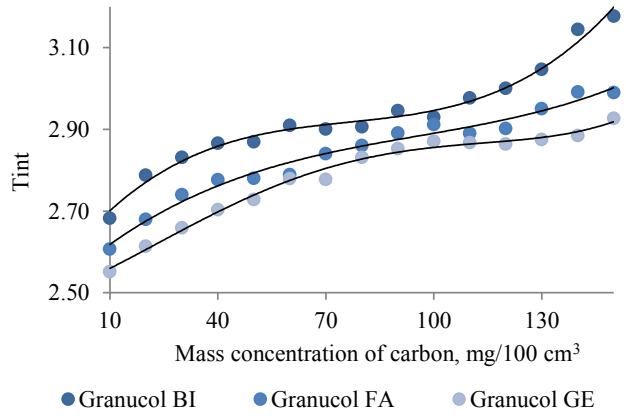

Fig. 4. Effects of the concentration and type of Granucol carbons on the tint of the wine material.

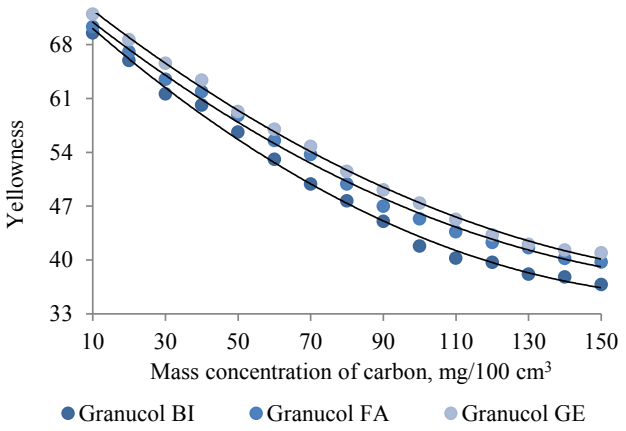

Fig. 5. Effects of concentration and type of Granucol carbon on the yellowness of the sea-buckthorn wine material.

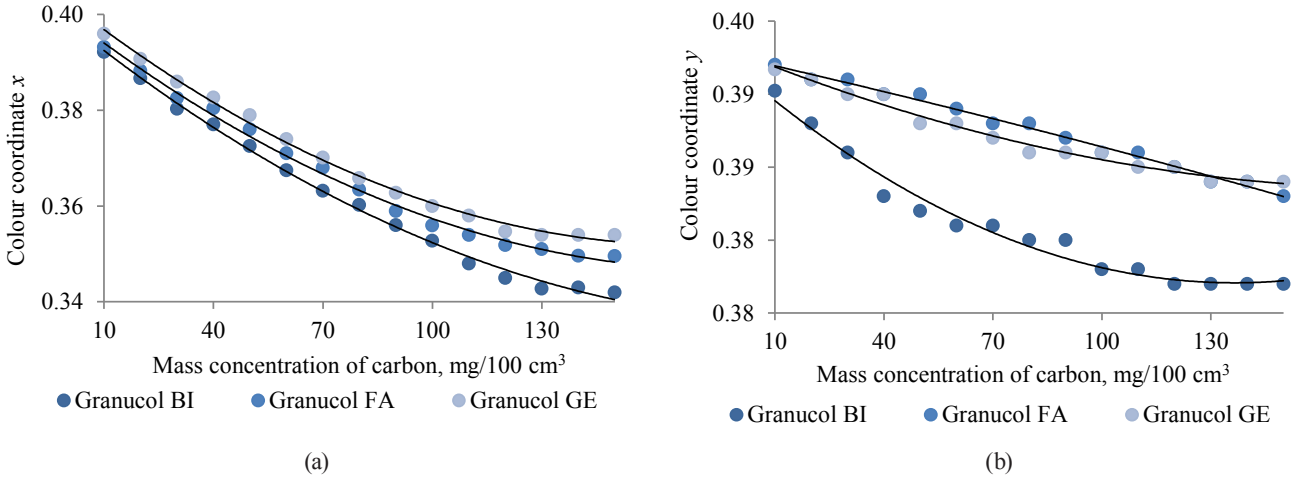

Fig. 6. Effects of the concentration and type of Granucol carbon on the displacement of the coordinates *X* and *Y* (according to the CIE 1931 chromatic system of coordinates).

to measure the optical density of the wine material in cuvettes with a path length of 10 mm. To define the intensity and tint of the colour, the optical density was measured at the waves of 420 and 520 nm. To obtain the trichromatic coordinates, we calculated the transmittance at 445, 495, 520, and 650 nm. The results were calculated according to the OIV methods [41, 42].

The following dependency graphs feature the colour intensity, tint, and yellowness according to the concentration of Granucol carbons (Fig. 3, 4).

The physical-and-chemical analysis and simple visual observation proved that Granucol carbon lowered the colour intensity. A larger mass of Granucol carbon changed the colour of the wine material from intense amber to light yellow. Granucol FA and Granucol GE also reduced the intensity of colour. However, the wines visually maintained the brown tint, which made them less attractive.

Yellowness is another factor that characterises the state of wine and wine materials, but fixed standards have been established for grape wines only [43]. Currently, yellowness is not used for sea-buckthorn wines assessment or for fruit wines in general. Nevertheless, we calculated the index of yellowness of our samples. Fig. 5 shows the changes of yellowness according to the concentration and type of Granucol carbon.

Remarkably, Granucol BI proved to be the most effective type of carbon to improve the wine colour: not only did it lower the amount of phenolic substances, but it also improved it by making the wine more visually attractive. Granucol FA and Granucol GE also improved the colour and removed partly the brown tint, but their amounts were higher.

The trichromatic colour coordinates of wine (*xyz*) and the subsequent coordinates *X* and *Y* were calculated according to the CIE Lab system of coordinates. Granucol carbon changed the coordinate *X* (the chromatic green-red axis) and produced almost no change on the coordinate *Y* (the chromatic yellow-blue axis) (Fig. 6a and 6b).

The beneficial effect of Granucol carbon on the aroma and taste were also quite remarkable (Fig. 7).

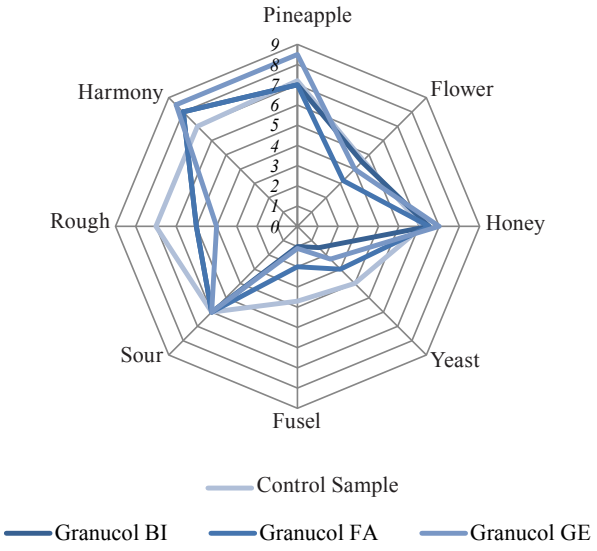

Fig. 7. Effects of Granucol carbon on the organoleptic properties of sea-buckthorn wine.

CONCLUSIONS

The present research proved that the activated carbon of the Granucol series can improve the sensory properties (taste and colour) of sea-buckthorn wine. The experiment demonstrated the effect of the concentration of carbons on the chromatic properties of wine. Granicol BI proved to be the most effective type of carbon to remove browning caused by oxidation, and Granucol GE greatly improved the sensory perception of taste and aroma.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this article.

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Immobilisation of bifidobacteria in biodegradable food-grade microparticles

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Abstract: The present research features a natural polymer that can be used for immobilisation of bifidobacteria as well as a method of immobilisation. We described a modified method of microencapsulation of probiotics using sodium alginate. The experiment studied the effect of encapsulation on probiotic stability and involved an *in vitro* model of human digestive tract. The test sample of microencapsulated *Bifidobacterium bifidum 791* showed a decrease in the activity from 3.0×10^7 to 2.2×10^5 CFU/ml in a mouse model with pH 1.2. By contrast, the control sample, unprotected by biodegradable polymer microcapsules, demonstrated a higher death rate of bifidobacteria: from 2.6×10^8 CFU/ml to 5.0×10^3 CFU/ml. The control sample demonstrated the same downward trend in *in vitro* gastrointestinal models with pH values of 4.5, 6.8, 7.2, and 5.8. Because the total plate count fell down to 4.0l g CFU/ml in acidity gradients, the titres of the initial microencapsulated biomass had to be increased up to > 10^9 CFU/ml. According to the results of scanning electron microscopy, the new type of microcapsules obtained by using a resistant starch had a closed surface. Prebiotics increased the resistance of bacteria to low pH and bile salts. Bifidobacteria encapsulated with natural biodegradable polymers proved to be well-tolerated and harmless for mice. The experiment revealed that biodegradable polymer microcapsules did not cause any chronic or acute toxicity when administered orally at 2×10^7 CFU per 1 gram of animal mass. The microcapsules demonstrated neither dermonecrotic properties nor any irritant effect on the ocular mucosa and, thus, can be used for food enforcement.

Keywords: Microencapsulation, bifidobacteria, food products, yogurt, sheep milk

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INTRODUCTION

The functional food market keeps holding the leading positions around the world as consumers tend to choose products that taste better and provide additional health benefits. Most consumers would like to prevent some diseases and cure the ones they already have. Therefore, they buy products with bioactive supplements that are able to support their health physiologically. It has been scientifically proven that non-microbial and microbial functional products have a therapeutic effect and can be used in preventive medicine. However, these biologically active ingredients are prone to rapid degradation during food processing, storage, and gastrointestinal transit. One of the best ways to prevent the degradation of these non-microbial and microbial bioactive components is to encapsulate them.

Recently, the popularity of functional foodstuffs on the global food market has increased significantly. The turnover of the global functional food market will reach several hundred billion dollars in the nearest fu-

ture. In addition to the positive effect they exert on human health, functional foods correspond to and satisfy all basic nutritional needs. Functional food products with probiotics and prebiotics have gained significant market share worldwide, especially in Europe, Asia (Japan), Australia, and, more recently, in the United States.

Despite all their fundamental differences, probiotic and prebiotic approaches to functional foods are equally beneficial for gastrointestinal tract (GIT). As a result, a symbiotic approach, i.e. a combination of probiotic and prebiotic approaches, is becoming more and more popular. Therefore, a number of symbiotic products are currently being developed for functional food markets.

Low survival rate of potential probiotics during storage and intestinal passage may limit the positive qualities of food products. Microencapsulation helps reduce the adverse effects on the viability of probiotics in GIT, as well as during food or nutraceutical processing, storage, and consumption. Microencapsulation separates and protects probiotic cells from the environment before their release.

There are various methods of gel microencapsulation that involve polymers: extrusion method, emulsification method, spray drying technology, etc. The main advantage of microencapsulation is in the controlled release of bacteria.

Microencapsulation is the process of enclosing substances in microcapsules, i.e. a material or a mixture of materials covered, or encapsulated, by another material or system. The coated material is called active, or base, material. It can be solid, liquid, or gaseous. The coating material is called shell, wall material, carrier, or encapsulating agent. Microparticles have a multicomponent structure with a diameter of 1–1,000 micrometers [1]. As a rule, microspheres are described as a matrix system where the active ingredient is dispersed/dissolved in a carrier matrix. Microcapsules have, at least, one discrete domain of the active agent, sometimes more (reservoir system) [2]. As a result, every microcapsule consists of a layer of an encapsulating agent that isolates and protects the active substance from any negative impact. Microcapsules can have a regular (spherical, tubular, or oval) or irregular shape [3].

An analysis of scientific resources resulted in the following list of substances used for microencapsulation of probiotics in food industry: sodium alginate, pectin, chitosan, carrageenan, gelatin, xanthan-gelatin mixture, and cellulose acetyl phthalate. All these substances help mitigate the process of immobilisation, thus, preserving the biological properties of substances and cell integrity. The most common encapsulating material is sodium alginate: it is simple, biocompatible, non-toxic, and cost effective. Alginate is a polysaccharide extracted from algae. It consists of β-d-mannuronic and α-l-guluronic acids. Different amounts and sequential distribution of β-d-mannuronic and α-l-guluronic acids in the chain can affect the functional properties of alginate as an auxiliary material [17].

If a polymer base is chosen as a shell, it results in the formation of microcapsules of various sizes, as well as in a good packing degree, molecular weight, structure, and shape, which guarantees targeted delivery of viable probiotics into the GIT as a part of food matrix.

When microencapsulating probiotics, one should take into account the chemical nature of coating materials. The use of microencapsulation techniques increases the viability of probiotics, both within food products and during their passage through the GIT. However, coating materials behave differently, and, therefore, their ability to protect living microorganisms and deliver biologically active substances also varies. In addition, the effectiveness of material depends not only on its encapsulating properties and strength, but also on its low cost, availability, and biocompatibility [18].

Microcapsules are currently applied in food [4], textile, pharmaceutical [5, 6], cosmetic, and agrochemical [7] industries. This method allows the producers to improve and/or modify the characteristics and properties of the active material, as well as its protection, stabilisation and slow release.

Microencapsulation can modify the colour, shape, volume, pressure sensitivity, heat sensitivity, and photosensitivity of the encapsulated substance [8]. In addition, microencapsulation:

– protects the base material from ultraviolet rays, moisture, and oxygen;
– increases the shelf life of the volatile compound;
– reduces the rate of evaporation or transfer of active material from the core to the medium;
– prevents chemical reaction; reduces the problems of fine powders'agglomeration;
– improves the processing properties of sticky materials;
– controls the release of substances; and
– reduces toxicity.

Thus, a research in the following spheres seems very promising: immobilizing methods of bifidobacterial cells and their use in the development of enforced dairy products from goat or sheep milk. Microencapsulation of bifidobacteria is important since it allows one to preserve the useful properties of bifidobacteria in foodstuffs. In addition, it helps to protect the viable cells from gastric juice, bile, and other external conditions.

The research objective was to provide a scientific basis for choosing a natural polymer as a method of immobilisation of bifidobacteria; to evaluate their physical and chemical characteristics; to study the process of microencapsulation of probiotics with prebiotics; to study the morphological features of microparticles, formed by natural biodegradable polymer (sodium alginate), using optical and electron microscopy.

STUDY OBJECTS AND METHODS

The research featured extrusion technique in alginate gel microencapsulation of bifidobacteria. Thus, there were two study subjects: microcapsules with bifidobacteria and resistant starch (Hi-maize) as a part of the capsular structure and microcapsules without starch. Dehydrated and hydrated microcapsules were assessed for

average diameter, size, and general morphology characteristics.

To obtain a $\geq 10^9$ liquid bifidobacteria concentrate, we used a serial passage. For the first passage, we prepared a culture medium. A sublimated *Bifidobacterium bifidum* 791 was inoculated into the culture medium and incubated at 37–38°C to obtain microbacterial mass with the bifidobacteria content of 10^8 CFU/ml. During the second passage, 10% of the cultured *Bifidobacterium bifidum* 791 was inoculated into the second culture medium at 37–38°C. During the third passage, 10% of the cultured *Bifidobacterium bifidum* 791 was inoculated into the third culture medium at 37–38°C and incubated for 6 hours. Next, it was centrifuged at 4°C for 20 min at 5,000 rpm. The resulting bacterial concentrate contained at least 10^{10} CFU/ml and was used to obtain microcapsules.

The microcapsules were obtained using extrusion technique. Two kinds of solution were prepared: the first solution contained 1% of sodium alginate and 1% of prebiotic Hi-maize, the second – 1% of sodium alginate. After the polymers dispersed completely, *Bifidobacterium bifidum* 791 was added to the solutions. The multicomponent composition was sprayed from 30 cm into 0.1 M of $CaCl_2$ using an airbrush (model EW 110) with a 0.3 mm nozzle that was attached to an air compressor (model Jas – 1203). The resulting particles were stirred for 30 min in a $CaCl_2$ solution to ensure complete gelation. After that they were removed from the solution.

To define the specific activity of the encapsulated probiotics, we used Procedural Guidelines 1.2.2566-09*.

The acidic gastric environment was modeled by adding two components into the sterile physiological solution. The first component was 0.5 mg/ml of acidin-pepsin manufactured by RUP Belmedpreparaty (Minsk, Belarus), registration number LS-001355. Each tablet contained 50 mg of proteolytic pepsin enzyme and 200 mg of acidin. The second component was a 0.1 mol solution of HCl (pH $\leq$ 2.0), which corresponds to the average gastric juice acidity [State Pharmacopoeia CCC ed. XI]. The small intestine environment was modeled by adding 2.5 mg/ml of panzinorm forte 20,000 produced by OOO KRKA-RUS (Istra, Moscow region, Russia), registration number P No. 014602/01. The pH level was adjusted by sterile 0.1 mol solutions of NaOH and HCl.

As a rule, survival studies of probiotic microorganisms are performed on a model *in vitro,* simulating the process of digestion in the human body. During the first stage, microorganisms are incubated at 37 ± 1°C first in an acidic model environment with acidin-pepsin (pH 2.0) and then in an alkaline model with panzinorm forte 20,000 (pH 6.8–5.8). The incubation time equals the period it takes mixed food to pass through the GIT. After that, the number of surviving microorganisms is assessed according to colony forming units of bifidobacteria (CFU/ml) in tenfold limiting dilutions, as recommended in Procedural Guidelines 4.2.999-00**.

To study the protective properties of microcapsules, we used solutions with the following pH gradients: pH 2.0 – gastric environment model (exposure time = 30–120 min); pH 4.5 – duodenum environment model (15–60 min); pH 6.8 – jejunum environment (60–120 min); pH 7.2 – ileum environment (60–120 min); and pH 5.8 – large intestine environment (18 hours). The temperature was 37 ± 1°C. The sample vials were intermittently stirred with circular motions. At each time point, we tested the probiotic survivability using the titration method of tenfold serial dilutions from 10^{-9} to 10^{-1} CFU/ml in two parallel rows of test tubes. The cell cultures were thermostated at 37 ± 1°C for 72 hours.

If no significant differences were registered in the number of CFU/ml of control and test samples, it was concluded that the concentration of the substance had no effect on the *in vitro* GIT model. If the CFU/ml in the test samples decreased significantly (up to one logarithmic order) in comparison with the control sample, the effect was declared inhibitory.

The general morphology of the microcapsules was determined with the help of a scanning electron microscope (SEM) (MIRA3 TESCAN). The microcapsules were placed on the substrate of the mechanical microscope stage using a double-sided gold-flashed tape. The accelerating direction of the microscope was 5 kV. The diameters of the microcapsules were determined using the ImageJ software (NIH, USA). The average diameter was calculated by measuring 100 microcapsules.

Fifty white mice of the SHK line of both sexes were selected to assess the safety level of the microcapsules. The animals were provided by the Research Centre for Biomedical Technologies of the Russian Federal Medical-Biological Agency (certificate number 05815, 11.05.2017 – 19.10.2017; veterinary certificate 250 № 0860445, 11.05.2017). The mice had a 21-day quarantine.

The dermonecrotic properties (irritant effect on mucous tissues) were studied on rabbits of Soviet Chinchilla breed. The animals were provided by the Federal State Unitary Enterprise, Experimental production farm (Manihino branch) (veterinary certificate 250 №0819660, 11.22.2016). The rabbits were quarantined for 30 days.

Three series of experiments were conducted to estimate the levels of safety, acute toxicity, chronic toxicity, and dermonecrotic properties.

Sample groups were formed according to body weight and age factor. The animals were kept in a vivari-

* Procedural Guidelines 1.2.2566-09. Security assessment of nanomaterials based on in vitro and in vivo model systems. Moscow: Federal Center of Hygiene and Epidemiology Publ., 2010. 71 p.; General pharmacopoeia monograph 2.2.1.0005.15 Solubility. Moscow: Ministry of Health of the Russian Federation Publ., 2015. 4 p.

** Procedural Guidelines 4.2.999-00. Bifidobacteria count in fermented milk products. Moscow: Federal Center of Hygiene and Epidemiology Publ., 2001. 18 p.; Procedural Guidelines 2.2602-10 System of pre-registration preclinical study of drug safety. Selection, verification, and storage of master seed strains used in the probiotics production. Moscow: Federal Center of Hygiene and Epidemiology Publ., 2010. 61 p.; State Standard R 4.1.1672-03 Guidelines for quality and safety control of dietary supplements. Moscow: Federal Center of Hygiene and Epidemiology Publ., 2004. 240 p.; General Pharmacopeia Article 1.7.2.0009.15 Determination of the specific activity of probiotics. Moscow: Ministry of Health of the Russian Federation Publ., 2015. 24 p.

um according to Sanitary Rules 2.2.1.3218-14***.

The analysis of safety and acute toxicity of microcapsules was conducted according to Procedural Guidelines 4.2.2602-10****. As a result, three experimental groups of five animals were formed. According to the key selective factors, each laboratory rodent had to be clinically healthy, well-fed, active and mobile, with a good pelage, normally coloured mucous membranes, and formed stool. The average animal weight was 20 ± 5 g.

A paste of microcapsules with concentration of bifidobacteria 1×10^8 CFU of microbial cells per 1 g was diluted with saline C080812 (expiration date September 2017). The paste was prepared in three doses. The ratio of the paste and the saline in the first dose was 1:5 while the amount of microbial cells was 2×10^7 CFU/ml. In the second dose, the ratio was 1:10, and the amount of cells equalled 1×10^7 CFU/ml. As for the third dose, the ratio was 1:15, and the amount of microbial cells was 6.7×10^6 CFU/ml. The solutions were administered orally using a 1 ml feeding tube. Four hours before the manipulations, the animals had stopped taking food and water. Feeding was resumed two hours after the procedure. As a control measure, physiological saline C080812 (expiration date: September 2017) was orally administered to the control group. The control group of five mice was used simultaneously in parallel experiments in safety, acute toxicity, and chronic toxicity. All the animals belonged to the same lot.

The dermonecrotic properties of the microcapsules were tested on rabbits of Soviet Chinchilla breed.

In vivo dermal irritation tests were performed on the anterior segment of the eyes of three rabbits. The animals weighed 1,500–2,500 g and were kept in a standard vivarium at 22 ± 2°C with a 12-hour synchronised change of the light period (Sanitary Rules 2.2.1.3218-14)*****. The animals were grouped according to body weight and age factor. During the whole research period, the animals received briquetted feed produced by OOO Laboratorkorm.

Animal testing was performed according to State Standard ISO 10993-10-2011******.

The bifidobacteria microcapsules were tested for the chronic toxicity according to Procedural Guidelines 4.2.2602-10****. Three groups of six animals were formed. All animals received specialized briquetted feed produced by OOO Laboratorkorm and water.

Group no. 4 was experimental. These animals received an experimental preparation, i.e. microcapsules composed of natural biodegradable polymers containing bifidobacteria.

Group no. 5 was control group. Animals received an alternative preparation, i.e. Bifidobakterin produced by ZAO Ecopolis (Kirov, Russia), series 792 (release date: March 2017; expiration date: April 2019).

Physiological saline C 080812 (expiration date: September 2017) was orally administered to the animals of the control group. To analyse the chronic toxicity, the preparations were administered orally once a day for 14 days to six animals in the experimental groups. The amount was equivalent to that proposed for humans. The dose was calculated according to the number of bifidobacteria in Bifidobacterin as stated in the product label. The dose of bifidobacteria in the microcapsules was identical to that in Bifidobacterin. For a 14 g mouse the concentration was 2.14×10^5 CFU. The animal behaviour and state of health were registered during the 14 days of administration and 7 days after the trial. Death rate of animals, state of hair, activity, colour of mucous membranes, body weight, and bowel movements were daily recorded.

RESULTS AND DISCUSSION

There are a lot of modern methods of microencapsulation, and this number continues to increase as companies keep patenting more and more innovative microencapsulation technologies [10, 11]. The methods make it possible to encapsulate active material. However, the final choice of microencapsulation method depends on the type of encapsulated material, the release characteristics of the encapsulated compound, application, and regulatory requirements, which can affect the final characteristics and properties of the microparticles. The whole spectre of microencapsulation methods can be divided into three main categories: chemical processes, which included interfacial and *in situ* polymerization methods; physicochemical processes, which involved coacervation (phase separation) and evaporation/emulsified solvent extraction; and physicomechanical processes, which involved air suspension method, spray drying, spraying, spray cooling, and fluidized bed coating.

Table 1 shows various methods of microencapsulation. The data presented in the table characterize the effectiveness of each encapsulation process.

Although complex and simple coacervation are the most effective methods, they are more costly. Spray drying and extrusion are second best according to the efficiency rating. Spray cooling and molecular incorporation are the least effective encapsulation techniques.

Table 2 demonstrates some of the most important and common methods of microencapsulation, the size

Table 1. Efficiency characteristics of encapsulation methods

Microencapsulation method	Maximum load, %
Simple coacervation	< 60
Complex coacervation	70–90
Molecular inclusion	5–10
Spray drying	< 40
Spray cooling	10–20
Extrusion	16–40

*** Sanitary Rules 2.2.1.3218-14. Procedural Guidelines 4.2.999-00. Bifidobacteria count in fermented milk products. Moscow: Federal Center of Hygiene and Epidemiology Publ., 2001. 18 p..

**** Procedural Guidelines 4.2.2602-10. System of pre-registration preclinical study of drug safety. Selection, verification, and storage of production strains used in probiotics production. Federal Center of Hygiene and Epidemiology Publ., 2010. 60 p.

***** Sanitary Rules 2.2.1.3218-14. Sanitary and epidemiological requirements for the organization, equipment, and maintenance of experimental biological clinics (vivaria). Moscow: Official Publ., 2009. 7 p.

****** State Standard ISO 10993-10-2011. Medical devices. Biological evaluation of medical devices. Part 10. Tests for irritation and delayed-type hypersensitivity. Moscow: Standartinform Publ., 2014. 42 p.

Table 2. Size of particles obtained by various methods of microencapsulation; advantages and disadvantages of each method

Microencapsulation method	Particle size	Advantages	Disadvantages	Scientific resources
Simple coacervation	20–200	high encapsulating efficiency; effective particle size control	expensive method; particle aggregation; complex scaling; evaporation of volatile substances	[12, 13]
Complex coacervation	5–200	dissolving capacity of the active compound for further processing; product oxidation	expensive method; particle aggregation; complex scaling; evaporation of volatile substances	
Spray drying	1–50	cheap; easy scaling technique	uniform particles; low level of microcapsule loading, further processing is required	[12, 13]
Spray cooling	20–200	suitable for water soluble substances	high engineering costs	[18]
Film coating	> 100	low operating costs; high thermal efficiency; full temperature control	long process	[14]
Emulsification	0.1–100	small drops; limited particle size distribution; suitable for biodegradable and non-biodegradable polymer microparticles and a wide range of liquid and solid materials	low efficiency of encapsulation; expensive method	[12, 13]
Interfacial polymerization	0.5–1,000	easy scaling technique; high encapsulating efficiency	difficult to control; possibility of non-biodegradable and / or non-biocompatible monomers' formation	[13, 15, 16]
Extrusion	from 150 to 2,000 micrometers	easy scaling technique	formation of rather large particles	[15, 16]

of particles obtained by various methods of microencapsulation, as well as the advantages and disadvantages of every method.

According to Table 2, there are several advantageous techniques for immobilisation of bifidobacteria. However, extrusion proves to be the most acceptable variant, given the limitations described.

Polysaccharides are the most widely used materials for various encapsulation techniques. They are followed by proteins and lipids.

The following types of carriers were selected to obtain bifidobacteria microcapsules, based on the cost parameter, as well as on safety and technology indexes.

Alginate gels are quite suitable for encapsulation of eukaryotic and prokaryotic cells. Microencapsulation using alginate gel was evaluated as a possible method for improving the viability of probiotics during the low pH exposure and storage.

Table 3. Physicochemical parameters of biopolymers

Biopolymer sample	Score				
	Optimum pH range	Gelation conditions	Duration of dissolution, max, s	Moisture content, max %	Ash content, max, %
Potassium alginate	4.7–6.3	Exposure to gelling ions	720	10.0	23.0–25.0
Sodium alginate	4.5–6.5	Exposure to gelling ions	720	10.0	18.0–22.0

Table 3 presents some physicochemical parameters of natural non-toxic biodegradable biopolymers.

Thus, the analysis of the physicochemical properties presented in Table 3 allows one to conclude that all these samples of biological polymers can be used for the immobilisation of bifidobacteria.

As for the molecular aspect, the alginate creates a particularly strong molecular structure in the presence of Ca^{2+}. As a result, one can obtain cold-prepared, thermoreversible, and freeze-thaw resistant microcapsules.

Probiotics are living microorganisms that help consumers to improve their health. However, such kinds of microorganisms lose their viability and stability rather

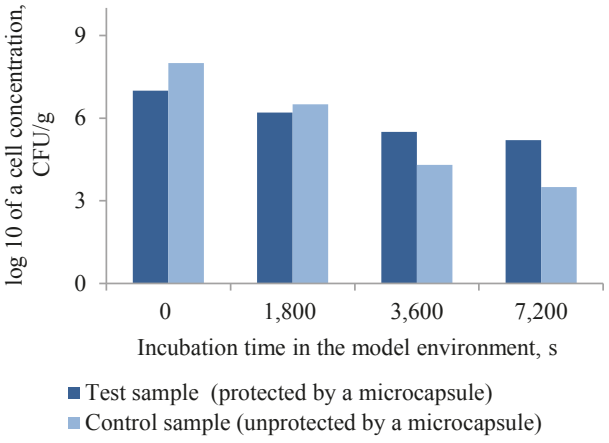


Fig. 1. Survival rate of bifidobacteria in the stomach model, pH 1.2.

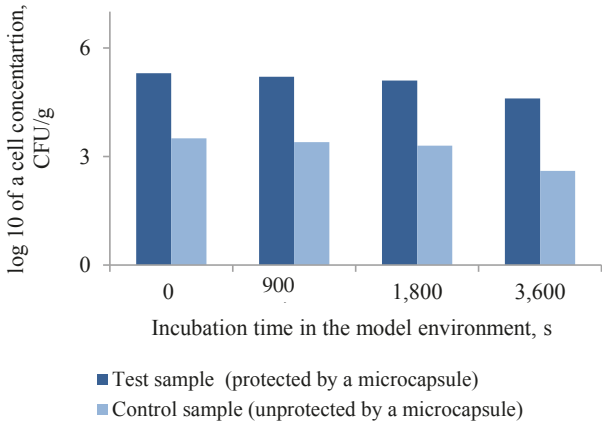


Fig. 2. Survival rate of bifidobacteria in the duodenum environment model, pH 4.5.

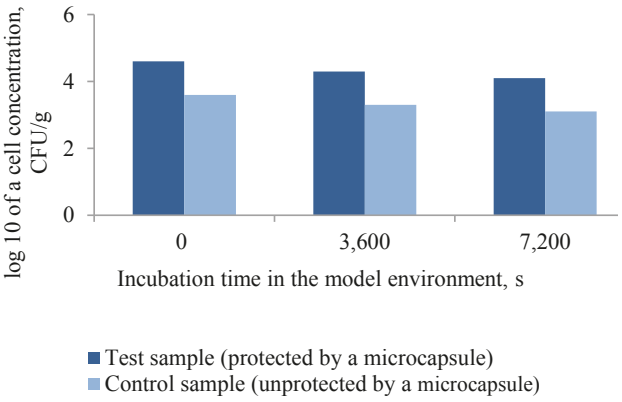


Fig. 3. Survival rate of bifidobacteria in the jejunum environment model, pH 6.8.

easily due to various physical and physiological conditions and factors.

The selected immobilisation method has a great effect on the viability of associated probiotic bacteria. It proved to be an effective method of probiotic viability improvement. Figs. 1–4 show some results of probiotic survivability in vitro in a GIT model.

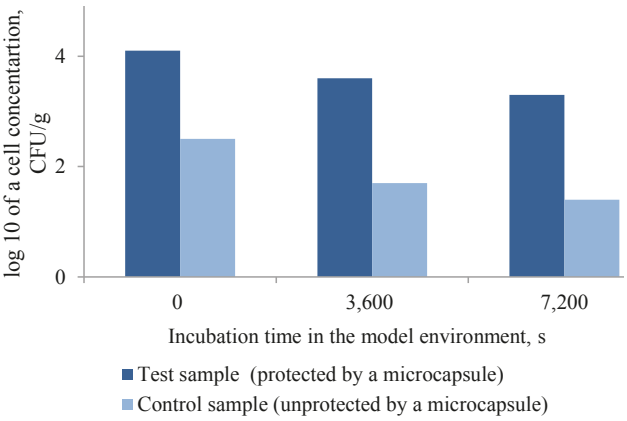
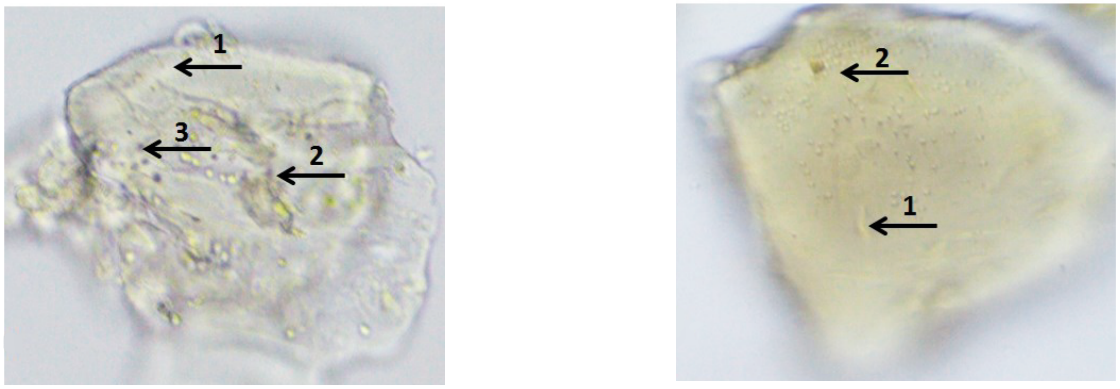


Fig. 4. Survival rate of bifidobacteria in the ileum environment model, pH 7.2.

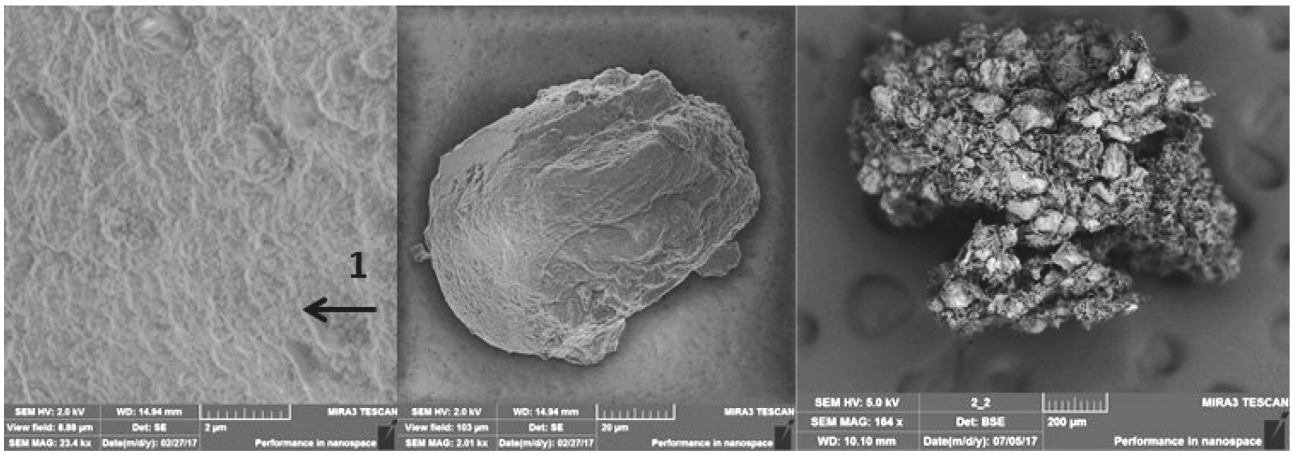
During the test on bifodobacteria survival rate in a simulated gastric environment at pH 1.2 (Fig.3), the microencapsulated test sample demonstrated a reduction from 3.0×10^7 to 2.2×10^5 CFU/g. The unprotected control sample showed a higher death rate – from 2.6×10^8 CFU/ml to 5.0×10^3 CFU/g. The same downward trends in the viability of unprotected bifidobacteria were registered in *in vitro* GIT models with pH 4.5; 6.8; 7.2; and 5.8 (Figs. 1–4). This confirms the protective effect of the biodegradable natural polymer on bifidobacteria during their passage through the *in vitro* gastric model. However, because the total plate count fell down to 4.0 lg CFU/g in acidity gradients (*in vitro* GIT model), the titers of the initial microencapsulated biomass had to be increased up to > 10^9 CFU/g. Probiotics must have a 10^6–10^7 level of living microorganisms per 1 gram of product when administered orally to maintain viability when passing through the GIT.

Alginate immobilisation of bifidobacteria protects them from aggressive external factors. To increase the stability of bifidobacteria, resistant starch (Hi-maize) was introduced into the composition of the biodegradable microcapsules. If prebiotics are introduced into the walls of probiotic microcapsules, it provides an improved protection for active microorganisms.



(a) (b)

Fig. 5. Optical microscopy of alginate microparticles + Hi-maize. (a) and alginate microparticles: 1 – sodium alginate inside the particle; 2 – the microorganism inside the particle (200 ×); 3 – the prebiotic Hi-maize (200 ×); (b) Alginate microparticles: 1 – sodium alginate inside the particle; 2 – the microorganism inside the particle (200 ×).



(a) (b) (c)

Fig. 6. Morphology and microstructure of lyophilized microparticles (alginate + Hi-Maize), obtained using scanning electron microscopy. (a) Surface of the microparticles with microorganisms (1 – the microorganism inside the particle); (b) microparticle (alginate + Hi-Maize); (c) particle distribution.

Some researchers also reported of a higher bacterial survival rate in alginate microcapsules containing prebiotics in a GIT model (fructo-oligosaccharides, galacto-oligosaccharides/inulin, fructo-oligosaccharides, monosaccharides, respectively) compared with alginate microcapsules without prebiotics [19, 20].

Some studies suggest that alginate-based microcapsules may provide a limited protection for probiotics due to its specific properties. For example, microcapsules obtained by extrusion using alginate as the main carrier and biopolymer are not stable in an acidic medium. Moreover, the microspheres obtained on the basis of alginate are characterized by a porous structure and provide an easy diffusion of acid into and out of the microspheres. These disadvantages can be effectively eliminated: alginate can be combined with other polymers or structurally modified using various additives [21].

Our method of obtaining microcapsules based on biodegradable non-toxic polymers of natural origin allows one to obtain microcapsules with a closed surface and specific sizes.

The optical microscopy of wet microparticles was performed using a microscope and a digital-still camera. The morphology of the lyophilized microparticles was evaluated using a scanning electron microscope. Microcapsules were mounted on aluminum plugs using a double-sided adhesive tape and then sprayed with gold.

The shape of the wet microparticles was close to spherical, and the core material was distributed throughout the matrix (Fig. 5). The optical micrographs show that alginate particles and microorganisms were found inside the microparticle. Thus, microencapsulation of *Bifidobacterium bifidum* 791 was effective for both treatments.

Scanning electron microscopy (Fig. 6) revealed that the morphology of freeze-dried microparticles had a high agglomeration of particles and a variety of particle size distribution for both treatments.

A sharp dehydration of lyophilized polysaccharide gels results in a porous matrix. In the process of lyophilization, all microcapsules were exposed to low temperatures. This led to the formation of ice crystals and the sublimation of ice under reduced pressure, resulting in a porous, dry product. The microparticles containing resistant Hi-Maize starch were more agglomerated if compared to the alginate microparticles.

The use of Hi-maize resistant starch in the process of microencapsulation did not significantly affect the diameters of wet microparticles.

As a result, the lyophilized microparticles had an average diameter of 150 and 97 micrometers for the matrix of microparticles alginate + Hi-maize and the alginate matrix, respectively. The structural changes caused by the process of freeze-drying increase the pore size, which results in a quick and full rehydration. Thus, freeze-dried microparticles swell quickly after being immersed in water and get larger than those wet microparticles that have not been lyophilized.

Nontoxicity and harmlessness of the new components, as well as the benefits to the human health, are the key factors in using additives in food dairy products.

For this purpose, acute toxicity, chronic toxicity, and dermonecrotic properties were tested in animals. The tests on the safety level and acute toxicity proved that

Table 4. Clinical score of animals (groups 1, 2, and 3) during the test on safety level and acute toxicity

Groups	Amount, CFU	The number of animals	Group mass before testing, g	Reaction	Observation time, 24-hour period	Results		Group mass after testing, g
						survived	died	
Group 1	2.0×10^7	5	118.5	negative	7	5	0	145.1
Group 2	1.0×10^7	5	113.2	negative	7	5	0	138.4
Group 3	6.7×10^6	5	115.5	negative	7	5	0	144.1
Control	–	6	121.4	negative	7	6	0	148.4

microcapsules made of natural biodegradable polymers with bifidobacteria were harmless to white mice of the SHK line of both sexes.

The mice were observed for 7 days. The experiment lasted 14 days. All animals survived the tests.

All the animals looked healthy and active, had a good appetite and a nice white thick tight pelage. The abdominal zone was not enlarged. The urinary frequency and urine colour corresponded to the physiological norm. The colour of the mucous membranes and the bowel habits remained the same during the entire time of the experiment. The behaviour of the test animals did not differ from that of the control group. Table 4 demonstrates the results of weighing.

On day 14 after the administration of the preparations, the animals were euthanized with chloroform, and further morphological studies of the internal organs followed.

A macroscopic examination did not register any effect of the preparations on the state of the internal organs of mice; no differences were found between the control and experimental groups.

The location of the internal organs was proper. Free fluid in the pleural and abdominal cavities was not detected. The lumen of the trachea and bronchi was unobstructed; the mucous membrane was moist and slimy. The spleen was elongated, not enlarged, with a dense texture and smooth surface. The liver was not enlarged, had a proper shape, with a dense homogeneous smooth and slimy texture without inclusions.

When administered orally, the dose of LD_{50} was not determined since the administered doses caused no clinical signs of poisoning (dose limitation was due to the possibility of administering a concentrated preparation through a probe).

When determining the chronic toxicity of microcapsules, the biodegradable microcapsules did not produce any chronic toxicity effect on the white mice of the SHK-line of both sexes when administered orally.

When determining the dermonecrotic properties of microcapsules, the assessment of the local irritant effect on the ocular mucosa was carried out on rabbits: 1–2 drops (0.1 ml ≤ 100 mg) of suspended microcapsules were introduced into the conjunctival sac of the left eye in diluted form. The ratio of the paste and the saline was 1:5 while the amount of bacterial cells equalled 2×10^7 CFU/g. Five minutes after application, the eyes were rinsed with distilled water. The ocular mucosa was inspected 1 hour after the introduction of the preparation and on the next day. The right eye of the animal served as a control sample. Observation of animals continued for 14 days. 24 hours after the preparations were applied to the rabbits' eyes, the following results were obtained: hyperemia – 0 points, swelling – 0 points, accumulation of serous secretions in the canthi – 0–1 points; cornea damages were not observed in any animal. The total score was 0–1. The results of irritation of the conjunctiva were assessed according to a 5-point scale, as recommended by Mikhailov [22].

On the second day after the exposure, the signs of eye irritation disappeared: the ocular mucosa recovered completely.

Thus, the preparation of biodegradable polymer microcapsules with bifidobacteria in diluted form 1:5 at a dose of 2×10^7 CFU/g had a slight irritant effect on the ocular mucosa. According to State Standard 1.12.007-76, it corresponds with the 4th hazard class.

CONCLUSION

The research featured a comparative analysis of various methods for obtaining immobilised probiotic cultures and their analogues.

The results of the analysis determined the choice of the method and the carrier for the immobilisation of bifidobacteria.

The morphological characteristics of microparticles were studied by using optical and scanning electron microscopy. The shape of the wet microparticles was close to spherical, and the core material was distributed throughout the matrix. The optical micrographs showed that alginate particles and microorganisms were found inside the microparticle.

The study also featured the effect of resistant starch on the process of immobilisation of bifidobacteria. The resistant starch (Hi-maize) in combination with alginate had a synergistic effect on gelation, providing additional protection for the probiotic cells.

We analysed the structural changes of microparticles caused by the process of freeze-drying. The scanning electron microscopy proved that the morphology of the freeze-dried microparticles had a high extent of agglomeration of particles, as well as a variety of particle size distribution.

All the characteristics of the obtained microcapsules underwent a comparative assessment. In the process of lyophilization, the microcapsules were exposed to low temperatures, which led to the formation of ice crystals and ice sublimation under reduced pressure, resulting in a porous, dry product. The microparticles containing resistant Hi-Maize starch were more agglomerated compared to the alginate microparticles. The lyophilized microparticles had an average diameter of 150 and 97 micrometers, corresponding to alginate microparticles with resistant starch (Hi-maize) microparticles with alginate matrix. The structural changes caused by the process of freeze-drying increased the pore size, which resulted in a quick and full rehydration.

The study proved the biodegradable polymer microcapsules with bifidobacteria to be well-tolerated and harmless for laboratory rodents. The experiment revealed that the microcapsules did not cause any chronic or acute toxicity if administered orally at a dose of 2×10^7 CFU per 1 g of animal mass. The microcapsules demonstrated neither dermonecrotic properties nor irritant effect on the ocular mucosa and can be used for food enforcement.

The research revealed good prospects for studying the properties and structure of microcapsules with immobilised bifidobacteria and their use in the food industry. The functional characteristics of a biopolymer particle ultimately depend on its composition, physicochemical properties, and structural characteristics. Therefore, it is a priority to study the most important characteristics of biopolymer particles and their connection with the physicochemical and sensory properties of food products.

Immobilisation of bifidobacteria in microcapsules makes it possible to preserve the useful properties of bifidobacteria in foods. In addition, it helps protect the viable cells from the negative impact of gastric juice, bile, and other external conditions.

Based on the above, it seems promising to continue the studies of immobilizing methods of bifidobacterial cells in the structure of natural biodegradable polymers and their use in the development of fortified dairy products.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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Quantitative and qualitative profile of biologically active substances extracted from purple echinacea *(Echinacea Purpurea* L.) growing in the Kemerovo region: functional foods application

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Abstract: Immunodeficiency causes a lot of modern diseases. Immunodeficiency, in its turn, is caused by such factors as polluted environment, chronic stress, sedentary lifestyle, unbalanced diet, etc. All these factors weaken respiratory organs and gastrointestinal tract, disturb hormonal regulation, and destabilize immune defence. Food industry responds to these challenges by developing functional foods and dietary supplements from medicinal plants. Dietary supplements made from natural plant extracts have more advantages than their numerous synthetic analogues. They produce a mild therapeutic effect and no pronounced side effects. Purple Echinacea (*Echinacea purpurea* L.) possesses immunomodulatory, anti-inflammatory, antiviral, and tonic properties. However, climatic and soil conditions are known to affect the qualitative and quantitative profile of biologically active substances. The present paper describes the micronutrient profile of various parts of *Echinacea purpurea* grown in the Kemerovo region. The study employed a complex of physical and chemical methods. The research featured leaves, roots, and flowers, as well as components extracted from the plant with the help of a 70% ethanol solution. The latter was chosen for its universal properties in micronutrient extraction. The methods included high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and IR spectroscopy. A set of triple experiments showed that the extracts contained substances with anti-inflammatory, antioxidant, and immunomodulating properties. Thus, Echinacea extract can be recommended for functional foods and dietary supplements.

Keywords: *Echinacea purpurea*, quality extraction, extract, biologically active substances, biologically active substances, qualitative and quantitative identification, chromatography

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INTRODUCTION

One of the biggest problems the humanity is currently facing is human-environmental interactions in the aspects of human health and homeostasis. Polluted environment damages immune status irrespectively of social stratum. This problem is especially relevant for regions with bad ecology and harsh climate [5]. Functional foods and dietary supplements with immunostimulant plant agents have become a popular preventive action against immunodeficiency [20]. Functional foods with targeted properties possess a high degree of usefulness and safety, which allows them to substitute pharmaceutical products, to a certain extent [1–3].

The alarming health status of population demands that Russian scientists started using medicinal plants since they are one of the largest domestic resources in the sphere of medicine. Biologically active substances obtained from medicinal plants can be used to treat even severe diseases. Medicinal plants have become a popular source of raw materials for preventive medical treatment. All these make studies of biologically active substances, extracts, and botanical medicines extremely relevant.

Unlike their artificial analogues, botanical medicines produce a mild therapeutic effect and no pronounced

side effects [13]. Despite the growing consumers' interest, the share of botanical medicines and herbal repertories on the Russian pharmaceutical market remains small: 11–12 million US dollars, or 0.5–1.5% [16]

Botanical medicines are getting more popular in Europe as well. For instance, sales of galenicals (mostly extracts) grew by 2% (440 million Euros) in France between 2009 and 2010. And if compared to 2005, the sector of painkillers grew by 12.3%, sedatives and soporifics – by 21.5%, cough mixtures, cold remedies, and antiallergic drugs – by 0.5%, and digestants – by 7.6% [17].

Medicinal plant materials contain a variety of biologically active compounds from various chemical classes of natural substances, i.e. terpenoids, polysaccharides, phenolic compounds, alkaloids, etc. Each class and group of BAS has a specific spectrum of biological activity, which is typical of the whole group. However, this spectrum may be variable for each subgroup of biologically active compounds. In some cases, there may also be fundamental differences for each individual substance, depending on its specific chemical structure [7, 8, 34].

The list of advantageous Siberian medicinal plants includes butterfly orchid (*Platanthera bifolia* L.), yellow seet-clover (*Melilotus officinalis* L.), skullcap (*Scutellaria moniliorhiza* L.), roseroot (*Rhodiola rosea* L.), maral root (*Rhaponticum carthamoides* L.), various sorts of milk-vetch (*Astragalus* turczaninowii L., *Astragalus danicus* L., etc.), hedisarum (*Hedysarum turczaninóvii* L.), etc.

To create new efficient and safe substances and functional foods, one has to use techniques based on the most recent knowledge about life and living systems. The basic idea behind all biotechnologies is how to use bio-objects in order to produce efficient and safe functional products. The share of vegetative BAS on the market of functional foods is 60–65% [1].

To study a group of biologically active substances and predict its toxic and pharmaceutical properties, one should start with a phytochemical analysis. A study of chemical formula of vegetative organic compounds begins with a series of identification tests that make it possible to define groups of biologically active substances [19]. The thin layer chromatography (TLC) method helps to refer a micronutrient to a particular group. It is often used to identify medicinal plant materials. Hence, the method defines the further research, as well as the choice of solvent and stationary phases [11, 13]. The method of high performance liquid chromatography (HPLC) makes it possible to perform a complete chemical analysis of plant samples, including identification and potency assay [6, 18].

Purple Echinacea (*Echinacea purpurea* L.) possesses immunomodulatory, anti-inflammatory, antiviral, and tonic properties [23]. It is a valuable medicinal plant from the *Asteraceae* family. The plant originated in North America, where it grows in the wild on fields, limestone wastelands, stony hills, and dry prairies of central and southern states. Some sources define it as *Rudbeckia purpurea,* although modern botany separates these two species [21].

Echinacea purpurea is a perennial herb. It is 50–100 cm tall and has one or several cylindrical ribbed ramose caulis. For medicinal purposes, the plant is harvested during its blooming stage, while its roots are usually dug in autumn [15, 24].

One of the main groups of biologically active substances found in *Echinacea* is phenylpropanoids, namely, derivatives of cinnamic acids. Another typical component is chicory acid, which is responsible for the immunomodulating and antiviral properties of Echinacea-based pharmaceuticals. The amount of chicory acid depends on the age of the plant [12, 14]. Phenylpropanoids contained in Echinacea include caffeic and chlorogenic acids. Virus-neutralising and immunostimulating properties are due to the presence of saponins. Inulin can be found in roots and, in lesser amounts, in leaves and stems. It possesses anti-inflammatory properties. The alkamides are responsible for the analgetic effect and improve the immune system. The same is true for vitamins A, E, and C. In spite of the fact that the pharmaceutical effect of separate components is relatively low, the medicinal effect of cumulative preparations, e.g. potions, extracts, or juices, is rather high [9, 10, 12]. Hydroalcohol potions, alcoholates, extracts, and juices are used to boost immune system by improving phagocytosis, bacericyte and cytotoxic properties of macrofags, and antibody synthesis.

Chemical industry produces various artificial substitutes, e.g. flavouring agents, preparations, active components, etc. Still, natural plant extracts remain in demand in food, cosmetics, and pharmaceutical industries [22, 25]. *Echinacea purpurea* makes part of many herbal immunoamplifiers.

Chemical composition of various plant parts depends on the climate and soil of the region where the plant grows. Hence, the research objective was to study the profile of the biologically active substances found in *Echinacea purpurea* that grows in the Kemerovo region. A set of physical and chemical methods helped to substantiate their use in functional food production.

STUDY OBJECTS AND METHODS

The research featured medicinal herbs of *Echinacea purpurea*. The averaged samples originated from the village of Novostroyka near the city of Kemerovo (Kemerovo region, GPS coordinates: 55°15'14"N, 86°13'05"E). The local soil can be characterized as black, leached, and argillaceous, with enough macroelements for medicinal herbs. The amount of humus was found to be 7.7%, nitrate nitrogen ($N\text{-}NO_3$) – 45 mg/100 g; labile phosphorus (P_2O_5) – 88 mg/100 g; exchange potassium (K_2O) – 142 mg/100 g of soil. To identify soil contamination and plant mass material with heavy metals, the averaged samples were sorted according to the state standards and approved techniques [28].

The atomic absorption method was used to determine heavy metals (Zn, Pb, Co, Ni, Cd) for an averaged soil sample [26, 27]. The samples were taken from the roots at the depth of 0–20 cm, from the rhizomes, and from the herbs. Heavy metals were extracted for 24 hours using an ammonium acetate buffer with a pH of 4.8. The soil-solution ratio was 1:10. The sample prepa-

ration of rhizomes, leaves, stems, and flowers was conducted separately and took 24 hours. Dry ashing was followed by extraction with diluted nitric acid (1:1).

In the Russian Federation, there are no current standards for the toxicological assessment of medicinal raw materials for heavy metals. Hence, most researchers have to use regulations adopted for plant-based dietary supplements [30, 31].

The research featured various parts of *Echinacea*, as well as components extracted from it with the help of a 70% ethanol solution. The latter was chosen for its universal properties in extracting of a wide range of biologically active substances.

The methods included high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and IR spectroscopy.

Statistical data processing was performed with the help of Microsoft Excel. The assay content of biologically active substances was defined with the help of standard curves. The concentration range was 0.5–150.0 mkg/ml.

The research involved five consecutive stages.

1. Extraction of the samples and preparation for screening and analysis of biologically active substances (secondary metabolites).

To define the sum of biologically active substances, the field test samples were air-dried (0.5 kg). An averaged sample of herbs (shoots, leaves, flowers) and foot ends (rhizomes and roots) were extracted and ground. After that, the plant material underwent a complete extraction with ethanol with the ratio of 1:10 at 10°C for 48 hours. The extract was a green-brown transparent liquid with a specific smell. The extracts were kept in the dark at 4–6°C.

2. Preparation of samples for HPLC analysis. The ground plant material (0.5 kg) underwent extraction with 70% ethanol in ratio 1:10 in a sonication bath (100 W, 35 kHz) at 40°C. The process lasted 30 min and was conducted twice. The extract was filtered through 0.2 micron membranes. Then, a vacuum rotary evaporator was used to concentrate the permeate in order to get water residue. After that, the permeate underwent a liquid-phase extraction with hexane (fraction 1) and an ethylacetat-ethanol mix (5:1). Fraction 2 was chromatographed with sorbent LH-20 with column of 10×350 mm by using a chromatograph (Bio-Rad, USA). The elution was conducted with isopropylalcohol at the gradient of 20–90%.

The extract was filtered and condensed with an 8 vacuum rotary evaporator at 72 Mbar to a thick consistency. The thickened suspension was then diluted four times with water and left for 12 hours at 40°C. The tarry residue was removed by filtration. After that, the permeate was treated with chloroform and ethyl acetate. The extract was then drained with anhydrous sodium sulphate. It was concentrated with the help of a rotary evaporator at 400°C, 240 mbar. The fractions were applied to column with an LH-20 sephadex (Pharmacia,). The fractions were mixed with a small amount of sorbent, loaded into the column, and eluted with aqueous alcohols at a ratio of 5:5; 6:4; 7:3; 8:2; and 9:1, as well as with absolute ethyl alcohol. The fractions were collected by 10–15 ml. The composition of the eluate was controlled with the help of TLC. If the fractions contained the same components, they were put together and condensed using a vacuum rotary evaporator.

After that, we defined the substances that could be classified as biologically active substances according to qualitation tests and chromatograms. Their structure was defined according to UV and IR Fourier spectra. UV-spectra were measured using СФ-2000 spectrophotometer both as pure components and with chemical reagents to specify the location of hydroxyl groups and clycosidation.

3. Preparation of samples for IR Fourier-transform spectrometry.

Two mg of a dried sample was ground in an agate mortar together with potassium bromide at a ratio of 1:100 (Fluka, Germany). A disk was formed in a press at 4,000 psi. IR spectra were measured by a single-beam interferometer with a ФСМ-1202 Fourier spectrometer (Infraspek, St-Petersburg, Russia). The spectra were registered in the range of 4.000–400 cm^1 with the resolution of 4 cm. The FSpec software 4.0.0.2 was used to process the data.

4. TLC stage. TLC analysis was performed on TLC aluminium foil analytical plates. It was followed by densitometry using a Sony densitometer (HDR-CH 405, OOO IMID, Russia). Photofixation was conducted at the waves of 254 and 365 nm and at a visual band after specific derivatization. The elution involved the following fluid systems: n-butanol – glacial acetic acid – water at the ratio of 60:15:25 and ethyl acetate – formic acid – glacial acetic acid – water at the ratio of 100:11:11:26.

In the preparative variant the chromatographic zones were cut out and subjected to further analysis.

5. HPLC conditions. The substances were separated using a Shimadzu –20 Prominence chromatograph with a photodiode array and a Shimadzu refractometric detector. The Kromasil –18 column was 250×4.6 mm, particle size – 5 µm. A mix of solvents was used as eluent components, namely methyl syanide MECN (solvent A) and 0.1% aqueous formic acid (solvent B). During separation, a gradient elution mode was used with the following isocratic components: 0 min – 20% A, 4 min – 55% A, 14 min – 55% A, 16 min – 20% A. The flow rate was 0.5 ml/min, the column temperature was 24°C, the sample volume was 20 µl, reference wave lengths were 254 and 330 nm.

Two approaches were used for identification.

1. UV spectra and retention time of peaks were compared with the reference samples. The chromatograms were developed using programme.

2. HPLC and/or TLC were used together with IR Fourier-transform sepctrometry. The column temperature was 40°C, while the volumetric flow rate of the eluent phase was 0.4 ml/min. A 0.1% water solution of formic acid (solvent A, v/v) and a 0.1% solution of formic acid in MECN (solvent B, v/v) were used as eluent. HPLC separation was conducted by gradient eluation. The eluent composition was as follows (solvent B, by volume): 0–1 min - 15%, 1–5 min – 30%, 5–15 min –

Table 1. Content of heavy metals in soil and in plant raw material of Echinacea, mg/100 g

Elements	Soil *			Plant raw material		
	MPC, TPC	Assigned value	MPC for dietary supplements [32]	Assigned value		
				roots and rhizomes	stems and leaves	flowers
Zink (Zn)	23.0	2.00 ± 0.12	–	0.93 ± 0.09	0.25 ± 0.007	0.09 ± 0.003
Lead (Pb)	3.2	0.82 ± 0.08	6	0.46 ± 0.06	0.18 ± 0.007	0.02 ± 0.002
Cobalt (Co)	5.0	1.15 ± 0.08	–	0.44 ± 0.06	0.21 ± 0.008	0.02 ± 0.002
Nickel (Ni)	4.0	1.78 ± 0.12	–	0.55 ± 0.06	0.34 ± 0.05	0.02 ± 0.002
Cadmium (Cd)	1.0	0.56 ± 0.06	1	0.22 ± 0.008	0.11 ± 0.007	–

Note: content of heavy metals measured in the active form

30 → 38%, 15–15.5 min – 48 → 45%, 15.5–23 min – 45%, 23–23.5 min – 45 → 95%. Each chromatographic fraction was analyzed after accumulation.

A quantitative assay of the secondary metabolites (flavones) was conducted with the help of standard curves at the concentration range of 1.9–235.0 mkg/ml. The equation for the standard curves was as follows:

$$y = a \times x \times X, \quad (1)$$

where X is standard concentration (mkg/ml); y is HPLC peak response (cu); and a is proportionality factor.

Formula (2) connects the peak response and the dry weight unit:

$$C = S / (a \times m \times 1000), \quad (2)$$

where C is dry flavones in the portion of dry material (mg/g); m is mass of dry weight (g); a is proportionality factor from the standard curve equation. A coefficient of 1,000 is necessary to transform C into mg/g dimension.

The concentration of chlorophylls *a* and *b* and carotinoids was measured using a UV 1800 Shimadzu spectrophotometer. The stems and leaves were used as assimilative bodies. A 0.1 g portion of plant material was ground, rubbed, put into a vile with 10 ml of 98% ethanol and was stored in a dark place. After 12 hours it was measured for pigments using spectrophotometry at the wave length of 649 nm, 665 nm, and 440.5 nm. The concentrations of chlorophylls *a* and *b* and carotinoids were measured as follows (mg/g):

$$C_{\text{chlorophyll}}a = 13.7 \cdot D_{665} - 5.76 \cdot D_{649,} \quad (3)$$

$$C_{\text{chlorophyll}}b = 25.8 \cdot D_{649} - 7.6 \cdot D_{665,} \quad (4)$$

$$C_{\text{carotinoids}} = 4.695 \cdot D_{440.5} - 0.268 \cdot (C_{\text{chlorophyll}}a + C_{\text{chlorophyll}}b), \quad (5)$$

where D_{649}, D_{665}, $D_{440,5}$ are absorbency at the wave lengths of 649 nm, 665 nm, and 440.5 nm, respectively.

RESULTS AND DISCUSSION

As a rule, pollutants enter the plant tissue from soil through the root system. They also enter the plant from the dust that settles down on the surface of its aerial organs and penetrates into the intercellular spaces through natural passages, e.g. stomata, pores, or lenticels [29, 32]. Therefore, herbal raw materials must be environmentally pollution-free. The analysis of soil samples and plant material confirmed this requirement (Table 1).

Table 1 shows that the content of heavy metals (Zn, Pb, Co, Ni, Cd) in the soil does not exceed the maximum permissible level. For food plants, including medicinal ones, the MPC content for heavy metals (HM) is stated in Sanitary Regulations and Norms 2.3.2.1078-01* for dietary supplements. However, the document features only lead and cadmium, which belong to technogenic metals and are of no biological significance for plants [31].

According to the assigned value analysis of the heavy metals, they are mostly accumulated by roots and rhizomes, and to a lesser degree – by flowers. However, the content of standardized elements (Pb, Cd) in different parts of the plant is significantly below the permissible level (Table 1). In general, the results indicate that the content of HM in the raw material corresponds to the standard indicators and can be used to obtain biologically active substances for dietary supplements and food.

Photosynthesis plays the key role in plant growth and development. Therefore, it determines the formation of the secondary metabolites, including those with biologically active properties. Chlorophylls *a* and *b,* as well as carotenoids, are involved in photochemical reactions. A high content of chlorophyll *a* and the chlorophyll *a/b* ratio may indicate a high potential photochemical activity of the leaves, and, consequently, a more active accumulation of biologically active substances [33].

Hence, it seemed important to investigate the quan in the leaves of *Echinacea purpurea* (Table 2).

The experimental data indicate that Echinacea leaves have the highest amount of chlorophyll *a* in their pigment complex. Apparently, chlorophyll *a* has the greatest stability among other pigments of photosynthesis

Table 2. Content of photosynthetic pigments in leaves of *Echinacea purpurea*

Indicator	Assigned value
Chlorophyll a, mg/g	0,700 ± 0.01
Chlorophyll b, mg/g	0,282 ± 0.01
Chlorophyll amount (a + b), mg/g	0,990 ± 0.02
Chlorophyll ratio a/ b	2.480 ± 0.01
Carotinoids, mg/g	0.190 ± 0.01
Ratio of chlorophylls and carotinoids, chlorophyll/carotinoid	5.210 ± 0.01

Note: mean values of triple consecutive tests

* Sanitary Regulations and Norms 2.3.2.1078-01. Sanitary rules and Regulations 2.3.2. 1078-01. Hygienic requirements for safety and nutritional value of food.

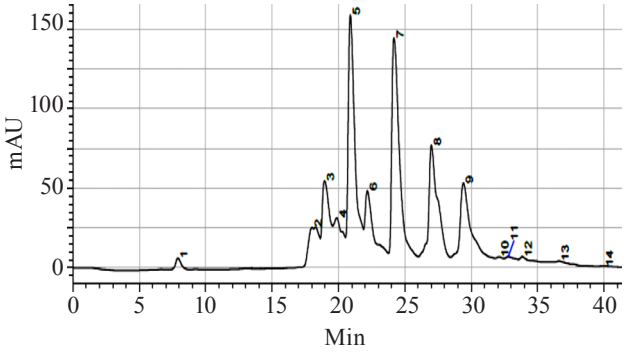

Fig. 1. HPLC chromatogram of the ethanol extract from *Echinacea purpurea* stems and leaves.

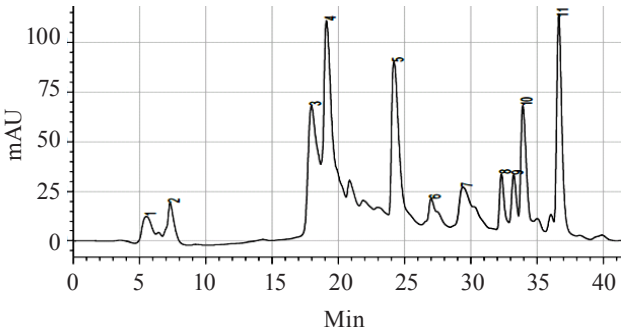

Fig. 2. HPLC chromatogram of the ethanol extract from *Echinacea purpurea* rhizomes and roots.

Table 3. Residence time Content of the main biologically active substances in the ethanol extracts from stem and leaves

Component	Concentration range mkg/ml	Correlation coefficient	Residence time t_R, min
Tetradec-8Z-en-11,13-diyn-2-one		0.9700	7.87
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one		0.9600	18.07
3,4-dioxybenzoic acid		0.9800	19.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one		0.9894	19.87
Echinacoside	0.50–50.03	0.9881	20.97
Caftaric acid	1.01–10.07	0.9602	22 22
Chlorogenic acid	0,103–50.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Caffeic acid	0.501–100.02	0.9831	29.39

Table 4. Content of the main biologically active substances in the ethanol extracts of *Echinacea purpurea* stem and leaves

Component	Content, mg/g of dry weight
Tetradec-8Z-en-11,13-diyn-2-one	1,098 ± 0.01
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	0,911 ± 0.01
3,4-dioxybenzoic acid	5.84 ± 0.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one	1.03 ± 0.01
Echinacoside	24.42 ± 0.01
Caftaric acid	5.21 ± 0.01
Chlorogenic acid	28.5 ± 0.01
Chicoric acid	17.56 ± 0.01
Caffeic acid	13.88 ± 0.01

and optimizes the photosynthetic processes. As the data show, the amount of chlorophylls (*a* + *b*) significantly exceeds the content of carotenoids – by 5.2 times. The pigment complex of Echinacea leaves has a rather high ratio of chlorophylls *a/b*. High ratios of chlorophyll *a/b* are characteristic of chloroplasts. The proportion of stromal thylakoids in chloroplasts prevails, and they have a greater light absorption and a better membrane protection of from photodamage.

Thus, a high content of chlorophyll *a* and a high chlorophyll *a/b* ratio indicate a high potential for photosynthesis of Echinacea leaves. Indirectly, it may indicate a more intensive synthesis of secondary metabolites, in particular, those with biologically active properties.

The main active biologically active substances of Echinacea plants are hydroxy acids and polysaccharides. For a more complete assessment of the Echinacea biologically active substances, we conducted a physico-chemical study of its main components, i.e. rhizomes, roots, stems, leaves, and flowers.

Table 5. Residence times of the main biologically active substances in the samples of chromatographic fractions of ethanol extracts of *Echinacea purpurea* rhizomes and roots

Component	Concentration range, mkg/ml	Correlation coefficient	Residence time t_R, min
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one			5.52
Tetradec-8Z-en-11,13-diyn-2-one			7.87
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one			18.07
Cynarine	1.03–150.00	0.9800	19.01
Echinacoside	0.50–5.03	0.9881	20.97
Chlorogenic acid	0,103–150.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Isobutylamide dodeca-2E,4E,8Z,10Z-tetraenic acid			29.39
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one			33.24
Isobutylamide undeca-2E-en-8,10-diynic acid			33.88
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid methylbutylamide dodeca (2E), (4Z)-dien-8,9-diynic acid			36.56

Table 6. Contents of the main biologically active substances in the ethanol extract of *Echinacea purpurea* rhizomes and roots

Component	Content, mg/g of dry weight
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one	2.67 ± 0.01
Tetradec-8Z-en-11,13-diyn-2-one	3.31 ± 0.01
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	15.84 ± 0.01
Cynarine	23.68 ± 0.01
Echinacoside	16.65 ± 0.01
Chlorogenic acid	3.38 ± 0.01
Chicoric acid	3.43 ± 0.01
Isobutylamide dodeca-2E,4E,8Z,10Z-tetraenic acid	3.51 ± 0.01
8-hydroxypentadien-9E-en-11,13-diyn-2-one	3.40 ± 0.01
Isobutylamide undeca-2E-en-8,10-diynic acid	8.88 ± 0.01
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid	15.19 ± 0.01

For the phytochemical characteristics of the extracts, we chose those groups of compounds that were more likely to be present in the hydrophilic extracts in question.

To study the content of biologically active substances, we analyzed the extract obtained by using a 70% ethanol solution of the stem and leaves. To determine the content of the main groups of biologically active substances, we used HPLC and TLC, accompanied with an IR Fourier-transform spectrometry.

Fig. 1 and Tables 3 and 4 present a detailed analysis of the composition of the extract obtained from the stems and leaves of *Echinacea purpurea*.

The analysis showed that the leaf extract of *Echinacea purpurea* contained such biologically active substances as acetylene and alkene derivatives, as well as phenylpropanoids, which are derived from caffeic acid. The fact that the extracts from leaves and stems contain phenylpropanoids confirms the biological value of the plant. This group of organic compounds is a rich source of substances with adaptogenic, tonic, immunomodulating, hepatoprotective, and antioxidant properties.

The next stage involved an analysis of the extract obtained from the rhizomes and roots of *Echinacea purpurea*. The extraction was performed with a 70% ethanol solution.

To determine the content of the main biologically active substances, we used HPLC and TLC, accompanied with IR Fourier-transform spectrometry. Fig. 2 and Tables 5 and 6 show the results of the chemical and physical analysis of the extract from rhizomes and roots.

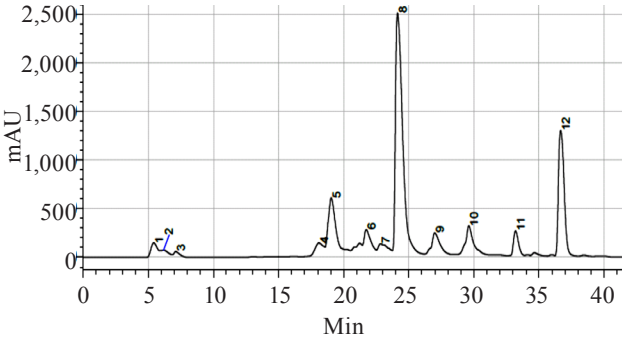

Fig. 3. HPLC chromatogram of the ethanol extract from Echinacea flowers.

Table 7. Residence times of the main biologically active substances in the samples of chromatographic fractions of ethanol extracts of *Echinacea purpurea* flowers

Component	Concentration range mkg/ml	Correlation coefficient	Residence time t_R, min
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one			5.46
8- hydroxy-pentadec-9Z-en-11,13-diyn-2-one			6.03
Tetradec-8Z-en-11,13-diyn-2-one			7.87
3,4-dioxybenzoic acid	1.03–150.00	0.9800	19.01
Echinacoside	0.50–5.03	0.9881	21.78
Chlorogenic acid	0,103–150.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Vanillic acid			29.70
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one			33.19
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid			36.56

Tables 5 and 6 and Fig. 2 show that the extract obtained from Echinacea rhizome and roots contain alkylamides and phenylpropanoids.

Alkilamides demonstrate a great variety. Despite their relatively simple molecular structure, these substances have a wide spectrum of biological activity. They have an immunomodulating, antimicrobial, antiviral, insecticidal, diuretic, and antioxidant properties. In addition, they can potentiate antibiotics and inhibit prostaglandin synthesis.

The final stage of the study featured the extract obtained from Echinacea flowers. The extraction was performed with a 70% ethanol solution.

For the physical and chemical evaluation of the flower extracts, we used HPLC and TLC, accompanied

Table 8. Content of the main biologically active substances in the ethanol extract of *Echinacea purpurea* flowers

Component	Content, mg/g of dry weight
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	2.53 ± 0.01
8- hydroxy-pentadec-9Z-en-11,13-diyn-2-one	1.65 ± 0.01
Tetradec-8Z-en-11,13-diyn-2-one	1,006 ± 0.01
3,4-dioxybenzoic acid	2.45 ± 0.01
Echinacoside	10.19 ± 0.01
Chlorogenic acid	45.32 ± 0.01
Chicoric acid	4.56 ± 0.01
Vanillic acid	5.94 ± 0.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one	3.22 ± 0.01
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid	19.19 ± 0.01

with an IR Fourier-transform spectrometry. Fig. 3 and Tables 7 and 8 show the results of the analysis.

The data show that the flower extract contains all the necessary biologically active substances. The most valuable substances are polar derivatives of caffeic acid and chlorogenic acids.

Chlorogenic acids possess strong antioxidant, antimicrobial, and anti-fungal properties. Therefore, they are considered valuable biological active compounds.

CONCLUSION

The experiment revealed that the soils associated with *Echinacea purpurea* in the Kemerovo region demonstrated no excess MPC of heavy metals (Zn, Pb, Co, Ni, Cd). The soils proved to be pollution-free, which makes them suitable for medicinal plants.

The content of such standardized elements as Pb and Cd in various parts of *Echinacea purpurea* is significantly below the permissible level. It makes this vegetable raw material environmentally friendly. It can be used as a source of biologically active substances to produce dietary supplements and functional foods.

The extracts obtained from Echinacea rhizomes, roots, stems, leaves, and flowers were used to study biologically active substances. A 70% ethanol solution was used as an extractant. It allowed for the maximum extraction of biologically active substances.

In order to study the quantitative and qualitative profile of Echinacea biologically active substances, a physical and chemical analysis of these extracts was performed using HPLC, TLC, and IR Fourier-transform spectrometry.

We conducted a comparative analysis of the composition of the biologically active substances in different parts of the plant. It showed that the leaf part of the plant was rich in phenylpropanoids. These compounds exhibited immunomodulatory and antioxidant properties.

The root of the plant mainly contains such significant biologically active substances as alkylamides, which possess immunomodulatory, antimicrobial, and antiviral properties.

The analysis of the ethanol extract of Echinacea flowers showed that it was rich in chlorogenic acid, which is responsible for the antioxidant property in this group of plants.

The experimental contribute to the formation of a data base on the chemical composition of medicinal raw materials that grow in various geographical zones of Russia. The research expands the existing profile of biologically active substances obtained from *Echinacea purpurea* that grows in the Kemerovo Region.

The experimentally established qualitative and quantitative profile that allows us to recommend it for the production of dietary supplements and functional foods.

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System modelling of non-stationary drying processes

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Abstract: The kinetics of the drying process in continuous drum dryers differs from the drying of single objects in a batch mode. Drying process is affected by too many factors; hence, it is practically impossible to obtain an analytical solution from the initial equations of heat and mass transfer, since the duration of drying depends on the operating parameters. Therefore, it is of high theoretical and practical importance to create a highly efficient rotary drum dryer. Its design should be based on an integrated research of non-stationary processes of heat and mass transfer, hydrodynamics of fluidized beds, and drying kinetics in the convective heat supply. The experiment described in the present paper featured sunflower seeds. It was based on a systematic approach to modelling rotary convective drying processes. The approach allowed the authors to link together separate idealized models. Each model characterized a process of heat and mass transfer in a fluidized bed of wet solids that moved on a cylindrical surface. The experiment provided the following theoretical results: 1) a multimodel system for the continuous drying process of bulky materials in a fluidized bed; 2) an effective coefficient of continuous drying, based on the mechanics of the fluidized bed and its continuous dehydration. The multimodel system makes it possible to optimize the drying process according to its material, heat-exchanger, and technological parameters, as well as to the technical and economic characteristics of the dryer.

Keywords: System modelling, continuous drying, heat and mass transfer, drum dryer, fluidized bed of wet solids

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INTRODUCTION

The general theory of heat and mass transfer in capillary porous and other dispersed media belonged to Prof. Lykov. It was based on the thermodynamics of irreversible processes and the theory of generalization of variables. According to Prof. Lykov, sets of equations are to be solved as a single complex process [1].

The theory of heat and mass transfer is based on solving sets of linear equations with boundary conditions, which corresponded to constant and variable potentials in a medium that varied according to established laws. Thus, it was intended for stationary material and medium [2].

To intensify and improve technological processes, one needs reliable, physically valid modelling methods [3]. This is especially important for energy-intensive drying processes of wet solids in a fluidized bed that is moving along a cylindrical surface, e.g. drum dryers [4].

In this paper, modelling means a physical analysis of heat and mass transfer, as well as the hydrodynamics of the processes that occur a rotary drum dryer, their mathematical description, and possible solutions by analytical or numerical methods involving various software [5]. The analytical and numerical methods were based on preliminary data on the kinetics of drying and heating of individual particles. Such information was obtained either from the available model representations or from experimental data. In most cases, preference was given to direct experimental data, which took into account possible effects of anisotropy of heat and mass transfer properties and irregular geometric shape of particles of real solids.

An adequate description of the continuous drying technology of wet solids in a fluidized bed requires an integrated approach to the problem. Such an approach requires a system analysis of hydrodynamic, diffusion, and thermal processes complicated by an overlap

of various phenomena. A complete theoretical picture of a continuous drying process should be based on a mathematical model that would link a set of typical structures, or idealized models, each of which reflects a particular type of transfer or transformation. The optimal way to develop a new drying technique is to combine the multimodel system of drying processes with experimental studies on the kinetics of moisture removal in a fluidized bed [6].

The research objective was to develop a multimodel system for the continuous process of drying and heat transfer in a fluidized bed of wet solids.

STUDY OBJECTS AND METHODS

The study was based on a complex of general and specific scientific methods. The general scientific methods involved analysis and synthesis, testing a theory with practice, interpretation of the results obtained, etc. The specific scientific methods included the abstract logical method, the method of modelling, the empirical method, the method of statistical probability, etc. The theoretical and methodological foundation of the research included studies conducted by Russian and foreign experts in the field of drying, such as Ginzburg, Frolov, and Lykov.

The research featured sunflower oil seeds.

The thermophysical characteristics of the vegetable raw material were determined according to the non-stationary thermal mode method of *two* temperature-time *points* developed by Volkenstein. The method of differential thermal analysis was used to identify the intervals of temperature zones of moisture evaporation with different forms and energy of moisture binding with the material. It was accompanied by the method of differential scanning calorimetry, which was used for quantitative measurement of heat flows that occurred when the trial sample and the control sample were undergoing a simultaneous programmed heating. The methods of high-performance gas chromatography, atomic absorption spectroscopy, infrared spectroscopy, capillary electrophoresis, and acid hydrolysis were used to determine the content of vitamins, amino acids, and other quality indicators of the wet solids. The measurement errors did not exceed the values established in the current standards for quantitative analysis of the quality indicators of the wet solids. The main part of the theoretical and experimental research was carried out on the premises of the Voronezh State University of Engineering Technologies (Voronezh, Russia) and the Bobrovsky Vegetable Oil Plant (Bobrov, Russia).

The research objective was achieved by the synthesis and analysis of classical and novel analytical and empirical methods in the sphere of heat and mass transfer and food dehydration studies. The obtained relations, the approximating equations, and the simulation results corresponded with the experimental data. The measurement results underwent statistical processing. The procedures and design solutions did not contradict the established methods of rational design and engineering. Modern computer mathematical programmes, instruments, and authentic trial equipment were used to conduct the experiments and test the physical and mathematical models of the drying and steam treatment processes.

The systematic approach in modelling the convective drying processes. The process of continuous drying is an original research subject that can be marked as a certain system *S*, while the model of the drying process *M* represents a different system. According to the systematic approach principle, the drying process interacts with the external environment *E*. Depending on the research objective, the study may feature different ratios between the subject and the external environment. These relations represent a model of relations of the external environment with the subject. Since our ideas of the subject and the external environment are also models, the following models can be proposed: M_S is the model of the subject S, M_F is the model of external environment at the inlet, M_T is the model of external environment at the output, M_{FS} is the model of the connections between the external environment and the subject at the inlet, and M_{ST} is the model of the connections between the external environment and the subject at the output.

The combination of all these models

$$M_{RS} = (M_F, M_{FS}, M_S, M_{ST}, M_T), \quad (1)$$

forms a multimodel system of transfer phenomena (Fig. 1).

A drum dryer as a subject of system modelling of non-stationary processes. Fig. 2 shows the experimental drum dryer used in the study of the continuous drying process.

The pressure type fan (*2*) was fixed on the angular steel frame (*1*). The fan was designed to supply atmospheric air through the duct (*4*) into the heater (*6*). The heater consisted of several sections. The flow rate of the air supplied to the heater was regulated by means of the gate valve (*5*). The temperature and the relative humidity of the air entering the drying chamber were measured with the dry (*7*) and the wet (*8*) thermometers.

At the core of the whole design there was a steel drum (*11*) with a diameter of 0.3 m and a length of 1.3 m. On the inner surface of the drum there was the channel nozzle (*12*). The nozzle contained longitudinal slots to feed the drying agent to the bed of wet granular material. The drying drum (*11*) was supported by two

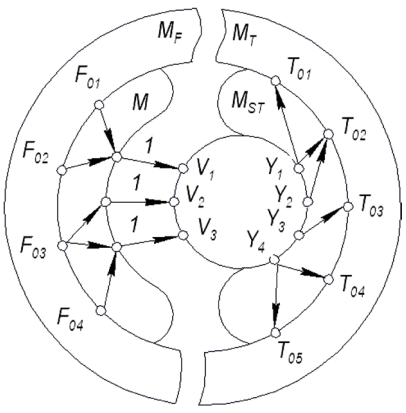

Fig. 1. Multimodel system of transfer phenomena.

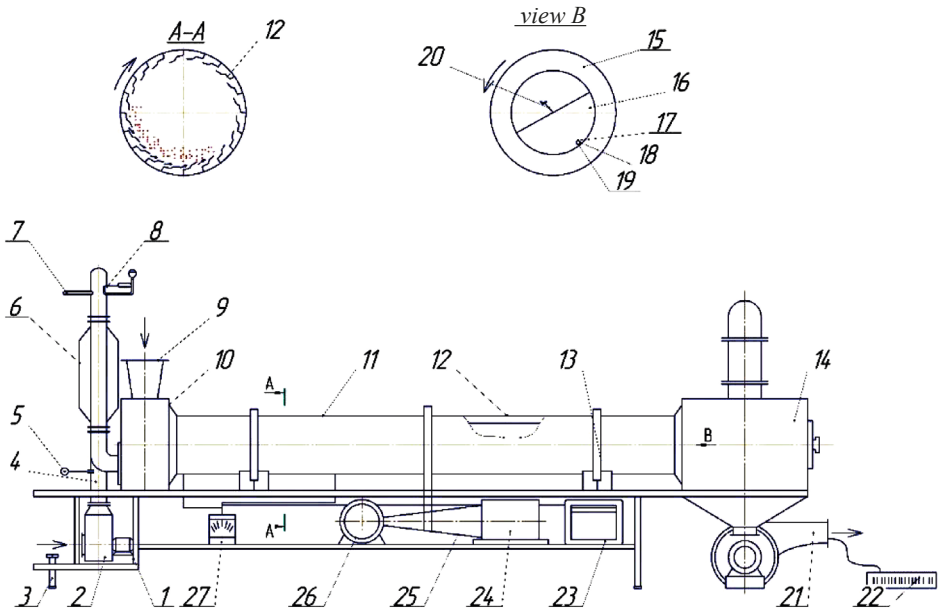

Fig. 2. Experimental drum dryer with a channel nozzle.

pairs of rollers (*13*). It was driven by the electric motor (*26*) and the gearbox (*24*), which were kinematically interconnected by the chain transmission (*25*).

During the drying process, the drying agent was supplied directly to the zone of the channel nozzles under the dryable material.

The pipe (*9*) was assembled on the body of the loading chamber (*10*). The pipe fed the wet bulk material to the drying drum. The spiral rotation of the drum moved the material to the discharge chamber (*14*).

The retaining ring (15) was assembled on the end surface of the drum. The ring slid in the groove of the fixed flange (*16*), through which the bulk material was continuously unloaded. During the drying process, the interior of the drum (*11*) was under a slight negative pressure due to the fact that the performance of the fan (*21*), which took away the waste drying agent, was several times higher than that of the delivery fan (*2*).

The temperature control of the drying agent was carried out directly in the bed of the wet solids. The thermocouples (*17*, *18*, and *19*) were installed on the bracket (*20*) (Fig. 2, view B).

The temperature was recorded with the electronic automatic self-recording potentiometer (*23*).

The relative humidity of the waste drying agent was measured with the hygrometer (*22*), a sorption-frequency single-channel digital device. The semiconductor thermoanemometer (*27*) measured the speed of the drying agent at the inlet and the outlet of the drum, as well as above and below the surface of the bed.

The angle of the drum, the frequency of its rotation, and the speed and temperature of the drying agent were set at the initial stage of the experiments. After that, there began a continuous supply of proportioned wet bulk material. Over the next 60–70 min, the material was sampled to measure its thermophysical characteristics, while the process of wet material feeding continued in the same mode.

After the sampling, the feeding of the wet bulk material to the drying drum and the movement of the drum stopped simultaneously. The speed and the temperature of the drying agent remained constant, according to the experimental conditions. After that, all the material was discharged from the drum into the receiving container, and its volume and weight were measured.

Taking into consideration the design feature of rotary drum dryers and the methods of continuous drying of wet solids in a fluidized bed, the model of continuous drying process can be represented as a multimodel system of transfer phenomena (Fig. 3).

The continuous drying process as a system of transfer phenomena. The model of the continuous drying process of wet solids in a fluidized bed displays the properties of the material and the coolant and the nature of non-steady processes that occur in the fluidized bed. It also makes it possible to improve the drying at various initial parameters of the material and the coolant.

The model of the product subjected to drying and the coolant connects the model of the system with the external environment and control actions. It also calculates the properties of the material and the coolant.

The following models of transfer phenomena correspond with the continuous process in a rotary drum dryer (Fig. 3):

– the model of the product subjected to drying;
– the coolant model;
– the model of the movement of the wet solids on the cylindrical surface;
– the model of the coolant fed to the fluidized bed;
– the hydrodynamic model of the fluidized bed;
– the model of the complex heat and mass transfer;
– the model of the drying process in the fluidized bed; and
– the model of the technical and economic characteristics.

The model of the product subjected to drying. If we consider the wet product as a subject of drying with

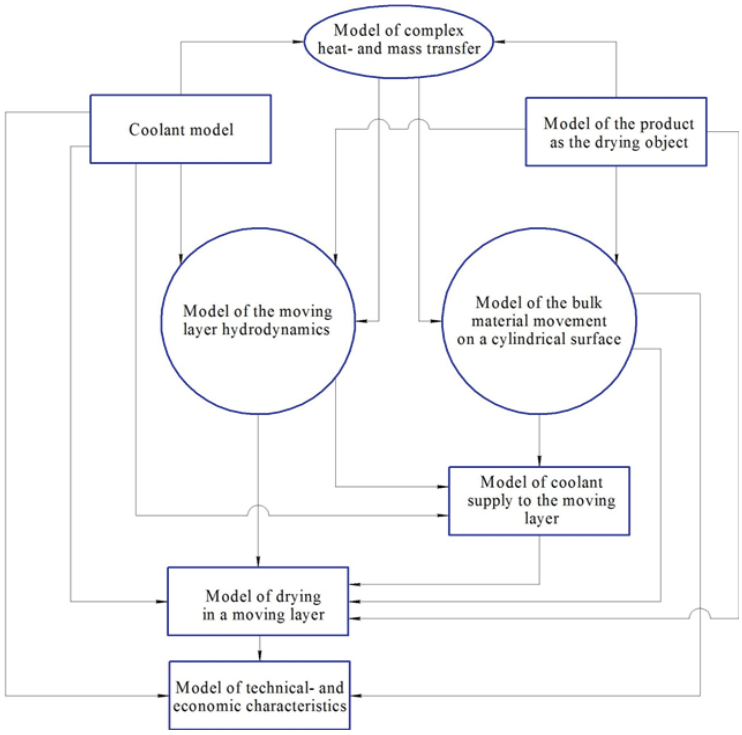

Fig. 3. Multimodel system of the continuous drying process of wet solids in a fluidized bed.

its moisture content, temperature, density of individual particles, and average linear dimensions, we can construct a mathematical model of the technological properties of the bulk material [8]. The model can describe the physical properties necessary for hydrodynamic and heat transfer processes. In this case, the linear dimensions of the bulk material determine its volume, surface area, equivalent diameter, sphericity (non-sphericity) coefficient, and the specific surface area. The equilibrium moisture, angle of repose, and the bulk density of the wet solids are determined depending on their humidity and the moisture of the drying agent. The specific heat is determined depending on the temperature of the product.

The model of the product subjected to drying. Let us consider the wet solids as a subject of drying, characterized by moisture content, temperature, density of individual particles of the product, and average linear dimensions. By doing so, we can construct a mathematical model of the technological properties of the wet solids [8]. The model describes the physical properties necessary for conducting hydrodynamic and heat transfer processes. In this case, the linear dimensions of the bulk material are determined by its volume, surface area, equivalent diameter, sphericity coefficient (non-sphericity), and the value of specific surface. Equilibrium moisture, angle of repose, and the bulk density of the wet solids are determined depending on their humidity and the moisture of the drying agent. The specific heat depends on the temperature of the product.

The coolant model. The wet and heated atmospheric air is considered as a binary mixture of the components of dry air and steam [9]. The parameters of the drying are calculated by the method of superposition of the component parameters. Tables and various functional and empirical relations are used to calculate the parameters of the components.

The model of the movement of wet solids on the cylindrical surface. In drum dryers, the material is dried in a fluidized bed while it is purged with a drying agent [5]. Fig.4 shows the flow chart of the bulk material in the transverse direction.

The angle of the bed in the cross section is determined by the angle of repose of the bulk material. It is assumed that the trajectory of the particles passes along a radial arc, whose radius equals the distance from the axis of the drum. When the particle gets to the end of the arc and surfaces, it falls down along the chordal surface of the flow.

The particle moves in the axial (translational) direction only if the flow surface has a certain axial angle, i.e.

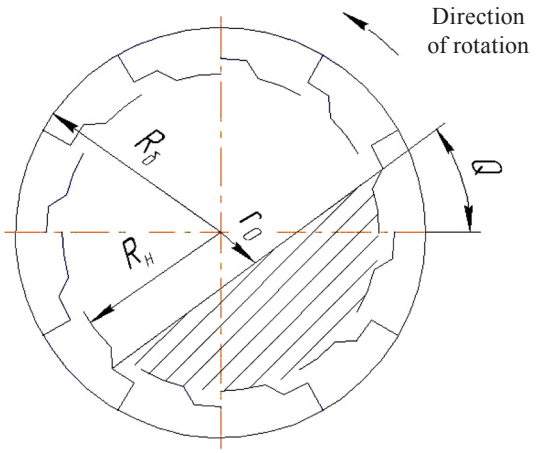

Fig. 4. Scheme of the movement of the wet solids in the rotating drum (cross section).

when there is a difference in the levels of the bed at the inlet and the outlet holes of the drum.

During translational motion, the trajectory of the particle follows the chordal plane. In this case, the trajectory is not perpendicular to the axis of the drum: it forms a < 90° angle with it and is perpendicular to the plane passing through the middle of the chords.

The model of the coolant fed to the fluidized bed. The drying agent enters the drying chamber of the drum dryer through the channels formed by the channel nozzles [6] and the outer cylinder of the drum through the side cavity of the drum (Fig. 5).

The side cavity of the drum was divided by a partition in such a way that the drying agent entered the channels under the nozzles that are situated under the material while the drum is rotating. The channel nozzle has a gap of constant width. The drying agent enters the drying chamber through this gap and contacts the layer of dispersed material.

Thus, the task is reduced to the calculation of gas distribution with an outflow through the lateral permeable surface, i.e. the layer of the dispersed material. It is possible to make an assumption that the parameters of the drying agent and the height of the bed are constant. Thus, one can calculate the hydrodynamics of the flow of the drying agent. In this case, the bed height may be its height in the middle section of the material flow. The parameters of the drying agent, such as density and kinematic viscosity, are assumed to be constant.

Two models were considered when choosing the mathematical model for the coolant supply from the channel nozzle slit through the bed of dispersed material. The first was the model for calculating the distribution of velocity and pressures along the z-shaped collectors; the second was the model of the constant-section air distributor with a longitudinal slit of constant width.

The hydrodynamic model of the fluidized bed. According to the hydrodynamics of the fluidized bed of the wet solids in rotating drum dryer, the drying agent flows around the particles in the fluidized bed of the granular product and between the particles of the material in the channels [6].

In this connection, we can point out external (flow-around), internal (filtration), and mixed hydrodynamics problem.

When solving the problem of heat and mass transfer, it is necessary to determine the active surface area of the product or the specific surface area of the bed and the speed with which the drying agent flows around the particles.

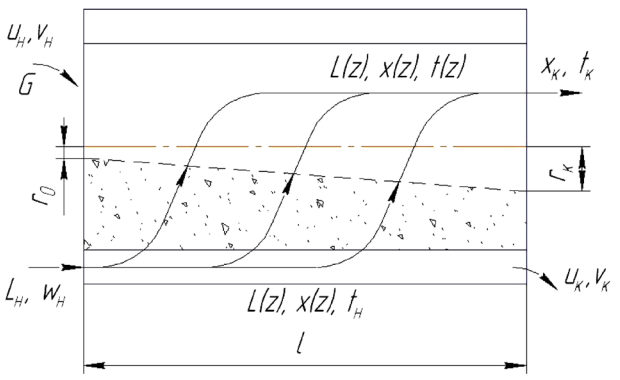

Fig. 5. Scheme of the coolant flow in the rotating drum dryer.

When solving the problem of the hydrodynamics of a bed, one has to determine the hydraulic resistance of the bed of the dispersed material. It is necessary to know the characteristics of the pore channel of the bed, its coefficient of the hydraulic resistance, and the flow rate of the drying agent in the channel.

For generality, it is assumed that the flow velocity around the particle and the flow speed in the channel are equal and related to the flow velocity in the direction of filtration in the whole liquidized bed by the ratio:

$$w_0 = \xi w. \quad (2)$$

The tortuosity coefficient ξ is calculated as follows:

$$\xi = 1 + (\pi/2 - 1)(1 - \varepsilon)^{2/3}. \quad (3)$$

The bed voidage ε is the ratio of voids between the particles in the layer and the volume of the bed. It can be expressed as the following ratio:

$$\varepsilon = 1 - \rho_d / \rho_p. \quad (4)$$

The specific surface of the material particles in the bed can be calculated through the specific surface of the particles S_s and layer porosity ε:

$$S_{sg} = S_s(1 - \varepsilon). \quad (5)$$

The loss of pressure during the movement of the drying agent through the granular layer can be calculated similarly to the pressure loss in pipelines:

$$\Delta = \lambda_g S_{sg} h \rho w^2 / (2\varepsilon^2). \quad (6)$$

The model of the complex heat and mass transfer. In the general case, when organizing continuous drying of wet solids in a fluidized bed, the coolant is supplied to the drying chamber at the expense of an external heat source (mechanical energy). As a result, there is a continuous forced flow of the heat transfer agent particles around the product. The process of continuous evaporation of moisture from the free surface of the moving material occurs, in this case, in the boundary layer and is caused by velocity and temperature gradients. As a result, there occurs a continuous diffusion flow of the medium. This continuous flow is constantly directed from the surface of the product into the depth of the coolant flow. The difference in the concentration of the vapor-gas mixture near the evaporation surface and in the main heat-wave flow of the coolant leads to a density difference in the vapor-gas mixture. It results in a continuous free natural heat and mass transfer.

Fig. 6 demonstrates the external heat and mass transfer under conditions of continuous dehydration as a combination of simple (marginal) modes of motion: forced, free, and diffusive.

To calculate the simultaneous continuous heat and mass transfer processes, we used the method based on the superposition of the absolute values of Nusselt num-

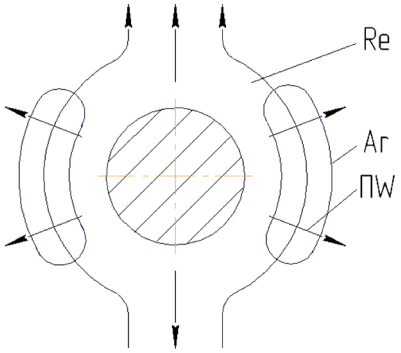

Fig. 6. Motion modes during continuous drying: Re – forced; Ar – free natural; PW – diffusive.

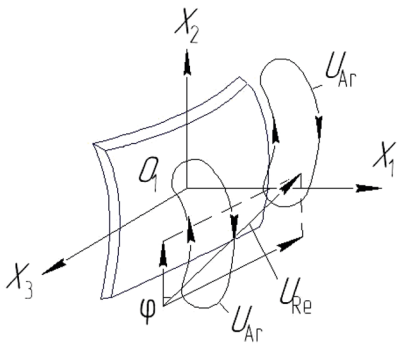

Fig. 7. The motion of the medium near the heat exchange surface in mixed convection.

ber [9]. In a complex continuous process, the absolute value of the transfer rate is determined according to the projection values of the transfer rate of simple processes onto two mutually perpendicular planes.

The velocity attitude of the forced motion of the medium U_{Re} makes an arbitrary angle φ with the planes, in which the velocity vectors of the free motion U_{Ar} lie.

Fig. 7 shows that the calculated dependence of the forced and the free motion with an arbitrary mutual orientation is

$$Nu = [(Nu^4_{ORe}\cos^4\varphi \pm Nu^4_{OAr})^{0,5}) + Nu^2_{ORe}\sin^2\varphi]^{0.5}. \quad (7)$$

When φ = 0, the forced and the free motions happen in the same plane:

$$Nu = (Nu^4_{ORe} \pm Nu^4_{OAr})^{0.25}. \quad (8)$$

When φ = π/2, the forced and the free motions happen in mutually perpendicular planes:

$$Nu = (Nu^2_{ORe} \pm Nu^2_{OAr})^{0.5}. \quad (9)$$

If the critical geometrical dimensions of the forced and the free motions are the same ($L = L_{Re} = L_{Ar}$), formula (8) coincides with the following relation:

$$Nu = \sqrt[4]{Nu^4_{ORe} + Nu^4_{OAr}}. \quad (10)$$

In accordance with the nature of the flow of the medium in the liquidized bed, the calculated relation of the intensity of the continuous transfer of the complex process meets conditions (8) and (10).

The absolute value of Nusselt number in forced motion conditions is calculated by the formula for the ball:

$$Nu_{OAr} = 2 + 0.56(Ar \times Pr)^{0.25}[Pr / (0.846 + Pr)]^{0.25}, \quad (11)$$

where $1 < Ar\ Pr < 10^5$. In conditions of free motion it is calculated by the formula for the sphere:

$$Nu_{ORe} = 2 + 0.03(Re^{0.54}Pr^{0.33} + 0{,}35Re^{0.58}Pr^{0.36}, \quad (12)$$

where $0{,}6 < Pr < 8\times10^3$ and $Re < 3\times10^5$.

In formulae (11) and (12), the critical value is the equivalent particle diameter of the wet solids.

The effective heat transfer coefficient α_{er} is used as a characteristic of heat transfer intensity. It takes into account the total amount of heat spent on continuous drying in a rotating drum dryer.

The effective heat transfer coefficient α_{er} is determined by a nonlinear differential equation, presented in dimensionless form:

$$dy/dx = A(y/x) + Rb, \quad (13)$$

where $y = t - T_i$; $x = u - u_e$; $A = \alpha S_s / c_m K$; $Rb = r(u_i - u_e / [c_m(t - T)]$ is Rehbinder number.

The solution for the equation (13) is determined by the following power function:

$$Rb = c[(t - T_i) / (T_f - T_i)]^m (\alpha S_s / c_t K)^n. \quad (14)$$

The effective heat transfer coefficient for the fluidized bed of wet solids under continuous dehydration conditions is determined by the following relation:

$$\alpha_{er} = (c_m K / S_s)[\ln Rb - \ln c + m\ln(t - T_i) / (T_f - T_i)] / n. \quad (15)$$

The model of the drying process in the fluidized bed. Let us consider the general case of an approximate mathematical description of a continuous process of moisture transfer:

$$dx_1/d\tau = -Kx_1(\tau),$$

$$dx_2/[G / U(\tau)]Kx_1(\tau), \quad (16)$$

where, in terms of optimal control methods [8], the parameters x_1 and x_2 are phase variables, and $U(\tau)$ is the control action.

The system of equations (16) is a combination of the kinetics of the continuous drying and the balance relation of moisture in the material and coolant.

Let us define the optimal control $U_{opt}(\tau)$ which transfers the continuous process from
– the given initial state:

$$x_1(0) = x_1^0 \text{ and } x_2(0) = x_2^0 \quad (17)$$

– and in the specified final state:

$$x_1(\tau_f) = x_1^f \text{ and } x_2(\tau_f) = x_2^f \quad (18)$$

so that the functional assumes the minimum value:

$$I = \int_0^{\tau_\kappa} U(\tau)\, d\tau. \quad (19)$$

Let us establish a function with auxiliary variables λ_0, λ_1 and λ_2:

$$H = \lambda_0 U - \lambda_1 K x_1 + \lambda_2 (GK/U_t) x_1 \quad (20)$$

and write the system of equations for the function λ_1:

$$d\lambda_1 / d\tau = -\partial H / \partial x_1 = \lambda_1 K - \lambda_2 \left(GK / U_T \right);$$
$$d\lambda_2 / d\tau = \partial H / \partial x_2 = 0. \quad (21)$$

The optimal control of the continuous process results from the condition that the function H has the maximum value:

$$\partial H / \partial U_T = \lambda_0 - \lambda_2 (GK / U_T^2) x_1 = 0, \quad (22)$$

Thus,

$$U_{opt}(\tau) = \sqrt{(\lambda_2 / \lambda_0) GK x_1}. \quad (23)$$

The following function solves the equations of the system (7), which describe the kinetics of continuous drying with the boundary condition (8):

$$x_1(\tau) = x_1^0 e^{-K\tau}. \quad (24)$$

In the rotating drum dryer, the dryable product is, as a rule, continuously loaded into the drying chamber on one side and unloaded on the other. The height of the bed at the input exceeds the height of the bed at the output, due to the rotation frequency and the horizontal angle of the drum. In general, the coolant is fed to the drying chamber and passes through the fluidized bed of the bulk material [5].

To study the dynamics of the continuous dehydration mechanism [9], the original scheme of interaction of the coolant flow and the fluidized bed of the wet solids moving along the cylindrical surface can be presented as (Fig. 8):

For practical calculations, the surface of the fluidized (sliding) bed is represented as a plane, and the cross section of the material flow is represented by a segment of a circle. To describe the continuous drying mode, the continuity equation for the flow of the dryable material can be represented as

$$q = \rho v S, \quad (25)$$

Note that the density of the dryable material is the function of its moisture content u:

$$\rho(u) = a - bu. \quad (26)$$

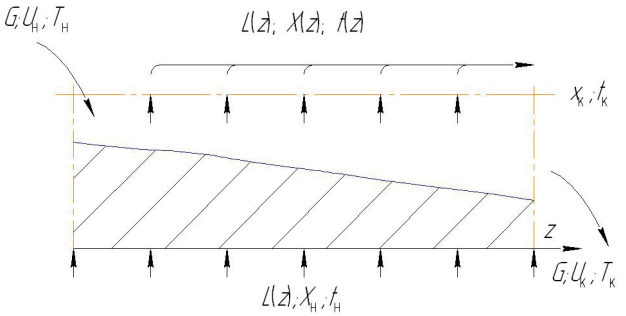

Fig. 8. Diagramme of interaction of the coolant flow and the fluidised bed of the dryable product moving along the cylindrical surface.

In rotary drum dryers, the warm-up periods are short, and the drying rate is constant. Hence, the drying rate can be represented as a function of moisture content [10]:

$$du/d\tau = -K(u - u_p). \quad (27)$$

For rotary drum dryers, $u = dz/dt$. In this case, if we take into account Eq. (25) and Eq. (26), the drying rate can be represented as follows:

$$du/dz = -(KS\rho/q)(u - u_p). \quad (28)$$

The heat is continuously supplied to the dryable product. It heats the material and evaporates the moisture:

$$dT/dz = [(\alpha S_s (t - T) / v) + r(du/dz)] / (c_m + c_w u), \quad (29)$$

$$S_s = S_p (1 - \varepsilon) / (V_p \rho_m). \quad (30)$$

In Eq. (29), we point out, first, the heat supplied to the material,

$$q_1 = \alpha S_s (t - T), \quad (31)$$

second, the heat spent on heating the mass of absolutely dry material and moisture contained in it,

$$q_2 = v(c_m + c_w u)(dT/dz), \quad (32)$$

third, the heat spent on the evaporation of moisture from the material,

$$q_3 = vr(du/dz). \quad (33)$$

Using the continuity equation (25), we express the equation for the temperature of the material through its moisture content:

$$dT/du = -[\alpha S_s (t - T)] / c_{am} K(u - u_e)] + r / c_{am}, \quad (34)$$

where $c_{am} = c_m + c_w u$.

To formulate the balance of the coolant, we divide the fluidized bed of the material of length l into a bed of infinitely small sections of length Δz. Let us assume that, within each of such moving sections, the material is perfectly mixed and has a constant temperature and moisture content. When moving from section to section, the temperature and the moisture contents of the material change to infinitely small values.

Let us single out one of these sections and consider the material flow in the moving element of the wet solids (Fig. 9).

The flow $G(1 + u)$ of the wet solids enters the volume of the moving element, and the flow $G(1 + u + du)$ comes out with its moisture content being $u + du$. The coolant supplied to the fluidized bed is determined by the expenditure function $L(z)$. When they enter the fluidized bed, the moisture content and the temperature of the coolant are constant throughout the whole layer. They are functions of the z coordinate at the output. The amount of moisture evaporated from the elementary volume of the material equals the amount of moisture absorbed by the

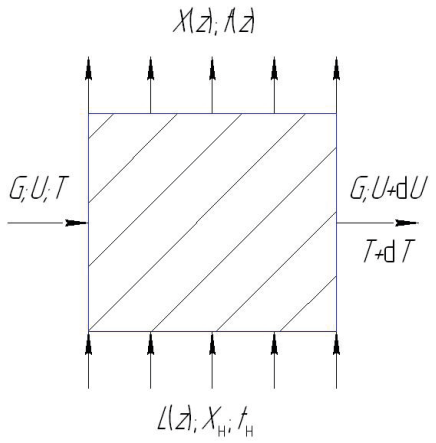

Fig. 9. Material flows in the moving element of the wet solids.

coolant that enters the elementary volume:

$$dL(z) / V_s = - G du. \quad (35)$$

The heat balance equation is similar to the moisture balance equation (26) for the elementary volume:

$$- d[L(I - I_i)] / V_s = G(di + r du), \quad (36)$$

where $i = (c_m + c_w u)$ is the enthalpy of the material; $I = c_{hc} t + (r + c_s t)x$ is the enthalpy of the coolant; c_{hc}, c_s is specific heat capacity of the coolant and the steam.

Thus, the continuous drying process on a cylindrical surface can be approximately described by a system of ordinary differential equations:

$$du/dz = - (KS\rho / q) (u - u_p), \quad (37)$$

$$dT / dz = [(\alpha S_s (t - T) / \nu) + r(du/dz)] / (c_m + c_w u), \quad (38)$$

with initial conditions

$$u(0) = u_i, T(0) = T_i \quad (39)$$

and a system of balance relations obtained after Eq. (35) and Eq. (36) were integrated with initial conditions $x(0) = x_i$ and $t(0) = t_i$:

$$x = x_i + (GV_s / L)(u_i - u), \quad (40)$$

$$t = \{I_i - rx - (GV_s / L) [(i - i_i) + r(u - u_i)]\} / (c_{hc} + c_s x). \quad (41)$$

Upon integrating Eq. (37), we receive an analytical expression for the moisture content of the material in the coordinates of the length of the rotating drum:

$$u(z) = (u_p + me^{-a}) / (1 + ue^{-a}), \quad (42)$$

where $m = a(u_i - u_e)/\rho(u_i)$; $n = b(u_i - u_e)/\rho(u_i)$; $\alpha = K\rho(u_e)V(z)/q_i$; $q_i = G(1 + u_e)$; $\rho(u_e) = a - bu_p$; $\rho(u_e) = a - bu_i$; $V(z)$ is the volume of the fluidized bed from the upload point to the coordinate z.

Unlike Eq. (37). Eq. (38) makes it possible to obtain an analytical solution only with the assumption that the heat capacity of the product remains average. If we agree that $c = c_m + c_w u_{am}$, where u_{am} is the average moisture content of the material, we obtain the following equation:

$$dT/du = - [\alpha S_s (t - T) / c_{am} K(u - u_e)] + r / c_{am}, \quad (43)$$

where $dT/du = \nu(dT/d\tau)$.

By integrating Eq. (43) with the initial condition $T(0) = T_i$, we get:

$$T(u) = T_i \varphi(u) + \{t + [rK(u - u_e) / \alpha S_s]\}[1 - \varphi(u)], \quad (44)$$

where $\varphi(u) = (u - u_e) / (u_i - u_e)$.

Formula (44) gives us values that are close to those we obtain after integrating Eq. (37), while the ratio error does not exceed 2.0%. This allows us to declare Eq. (43) and its analytical solution (44) applicable for analytical studies of continuous dehydration in a fluidized bed of bulk material.

Eqs. (37), (38), (40), and (41) make it possible to calculate the moisture content and the temperature of the dryable product in the fluidized bed along the length of the drying drum. We know the values of moisture content and coolant temperature only at the surface of the material. To calculate the parameters of the coolant at the outlet of the drying drum, we use the formulae for coolant mixing [11].

The equations for the moisture content and the temperature of the coolant at the outlet from the elementary volume of the fluidized bed (Fig. 9) can be formulated on the basis of Eqs. (31) and (32), if we assume that the temperature t_j and the moisture content x_j of the coolant at the outlet from the elemental volume are constant, and the coolant rate through this elementary volume is the difference in rates $dL_j = L_{j+1} - L_j$, where L_j is the coolant rate when it is supplied to the fluidized bed in the section $[0, z_j]$:

$$x_j = x_i + (GV_s / dL_j)(u_{j+1} - u_j), \quad (45)$$

$$Ij = I_i - (GV_s / dL_j)[(i_j - i_i) + r(u_{j+1} - u_j)], \quad (46)$$

$$t_j = (I_j - rx_j) / (c_{hc} + c_s x_j), \quad (47)$$

where $i_i = c_m T_i$; $j = c_m T_j$; $I_s = c_{hc} t_i + (r + c_s t_j)x_j$.

The values of moisture content and the temperature of the coolant at the outlet of the drying chamber are calculated from the parameter values of the coolant flows that are coming out of all the elementary volumes of the fluidized bed, according to using the mix formulae of the flows:

$$x_{\text{ê}} = (1 / L)\sum_{j=1}^{n} x_j dL_j; \quad (48)$$

$$I_к = (1 / L)\sum_{j=1}^{n} I_j dL_j; \quad (48)$$

$$t_f = (I_f - rx_f) / (c_{hc} + c_s x_f). \quad (49)$$

If we use Eq. (42) and take into account that the kinetics of continuous drying is described by Eq. (27), while the flow of the bulk material moving on the cylindrical surface is presented as an ideal extrusion model (25), we can determine the equation of the effective continuous drying coefficient:

$$K_e = \{q / [V_e \rho(u_e)]\} \ln[\rho(u_f)(u_i - u_e) / \rho(u_i) (u_f - u_e)]. \quad (50)$$

Relation (50) differs from others that determine the coefficient of drying. It is based on the analysis of the mechanics of a fluidized bed of wet solids on a cylindrical surface with regard to the continuous dehydration.

The model of the technical and economic characteristics. To assess the energy performance of a drying unit with the convective method of heat supply, one can assess the use of the drying agent. The energy losses are determined by the difference between the amount of the supplied and the usable energy [11, 12–15].

Various coefficients of efficiency are used as energy criteria. In the general case, they are defined as the ratio of the usable energy E_1 to the expended energy E_2:

$$\eta = E_1 / E_2. \quad (51)$$

For a convective drying process, the energy efficiency can be expressed by the following relation:

$$\eta_t = (t_1 - t_2) / (t_1 - t_0). \quad (52)$$

The thermoeconomic analysis combines exergy analysis and economic optimization. The criterion for the thermoelectric optimization is a composition of additive functions. These functions should quantify the exergy, the equipment costs, etc.

The most general formula for the so-called thermoelectric criterion is

$$\{\min C\} = \left\{ \min \left[\frac{\sum\limits_j c_{ei} e_i + \sum\limits_n k_n}{\sum\limits_k e_{nk}} \right] \right\}. \quad (53)$$

Table 1. Baseline input

Input parameter	Variants of the computational experiment					
	1	2	3	4	5	6
1.1. Rotary drum dryer						
Dryer drum length, m	1.2	1.2	1.2	1.2	1.2	1.2
Outer radius of the drum, m	0.15	0.15	0.15	0.15	0.15	0.15
Channel nozzle radius, m	0.115	0.115	0.115	0.115	0.115	0.115
Width of the slits of the channel nozzle, m	0.02	0.02	0.02	0.02	0.02	0.02
Number of slits channel nozzles, pcs.	12	12	12	12	12	12
Number of slits in the channel nozzle through which the coolant is supplied, pcs.	4	4	4	4	4	4
Coefficient of local resistance	0.16	0.16	0.16	0.16	0.16	0.16
1.2. Product						
Density of the material particles, kg/m^3	770	770	770	770	770	770
Geometrical dimensions of the material particles, m:						
length	10.7	10.7	10.7	10.7	10.7	10.7
width	5.0	5.0	5.0	5.0	5.0	5.0
thickness	3.3	3.3	3.3	3.3	3.3	3.3
Initial moisture content, kg/kg	0.105	0.105	0.105	0.105	0.105	0.105
Initial temperature, °C	19	20	14	17	13	14
Specified final moisture content, kg/kg	0.0547	0.0705	0.0546	0.0828	0.0574	0.0387
Set final temperature, °C	56	55	59	42	55	66
1.3. Coolant						
Barometric pressure, kPa	100.5	100.5	100.4	100.3	100.3	100.3
Outside temperature, °C	16	15	14	16	14	16
Outside air humidity, %	73.0	73.0	81.8	82.5	81.9	72.0
Temperature of the drying agent at the inlet of the drying chamber, °C	180	210	240	180	210	240
Moisture content of the drying agent at the inlet of the drying chamber, kg/kg	0.008413	0.007886	0.008295	0.009550	0.008314	0.008314
Temperature of the drying agent at the outlet of the drying chamber, °C	149	167	187	134	165	185
Specified moisture content of the drying agent at the outlet of the drying chamber, %	0.0243	0.0275	0.0332	0.0305	0.0294	0.0342
1.4. Process parameters						
Dum angle, rad	0.03490	0.03490	0.05236	0.05236	0.01745	0.01745
Drum rotation frequency, 1/sec	0.0250	0.0583	0.0250	0.0583	0.0417	0.0417
Product feed rate, kg/sec	0.0106	0.0174	0.0167	0.0348	0.0159	0.0121
Drying agent consumption, m^3/sec	0.04010	0.03868	0.04559	0.04396	0.04529	0.04199
Radius of the circle touching the bed at the input point, m	0.03	0.01	0.02	0.03	0.02	0.01
Consumption coefficient of the drying agent	0.6	0.6	0.6	0.6	0.6	0.6
Absolute roughness of the air duct wall, m	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}	0.1×10^{-3}
Filling rate of the drum, %	25	35	30	25	30	35

Table 2. The results of the computational experiment (output)

Input parameter	Variants of the computational experiment					
	1	2	3	4	5	6
2.1. Calculation results for the product model subjected to drying						
Volume of the particle, m^3	0.7506×10^{-7}	0.7506×10^{-7}	0.7506×10^{-7}	0.7506×10^{-7}	0.7506×10^{-7}	0.7506×10^{-7}
Surface area of the particle, m^2	0.1608×10^{-3}	0.1608×10^{-3}	0.1608×10^{-3}	0.1608×10^{-3}	0.1608×10^{-3}	0.1608×10^{-3}
Sphericity coefficient	0.53507	0.53507	0.53507	0.53507	0.53507	0.53507
Aspheric coefficient	1.87	1.87	1.87	1.87	1.87	1.87
Equivalent particle diameter, m	0.5234×10^{-2}	0.5234×10^{-2}	0.5234×10^{-2}	0.5234×10^{-2}	0.5234×10^{-2}	0.5234×10^{-2}
Equilibrium moisture content in the material, kg/kg	0.02183	0.02180	0.02183	0.02190	0.02193	0.02193
Angle of friction, rad	0.7345	0.7345	0.7345	0.7345	0.7345	0.7345
Loose weight density, kg/m^3	398.5	398.5	398.5	398.5	398.5	398.5
Specific heat capacity of the material, kJ/(kg×K)	1.5160	1.5164	1.5140	1.5152	1.5136	1.5140
Specific surface of the particles, m^2/m^3	2142.62	2142.62	2142.62	2142.62	2142.62	2142.62
2.2. Calculation results for the coolant model						
Moisture, %	1.33448	1.25207	1.31597	1.51116	1.31889	1.31894
Wet thermometer temperature, °C	45.2109	47.8192	50.2885	45.4979	47.9154	50.2922
Specific heat of dry air, kJ/(kg×K)	1.022	1.028	1.035	1.022	1.028	1.035
Specific heat capacity, kJ/(kg×K)	2.710	3.200	3.880	2.710	3.200	3.880
Specific evaporation heat, kJ/K	2015.20	1900.50	1766.00	2015.20	1900.50	1766.00
Coefficient of kinematic viscosity of the drying agent, m^2/sec	0.3029×10^{-4}	0.3136×10^{-4}	0.3032×10^{-4}	0.3000×10^{-4}	0.3111×10^{-4}	0.3029×10^{-4}
Drying agent density, kg/m^3	0.84373	0.84722	0.90496	0.85300	0.85459	0.90661
Specific volume of wet air, $m^3/(kg\cdot sec)$	1.31526	1.40113	1.49053	1.32022	1.40488	1.49211
Heat conductivity coefficient of the drying agent, W/(m·K)	0.3773×10^{-1}	0.3998×10^{-1}	0.4207×10^{-1}	0.377×10^{-1}	0.3998×10^{-1}	0.4207×10^{-1}
Prandtl number	0.7017	0.6946	0.6906	0.7041	0.6956	0.6907
Schmidt number	0.5638	0.5202	0.4512	0.5584	0.5160	0.4507
2.3. Calculation results for the model of the movement of wet solids along the cylindrical surface						
Specific consumption of the drying agent, kg/kg	62.9435	50.9856	40.1518	47.7182	47.4229	38.6292
Minimum design airflow per drying, kg/sec	3.0371×10^{-2}	2.7698×10^{-2}	3.0584×10^{-2}	3.3362×10^{-2}	3.2481×10^{-2}	2.8045×10^{-2}
Minimum estimated volume flow rate of the drying agent m^3/sec	0.03995	0.03881	0.04559	0.04405	0.04563	0.04185
Fictitious speed of the drying agent through the material bed, m/sec	0.2205	0.1941	0.2385	0.2417	0.2369	0.2108
Speed of the drying agent, reduced to the full cross section of the bed, m/sec	0.4570	0.4024	0.4943	0.5010	0.4910	0.4368
Porosity of the bed	0.4825	0.4825	0.4825	0.4825	0.4825	0.4825
Specific surface of the material in the bed, m^2/kg	1.5913	1.5913	1.5913	1.5913	1.5913	1.5913
Equivalent pore channel diameter, m	0.1740×10^{-2}	0.1740×10^{-2}	0.1740×10^{-2}	0.1740×10^{-2}	0.1740×10^{-2}	0.1740×10^{-2}
Tortuosity coefficient of the channels	1.3679	1.3679	1.3679	1.3679	1.3679	1.3679
Length of the pore channels, m	0.09375	0.1193	0.1070	0.09375	0.1070	0.1198
Equivalent Reynolds number	78.9714	67.1395	85.3087	87.4081	82.6012	75.4855
Hydraulic resistance coefficient of the bed	1.0080	1.1418	0.9516	0.9347	0.9746	1.0431
Bed resistance, Pa	507.731	447.649	601.354	572.002	573.984	515.488
Heat transfer coefficient, $kW/(m^2 \times K)$	0.206419	0.186365	0.224280	0.223457	0.220131	0.201593
Residence time in the drying chamber, min	7.899	6.710	6.016	2.389	6.315	9.723
Drying coefficient, 1/sec	0.1959×10^{-2}	0.1330×10^{-2}	0.2580×10^{-2}	0.2168×10^{-2}	0.2242×10^{-2}	0.2734×10^{-2}
2.4. Results of the calculation for the model of the coolant supplied to the fluidized bed						
Cross-sectional area of the air distributor, m^2	0.9712×10^{-2}	0.9712×10^{-2}	0.9712×10^{-2}	0.9712×10^{-2}	0.9712×10^{-2}	0.9712×10^{-2}
Perimeter of the air distributor, m	0.8350	0.8350	0.8350	0.8350	0.8350	0.8350
Equivalent diameter of the channel nozzle, m	0.04653	0.04653	0.04653	0.04653	0.04653	0.04653
Speed of the drying agent at the beginning of the drum, m/sec	4.1287	3.9852	4.6940	4.5261	4.6631	4.3233
Reynolds number	6342.55	5907.68	7202.19	7020.14	6973.78	6641.71
Average flow rate of the drying agent from the channel nozzle, m/sec	0.4177	0.4029	0.4749	0.4579	0.4718	0.4374
Coefficient of friction of the air nozzle	0.03705	0.03761	0.03609	0.03628	0.03633	0.03670
Coefficient of friction of the air nozzle friction	1.14102	1.14166	1.13992	1.14014	1.14019	1.14061
Total resistance of the air nozzle, Pa	8.2	76	11.4	9.6	10.6	9.7
Slit parameter	5.931	5.931	5.931	5.931	5.931	5.931
Air duct parameter	5.082	5.097	5.058	5.062	5.064	5.073
2.5. Results of the calculation for the hydrodynamics model of the fluidized bed						
Volumetric capacity of the dryer for wet material, m^3/sec	0.266×10^{-4}	0.4366×10^{-4}	0.4191×10^{-4}	0.8733×10^{-4}	0.3990×10^{-4}	0.3036×10^{-4}
Dryer productivity according to absolutely dry material, kg/sec	0.9593×10^{-2}	0.1575×10^{-1}	0.1511×10^{-1}	0.3149×10^{-1}	0.1439×10^{-1}	0.1095×10^{-1}
Dryer productivity according to the evaporated moisture, kg/sec	0.483×10^{-3}	0.543×10^{-3}	0.762×10^{-3}	0.6991×10^{-3}	0.6849×10^{-3}	0.7260×10^{-3}
Angle between the surface and the axis of the drum, rad	0.02775	0.02911	0.02819	0.02775	0.02818	0.02913
Radius of the circle touching the bed at the output of the product, m	0.06331	0.04494	0.05383	0.06331	0.05382	0.04497
Volume of the drum occupied by the bed, m^3	0.01246	0.01745	0.01496	0.01246	0.01496	0.01745
Area of the middle section of the bed, m^2	0.01039	0.01454	0.01246	0.01039	0.01246	0.01454
Radius of the circle touching the bed in the middle section, m	0.04646	0.02742	0.03677	0.04646	0.03676	0.02744
Thickness of the bed in the middle section, m	0.06855	0.08759	0.07823	0.06853	0.07824	0.08756
Width of the bed in the middle section, m	0.1516	0.1660	0.1593	0.1516	0.1593	0.1660

The rest Table 2

Effective area of the bed, m^2	0.1819	0.1932	0.1912	0.1819	0.1912	0.1992
Distance between the beginning of the drum and the middle section of the bed, m	0.5931	0.5982	0.5945	0.5930	0.5945	0.5982
Influence coefficient of the flow rate of the drying agent in the dense blown bed on the performance of the dryer	2.24200	1.41040	2.52000	2.40090	2.74770	1.98300
2.6. Results of the calculation for the model of complex heat and mass transfer						
Equivalent Reynolds number	71.1793	67.2305	81.9623	79.8905	79.3629	75.5839
Archimedes number	455.991	479.825	565.384	463.839	487.412	455.778
Limit value of Nusselt number for natural convection conditions	2.4107	2.4355	2.5338	2.4231	2.4459	2.5354
Nusselt number for simultaneous processes	5.5678	5.4071	5.8927	5.8149	5.7896	5.7035
Heat transfer coefficient, $kW/(m^2\times K)$	0.04014	0.04131	0.04737	0.04191	0.04423	0.04585
Specific heat flow, kW/m^2	5.4107	6.6998	8.9866	5.6374	7.1691	8.6980
Specific mass flow, $kg/(m^2\times sec)$	0.2685×10^{-2}	0.3525×10^{-2}	0.5089×10^{-2}	0.2797×10^{-2}	0.3772×10^{-2}	0.4925×10^{-2}
2.7. Results of the calculation for the model of drying in a fluidized bed						
The final design value of the moisture content in the material, kg/kg	0.0546	0.0704	0.0545	0.0828	0.0573	0.03856
The final design value of the material temperature, °C	56	55	59	42	55	66
The final calculated value of the moisture content in the drying agent, kg/kg	0.0243	0.0276	0.0332	0.0306	0.0296	0.0342
The final design value of the temperature in the drying agent, °C	156.664	171.018	191.130	138.289	171.987	193.168
Effective heat transfer coefficient, $kJ/(m^2\times K)$	1.4338×10^{-3}	1.0687×10^{-3}	1.3248×10^{-3}	2.4044×10^{-3}	1.4310×10^{-3}	1.0144×10^{-3}
2.8. Results of the calculation for the model of technical and economic characteristics						
Dryer productivity for moisture removal, kg/sec	0.4834×10^{-3}	0.5441×10^{-3}	0.7631×10^{-3}	0.7001×10^{-3}	0.6862×10^{-3}	0.7276×10^{-3}
Exergy of the drying agent at the inlet to the drying chamber, kJ	35.32	48.15	62.78	35.39	48.74	61.56
Specific exergy, kJ/kg	2227.66	2443.20	2515.93	1683.07	2289.72	2380.89
Energy efficiency	7.2	6.0	5.0	10.5	6.4	5.3
Capacity for evaporation according to evaporated moisture, kg/m^3	0.9696×10^{-2}	1.0913×10^{-2}	1.5307×10^{-2}	1.4041×10^{-2}	1.3763×10^{-2}	1.4593×10^{-2}

RESULTS AND DISCUSSION

The research was based on the informational and structural scheme of a convective drying unit model. For all its components, we developed the mathematical models in accordance with the analytical multimodel system for the continuous drying process of wet solids in a fluidized layer. As a result, we constructed an automated calculation system for the continuous process of convective drying, which can be applied to a rotary drum dryer (Tables 1 and 2).

The practical result of the study consisted in assessing and comparing the quality indicators of sunflower seeds of natural moisture. First, one sample of sunflower seeds was dried on an experimental drum dryer with a channel nozzle. Second, another sample was dried on an industrial drum dryer with a lifting vane system at the vegetable oil plant ZAO ZRM Bobrovsky. Finally, the experimentally obtained results were compared with the computed results obtained from the mathematical model.

The experimental data show (Table 3) that after the sunflower seeds were dried in a drum dryer with a channel nozzle, the difference between the maximum and minimum humidity of individual seeds decreased by 2.34 times. This can be explained by the same residence time in the drying zone and the uniform distribution of the coolant flow in the fluidized bed.

Table 3. Change in humidity of single sunflower seeds during the drying process in the drum dryer with a channel nozzle, %

Before drying		After drying	
min	max	min	max
14.02	14.69	5.19	5.51
12.28	12.68	4.19	4.34
10.04	11.08	3.65	4.10

The effect of the drying mode on the change in the quality of oil in the sunflower seeds (Table 4) was measured by changing the acid, peroxide, and iodine numbers at a different initial seed moisture. The heating temperature did not exceed the maximum permissible temperature for the particular humidity. It ensured the inactivation of enzymes, i.e. lipase and lipoxygenase.

Table 4 shows that the acid values of the oils in the studied modes were somewhat reduced. This can be explained by the fact that low molecular organic acids were distilled together with the water steam during the drying process. The peroxide numbers somewhat increased with increasing temperature, which can be explained by the catalytic effect of temperature on fat oxidation due

Table 4. Effect of drying process of sunflower seeds in a drum dryer with a channel nozzle on the quality of vegetable oil

Drying agent temperature, °C	Acid number, mg KOH	Peroxide number, % I_2	Iodine number, g I_2
10.56% moisture			
0	1.80	0.016	151.6
130	1.71	0.021	148.1
150	1.68	0.024	145.6
170	1.65	0.035	142.2
14.45% moisture			
0	1.82	0.015	149.8
130	1.71	0.017	149.2
150	1.65	0.019	147.9
170	1.63	0.030	144.5
18.42% moisture			
0	1.85	0.014	146.3
130	1.79	0.017	143.8
150	1.76	0.018	141.2
170	1.72	0.025	140.5

Table 5. Physical and chemical indicators of sunflower seeds and oil

Parameters	Drying method	
	In a drum dryer with a channel nozzle	In an industrial drum dryer
Drying agent temperature, °C	150	280
Seed moisture, %		
initial	14.35	14.35
final	7.15	7.20
Oil content on absolutely dry matter, %	55.82	56.36
Damaged seeds, %	4.15	6.15
Phosphatides in the oil, %	0.050	0.049
Acid number, mg KOH	1.65	1.87
Peroxide number, % I_2	0.019	0.025
Iodine number, g I_2	147.8	160.6

to the presence of oxygen in the air. The iodine numbers decreased with increasing temperature. This resulted from the chemical reactions of breaking double bonds in the carbon chain of the fats and the addition of organic compounds and radicals that were present in the air.

Table 5 features some results of the comparative production tests. They confirm the fact that the temperature of the drying agent destroys protein structure. The number of damaged sunflower seeds when dried in a dense ventilated bed of moving seeds is significantly lower than in the fluidized bed.

The analysis of the physicochemical parameters of the oil suggests that the structure of the drying agent largely determined the quality of the dried sunflower seeds: acid, peroxide, and iodine numbers decreased by 12, 24, and 9% respectively.

If we compare the data obtained from the practical tests and from the model (Table 1), we can conclude that the results are reproducible. The following optimal values were also obtained while solving the problem of convective drying optimization: the initial moisture content of sunflower seeds was 16–17%; the temperature of the drying agent in the bed was 66–67%; the consumption of drying agent was $(3.2–3.4)\times10^{-2}$ m^3/sec; the angle of the drum was 0.61–0.70 rad; the drum rotation frequency was 3.6–4.2 min. These results agreed with the data of the model presented in Table 2.

CONCLUSION

The proposed multimodel system of non-stationary drying processes for bulk materials has a number of advantages. First, it leads to a block-modular construction and expedient aggregation of rotary drum dryers. Second, it optimizes the allowances on the inputs and outputs of technological operations and links them together. Third, it develops requirements for the quality of raw materials and environmental conditions, in terms of the high efficiency of the organization of its processing.

Thus, when studying the specifics of heat transfer between the coolant and the solid particles of bulk material in a rotary drum dryer, most researches determine the average values of heat transfer coefficients. The proposed approach for calculating the effective heat transfer coefficient in a fluidized bed provides the required reproducibility and differs from the experimental data by no more than 2.0% (Table 2).

The energy performance of rotary drum dryers with a convective method of heat supply can be assessed according to the degree of the coolant use. The energy losses are determined by the difference between the amount of supplied and usable energy. It is more difficult to determine the optimal variant if it is necessary to satisfy several efficiency conditions. In this case, one should use compromise criteria, e.g. capital and energy costs, capacity, quality of the finished product, reliability of the management system, level of environmental safety, etc.

Nomenclature

τ – residence time of the bulk material in the dryer drum, sec;
G – dryer capacity, kg/sec;
ρ_{db} – dry bulk density, kg/m^3;
V_d – dryer volume, m^3;
G_1 – amount of material in the drum, kg;
G_2 – the amount of the material leaving the drum per unit of time, kg/sec;
V_m – volume of material unloaded from the drum, m^3;
$x_1(\tau)$, $x_2(\tau)$, – the moisture content of the product and the coolant, respectively, kg/kg;
K – drying ratio, 1/sec;
G – consumption of the dryable product, kg/sec;
$U(\tau)$, $L(z)$ – coolant flow rate, kg/sec;
q – moving mass flow, kg/sec;
ρ – density of the dryable product, kg/m^3;
u – material speed, m/sec;
S – section area of the fluidized bed, m^2;
a, b – constants determined experimentally;
u_e – equilibrium moisture content of the material, kg/kg;
T – product temperature, °C;
α – heat transfer coefficient, kW/(m^2×K);
t – coolant temperature, °C;
S_s – specific surface area of the material, m^2/kg;
r – specific heat of vaporization, J/kg;
c_m, c_w – specific heat capacity of dry material and water, kJ/(kg×K);
S_p – surface of the particle, m^2;
V_p – volume of the particle, m^3;
ε – porosity of the fluidized bed;
V_s – specific volume of the coolant, m^3/kg;
$x(z)$ – moisture content of the coolant, kg/kg;
V_f – the volume of the fluidized bed of the bulk material in the rotating drum, m^3;
$\rho(u_e)$, $\rho(u_i)$, $\rho(u_f)$ – product density corresponding to the equilibrium, initial, and final moisture content of the material, kg/m^3;
Nu_{ORe}, Nu_{OAr} – Nusselt numbers for forced and free movement forms, respectively;
c, m, n – constants of the equation;
n – drum speed per minute, min^{-1};
φ – drum angle, rad;

ψ – the angle between the surface of the bed and the axis of the drum, rad;
Θ – friction angle of the material, rad;
R – the radius of the channel nozzle of the drum, m;
r_0 – the radius of the circle touching the bed at the input, m;
A – coefficient that takes into account the effect of the average flow rate in the bed of the wet solids on the throughput of the dryer, A = f(Re) is determined experimentally;
ξ – tortuosity coefficient of the channel;
w – flow rate in the direction of filtration, m/sec;
ρ_d, ρ_p – bulk density and particle density, kg/m^3;
λ_g – the resistance coefficient of the granular bed;
w_w – flow rate in the direction of filtration, m/sec;
h – thickness of the bed, m;
ρ – density of the drying agent, kg/m^3;
t_1, t_2, t_0 – temperature of the drying agent at the inlet and the outlet from the drying chamber and the temperature of the outside air, °C.
C – unit exergy value;
e_i – energy of the consumed raw materials and exergy;
c_{ei} – unit cost of exergy of the raw materials and energy;
e_{nk} – exergy of the products;
k_n – capital and other associated expenditures for the n-subsystem.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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Effective technological scheme for processing triticale (*Triticosecale* L.) grain into graded flour

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Abstract: The present paper features Triticale grain processing. The research involved two Russian cultivars of Triticale grain, i.e. Ramzes and Saur. We investigated two schemes of processing these grain varieties into high-quality baker's grade flour. The first scheme was reduced and included only the processes of breaking and reduction, whereas the second scheme was more advanced and included breaking, sieving, sizing, and reduction processes. The paper gives a thorough description of the processing schemes, their parameters, and milling modes. A detailed analysis proved the high efficiency of the advanced scheme which presupposed the use of sieve purifiers. Their expediency was determined by the specifics of break dunst products at breaks I, II, and III. The Triticale flour varieties were produced by mixing various flows of the central, intermediate, and peripheral parts of the Triticale grain endosperm. The reduced scheme produced a 40% yield for the Ramzes variety (ash content = 0.70%, according to the State Standard 34142-2017*), while the advanced technological scheme resulted in a 63% yield. As for the Saur variety, the advanced scheme produced a total yield of 78%, which was 0.6% higher than in the reduced scheme. The advanced scheme resulted in a 46% yield of the T-60 flour variety, which had the lowest ash content among all the varieties of Triticale flour, whereas the reduced scheme failed to produce the flour of this variety. The experiment also involved the first-ever study of the rheological properties of Triticale flour varieties with Mixolab (Chopin Technologies, France). The study revealed significant differences in baking absorption, doughing time, batch, gluten, viscosity, amylase, and retrogradation. The best baking properties were displayed by T-70 and T-80 Triticale flours that were obtained from the central part of the endosperm, both in reduced and advanced processing schemes. However, the advanced scheme proved to be the most effective way of processing Triticale grain into baker's grade flour.

Keywords: Triticale grain, grain processing, reduced and advanced technological scheme, rheological and baking properties of flour

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INTRODUCTION

The use of non-traditional grain products, such as Triticale, in various sectors of the food industry is currently attracting increasing attention of Russian researchers and manufacturers. The interest can be explained by the increasing acreage, new Triticale varieties, and numerous studies of their technological, biochemical, and biological potential [1–7].

Triticale is a laboratory-made hybrid of wheat and rye. Its nutritional values are superior to those of both parental plants [1]. In Russia, Triticale grain is currently used in compound feed and alcohol production. However, Triticale grain can substitute wheat baking flour as a very advantageous source of raw materials in the production of various pastries, e.g. cookies, biscuits, waffles, muffins, crackers, etc. Triticale flour can be used in the production of instant noodles and quick breakfasts, as well as dietary, therapeutic, and prophylactic bread varieties, e.g. wholegrain and multigrain bread [8–13]. In addition, Triticale grain can be used to manufacture mass-market pasta products. Other promising research areas are the technology of processing Triticale grain and bran for starch [15], dietary fibre [14, 16], and bio-

* State Standard 34142-2017 Triticale flour. Specifications. Moscow: Standartinform Publ., 2010. 8 p.

logically modified products [17, 18]. However, there is currently no industrial production of high-quality Triticale flour in Russia.

Over the past decade, foreign scientists have focused mainly on the biology of Triticale cultivars, their biological safety and development, the origin of hexaploid triticale, industrial production of triticale, its competitiveness with wheat, genomics, and biotechnology [19–28].

Until recently, Triticale grain has been considered as an analogue of rye, at least according to its technological properties [13]. However, Russian breeders have made it possible to develop and introduce new promising Triticale varieties into agricultural practice. These varieties have a predominance of wheat genotype, which affects the phenotypic characteristics of Triticale kernels, i.e. size, shape (sphericity coefficient ≥ 0.8), colour, as well as structural, mechanical, and technological properties [1].

The recent studies conducted have made it possible to obtain new data about the technological properties, biochemical composition, and varietal characteristics of Triticale grain and its products. The studies resulted in new technologies of Triticale flours production, as well as a new grit variety with specific properties that will be in demand in the baking, macaroni, confectionery, starch, meat, and other food industries [2, 3, 5, 7, 29, 30].

The present research aims at developing an effective technological scheme for processing Triticale grain into high-quality baker's grade flour.

STUDY OBJECTS AND METHODS

The experimental studies were conducted by the departments of complex grain processing and safety of grain and grain products at the All-Russian Scientific Research Institute for Grain and Products of its Processing (V.M. Gorbatov Federal Scientific Centre for Food Systems of the Russian Academy of Sciences). The experiment involved samples of two Triticale varieties: Ramzes (harvest 2014) and Saur (harvest 2015). Both cultivars were bred at the Don Zonal Research Institute of Agriculture (Rostov region, Russia). Table 1 shows the initial quality indicators of Triticale grain. The grain was prepared for milling according to the previously established parameters of hydrothermal treatment [2].

The milling was carried out on two milling and sorting aggregates: with fluted rollers (RSA-4-2) and with frosted rollers (RSA-4). The intermediate products were enforced in a laboratory sieve purifier. The set of sieves and the speed of the air flow depended on the size of the initial product. The milling products were sieved on a laboratory plansifter for 90 seconds. The parameters and regimes of milling corresponded to the 'Rules for organizing and conducting the technological process at flour mills'.

A research conducted in the laboratory 'Technology and equipment of the milling industry' (2014–2015) showed that the processing of Triticale grain into baker's grade flour is more similar to that of wheat in its technological properties [1, 13]. The grit formation process is characterized by a significant number of grits that consist of pure endosperms. The research employed the method of analysis developed for intermediate products of grain milling at the All-Russian Scientific Research Institute for Grain and Products of its Processing. According to the analysis, Triticale products were divided into 3 groups: the actual grits (particles of pure endosperm), clots of endosperm and shell, and tail-end products that differed in shape and colour. The analysis proved the need for the introduction of sieve purifiers [5]. The analysis also revealed a high content of grits in intermediate products of the high-quality milling of Triticale grain. Hence, it was found recommendable to extract the grits. The use of sieve purifiers in the high-quality milling of Triticale grain made it possible to increase the yield of top-quality flours, as well as to obtain granular substances and middling that can be used for pasta manufacturing.

The expediency of the extraction and enforcement of large-size grits of 560–950 micrometres (μm) was proved only for break I. It was revealed that middle-size grits (315–560 μm) were suspended during breaks I and II, while the small-size grits (224–315 μm) were suspended during breaks I, II, and III. The composition of the intermediate products of the fourth break was characterized mainly by the presence of bran particles with high ash content. Hence, it was found impractical for enforcement in a sieve purifier.

Thus, we developed an advanced technological scheme for milling Triticale grain with a sieve purifier and sizing. The scheme was based on the principle of gradual milling and sorting. The construction of the technological scheme was determined by the requirements for the finished products (quality and yield of flour), variety of grain, and productivity. The reduced technological scheme included four break systems (br.), six reduction systems (red.), and one scratch system (scr.) [22]. The technological process of the advanced scheme included four break systems, two sizing systems (sz.), three sieving (SV), and six reduction systems (red.) (Fig.1).

The break process of the advanced scheme consisted of the stage of grit formation (breaks I – II) and a scratch stage (break IV and reduction system VI). The sieving process involved a separate enforcing of large-size grits of the first break (SV-1), medium-size grits of break systems I + II (SV-2), and small-size grits of I + II + III breaks (SV-3) [5]. The parameters of the sieving process were characterized by extracting the tail-e-nd fraction in an amount of at least 80% of the ini-

Table 1. Basic quality indicators of Triticale varieties

Triticale variety	Quality indicators				
	Mass of 1,000 kernels, g	Grain hardness, %	Grain-unit, g/l	Ash content, %	Moisture, %
Ramzes (2014)	31.8	18	625	2.07	10.2
Saur (2015)	33.2	44	661	1.99	9.3

tial mass. The through product of the sieve purifier SV-1 was directed to the frosted rolls of the first roller mill of the sizing system. The through product of sieve purifiers SV-2 and SV-3 were combined and directed for the milling to the roller mill of the first reduction system. Tailings from the first and second sieving systems, which made up 15–20%, were combined and sent for additional milling to the roller mill of the fifth reduction system. Tailings from the third sieving system were sent for additional milling to the roller grinding machine of reduction system IV.

The break systems used fluted rollers that were fluted back on the back. All the reduction and sizing systems used roller machines with frosted rolls. The modes of milling were characterized by a total 75% extraction of large-size dunst products and flour on grinding mill of breaks I, II, and III. The extraction mode on the grinding mill of the first break was 25–30%. The extraction mode on the grinding mill of sizing systems I, II, and III was 25–30%. The removal on the grinding mill of reduction systems I, II, and III was at least 50%.

The whiteness of Triticale flour was determined by measuring the reflectivity of a compacted smoothed flour surface with a photoelectric device. To determine the ash content, the flour and bran were burnt, and the mass of the non-combustible residue was measured. The baking absorption and the rheological properties were measured by recording the consistency of dough in the process of its formation from water and flour. The change in the consistency of the dough during kneading was measured with the help of a Mixolab system (Chopin Technologies, France). The baking properties were measured by using the method of laboratory bread ba-king from Triticale flour. The method involved the vo-lume (cubic centimetres) of bread made from 100 g of flour, as well as scoring the appearance and the bread crumb.

RESULTS AND DISCUSSION

The first stage of the research was devoted to studying the basic milling properties of the initial Triticale grain samples. After laboratory milling, we selected four flows of Triticale flour that were obtained both with reduced and advanced technological schemes.

Triticale flour varieties were formed by three flour flows: A, B, and C [3]. Stream A was the flour from the hcentral part of the endosperm obtained during reduction systems I, II, and III + sizing system I (advanced scheme) and the flour obtained on reduction systems I, II, and III (reduced scheme). Stream B was the flour from the peripheral part of the endosperm and the subaleurone layer obtained on the third and the fourth reduction systems and on breaks I, II, and III. Stream C consisted of endosperm fragments and shells from other technological systems.

Tables 2 and 3 present the quality indicators of Triticale flour flows of Ramzes and Saur varieties obtained according to different technological processing schemes.

Figs. 2 and 3 show cumulative ash curves of the reduction and quality formation processes for Ramzes and Saur flours. The cumulative curves (Figs. 1 and 2) demonstrate that the reduced scheme had three distinct stages of flour formation, where as the advanced technological scheme had two stages. A statistical analysis showed that cumulative curves can be represented as three and two linear segments for different milling schemes [3]. In case of Ramzes grain, the yield of T-70 Triticale flour (ash content ≤ 0.70%) was 40% for the reduced technological scheme. For the advanced technological scheme, the yield of T-70 flour was 63%. The overall yield of flour was higher by 3.4% according to the advanced scheme, as compared with the reduced scheme. However, the advanced scheme resulted in a 46% yield of T-60 flour variety, which has the lowest

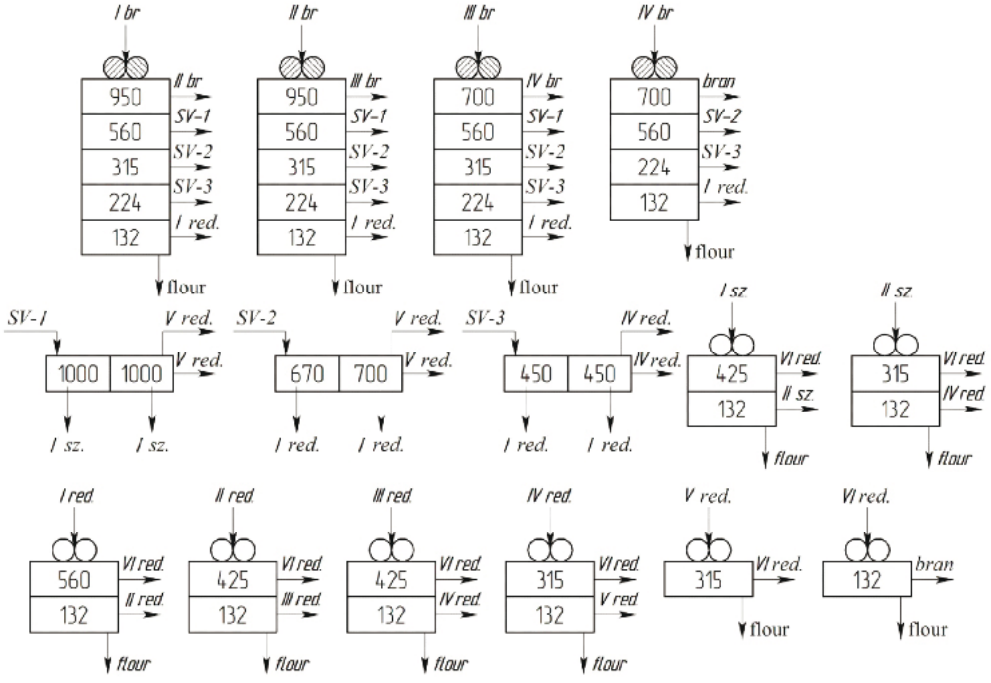

Fig. 1. Technological process of the advanced scheme for processing Triticale grain into high-quality baker's grade flour

Table 2. Quality indicators of Ramzes Triticale flour flows obtained according to different processing schemes

Product	Whiteness, conventional units		Ash content, %	
	Reduced scheme	Advanced scheme	Reduced scheme	Advanced scheme
		Flour from:		
break I	45.0	46.7	0.87	0.69
break II	52.0	55.5	0.69	0.57
break III	51.7	46.5	0.84	0.74
break IV	29.4	33.8	1.59	1.27
sizing system I	–	57.9	–	0.64
sizing system II	–	45.4	–	0.83
reduction system I	50.0	60.7	0.71	0.50
reduction system II	48.7	54.2	0.70	0.57
reduction system III	44.0	41.4	0.69	0.85
reduction system IV	34.8	23.6	0.77	1.29
reduction system V	26.7	6.1	0.88	1.70
reduction system VI	13.3	–1.8	1.27	1.83
scratch system I	–5.9	–	1.63	–
		Bran from:		
break systems	–	–	6.35	5.26
reduction systems	–	–	3.94	4.43

Table 3. Quality indicators of Saur Triticale flour flows obtained according to different processing schemes

Product	Whiteness, conventional units		Ash content, %	
	Reduced scheme	Advanced scheme	Reduced scheme	Advanced scheme
		Flour from:		
break I	45.3	42.5	0.67	0.77
break II	51.8	55.1	0.57	0.50
break III	53.1	40.1	0.56	0.82
break IV	38.3	22.6	1.05	1.91
sizing system I	–	62.7	–	0.63
sizing system II	–	55.5	–	0.65
reduction system I	48.4	65.1	0.59	0.54
reduction system II	50.6	60.8	0.58	0.53
reduction system III	42.7	53.3	0.77	0.60
reduction system IV	29.2	43.3	1.02	0.75
reduction system V	15.7	25.8	1.28	1.19
reduction system VI	1.6	6.3	1.73	1.53
scratch system I	–16.6	–	2.17	–
		Bran from:		
break systems	–	–	5.89	7.05
reduction systems	–	–	4.45	4.16

ash content (State Standard 34142-2017**). The reduced scheme resulted in 0% of T-60 flour.

When processing Saur grain variety, the yield of T-70 Triticale flour was 73% according to both schemes (Table 3). The overall flour yield increased by 0.6%. The advanced processing scheme resulted in obtaining 42% of Triticale flour with ash content ≤ 0.55%.

The second stage of the research featured the rheological properties [31] of ten separate flows of Triticale flour from Saur grain variety, obtained according to the advanced technological scheme by using a Mixolab system (Chopin Technologie, France). The Chopin+ protocol presupposes 5 research phases. Stage I lasts 8 min at 30°C; stage II lasts 15 minutes with a consistent increase in temperature at a rate of 4°C per minute from 30 to 90°C; stage III lasts 8 min at 90°C; stage IV lasts 10 min, with a consistent decrease in temperature from 90 to 50°C; stage V lasts 5 min at 50°C. The rotational input in the analyzed points of the graph, from the point of view of biochemical processes, characterizes: formation of the dough (C1); dough dilution (C2); the maximum rate of starch gelatinization (C3); and the beginning and the end of the retrogradation of starch (C4 and C5). α, β, and γ are the rates of biochemical reactions (calculated values). The analysis also included the following indicators: the baking absorption of the dough, %; dough formation time, min; dough stability, min. The data of the integral evaluation of the rheological properties of the dough are visualized on the graph of the rotational input versus time in a particular temperature mode (Fig. 4, Tables 4 and 5) [31–33].

** State Standard 34142-2017. Triticale flour. Specifications. Moscow: Standartinform Publ., 2010. 8 p.

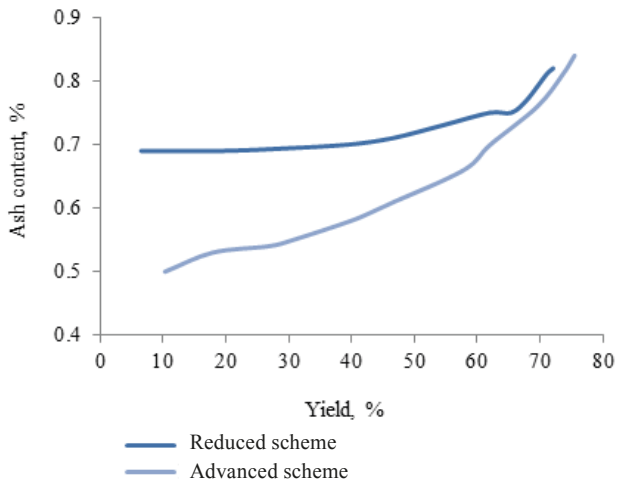

Fig. 2. Cumulative curves of ash content in Ramzes flour

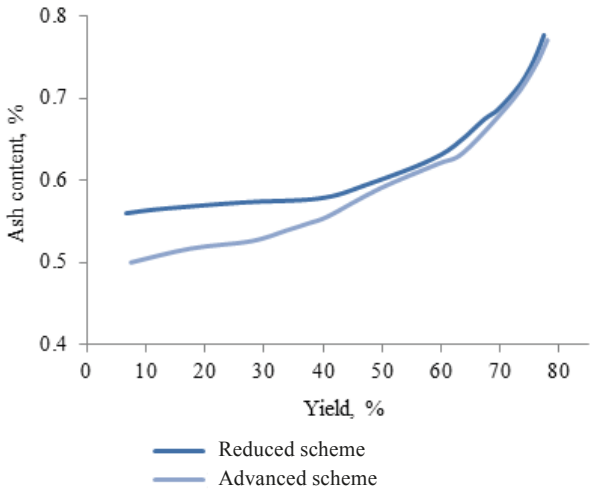

Fig. 3. Cumulative curves of ash content in Saur flour

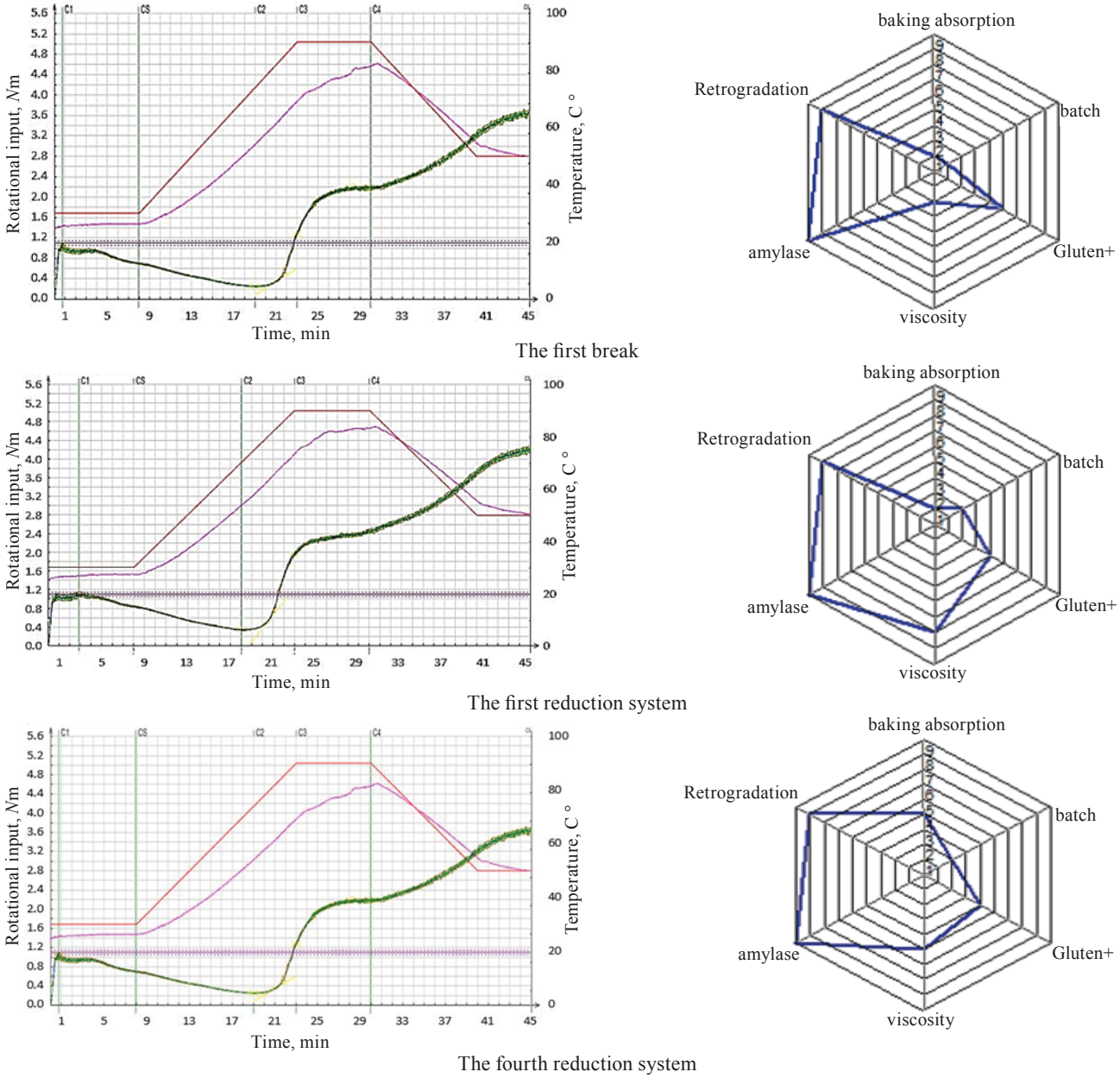

Fig. 4. Phases of rheological analysis of the dough and Mixolab profiles of Triticale Saur flour flows from different technological systems.

The rheological analysis made it possible to create graphical profiles inherent in each flow of Triticale flour. Fig. 4 shows the phases of the rheological analysis of the dough and the Mixolab profiles of the three flour flows: break I, reduction systems I and IV, since they demonstrated the greatest differences.

The viscosity value was different: 2, 7, and 5 scores for break I, reduction system I, and break system IV, respectively. It should be mentioned that viscosity depends on the state of starch, the activity of amylases, and the peripheral parts that contain non-starchy polysaccharides. The amylase index depends on the amylolytic activity of the flour. The higher it is, the lower the activity of enzymes. The starch retrogradation index is related to the rate of staling of the finished product. Its high value indicates a faster rate of staling.

Table 4 demonstrates that the baking absorption increased from the first reduction system to the sixth reduction system, which was connected with a larger number of peripheral water-absorbing particles in the flour. The first, second, and third breaks also showed an increase in baking absorption. The sizing system occupied an intermediate position between the break and the reduction systems. Its baking absorption was 55.0%.

During the first stage (C1), the flow stability was uneven. However, the stability time tended to decrease from reduction systems I – VI, which could also be connected with an increase in the content of peripheral particles and a decrease in the dough formation time.

At the second stage (C2) of the curve of the mixolabogram, one can observe the smallest rotational input, which is associated with the dough dilution and indirectly characterizes the state of the protein complex. Viscosity increased from breaks I – III. The sizing system had the lowest viscosity. The reduction systems demonstrated an increase in rotational input followed by its decrease, which was apparently due to an increase in the share of peripheral fractions in the flour of these systems.

During the third stage (C3), the starch granules broke down and gelatinized, which led to an increase in rotational input. There was a clear dependence of the increase in the rotational inputon the grain-size composition of the flour at the break system and its reduction at the reduction system.

During the fourth stage (C4), one could observe a gradual increase in the rotational input of the break systems and its decrease during the final reduction systems. The highest rotational input was registered during the second reduction system.

Table 6 visualizes the rheological characteristics of the flows as six consecutive indices: the index of water absorption index, the mixing index, the gluten index, the viscosity index, the amylase index, and the starch retrogradation index.

The fifth stage (C5) characterized the process of starch retrogradation during cooling and the rate of staling of the finished flour products. Here, the rotational input on the reduction systems fell significantly from 4.221 Nm on reduction system I to 2.731 Nm on reduction system I.

Table 4. Main parameters of the phases of rheological analysis of the dough of individual flows of Saur Triticale flour

Flour sample from:	Water absorption, %	Stability, min	C1	C2	C3	C4	C5
break I	54.2	4.42	1.030	0.253	1.273	2.178	3.644
break II	53.1	3.87	1.109	0.286	1.401	2.294	3.766
break III	53.5	4.78	1.226	0.335	1.809	2.245	3.529
sizing system I	55.0	5.23	0.992	0.299	2.031	2.438	3.859
reduction system I	54.1	5.62	1.093	0.335	1.947	2.449	4.221
reduction system II	55.0	5.72	1.159	0.367	2.035	2.490	4.150
reduction system III	57.0	5.42	1.156	0.372	1.820	2.441	3.957
reduction system IV	57.6	5.65	1.081	0.337	1.752	2.258	3.489
reduction system V	57.7	5.05	1.113	0.329	1.497	1.953	3.059
reduction system VI	58.3	4.62	1.236	0.354	1.875	1.826	2.731

Table 5. Calculated values of reaction rates * for individual flows of Saur Triticale flour

Flour sample from:	α, Nm/min	β, Nm/min	γ, Nm/min	Rotational input, Nm/min	Amplitude, Nm/min
break I	–0.036	0.130	0.088	3.644	0.134
break II	–0.056	0.160	0.078	3.766	0.141
break III	–0.064	0.374	0.010	3.529	0.140
sizing system I	–0.056	0.322	0.032	3.859	0.103
reduction system I	–0.060	0.286	0.038	4.221	0.108
reduction system II	–0.062	0.416	0.042	4.150	0.141
reduction system III	–0.056	0.292	0.020	3.957	0.168
reduction system IV	–0.058	0.316	0.020	3.489	0.073
reduction system V	–0.052	0.206	0.028	3.059	0.097
reduction system VI	–0.060	0.388	0.022	2.731	0.154

*) α is characteristic of the dilution reaction rate expressed by the angle of the tangent to the mixolabogram from the moment the temperature reaches 30°C to the point C2; β is characteristic of starch gelatinization reaction rate, expressed by the angle of the tangent to the mixolabogram on the C2 – C3 segment; γ is characteristic of the amylolysis rate, expressed by the angle of the tangent to the mixolabogram in the C3 – C4 segment.

Table 6. Indices of the Mixolab profiles for Saur Triticale flour

Flour sample from:	Indices of the Mixolab profiles					
	Water absorption index	Mixing index	Gluten index	Viscosity index	Amylase index	Starch retrogradation index
break I	1	1	5	2	9	8
break II	1	0	5	3	9	8
break III	1	2	2	6	8	8
sizing system I	2	1	5	8	8	8
reduction system I	1	2	4	7	9	8
reduction system II	2	2	3	8	9	8
reduction system III	3	2	3	6	9	8
reduction system IV	4	2	4	5	9	8
reduction system V	4	2	4	3	8	7
reduction system VI	5	2	3	3	8	7

The analysis of the graphical profiles (Fig. 4, Table 5) showed that the highest value of the baking absorption index was registered in the flour from the sixth reduction system. The high water absorption capacity could be explained by the fact that the system contained the largest number of peripheral parts of the kernels. The mixing index was related to the stability of the dough during kneading, which was 4.42 min for break I (1 score), 5.62 min for reduction system I (2 scores), and 5.65 min for reduction system IV (2 scores). The gluten index characterizes the stability of protein molecules when the dough was heated from 30°C to 60°C. It is rather difficult to interpret the gluten index since two very important phenomena occurred while the dough was being heated. First, starch granules began to swell; second, their structure remained unchanged, while the effect of α-amylase was insignificant. The consistency of the dough changes due to the changes in the structure of gluten proteins: hydrogen bonds break, stability of proteins improves, which is also related to their spatial structure, and, ultimately, the nature of these protein complexes [34, 35]. Such fractions of gluten proteins as gliadin and glutenin play a decisive role in gluten quality formation and its elastic properties. However, it is necessary to take into account the role of other compounds that interact with gluten proteins and affect the structure and properties of gluten. They are lipids, carbohydrates, and enzymes, namely proteases and their protein inhibitors, amylases, lipoxygenase [36].

The viscosity index scored 2 for the flour from break I, 7 for the flour from reduction system I, and 5 for the flour from reduction system IV. This indicator characterizes the phase at which the greatest number of physicochemical and biochemical parameters start to interact. It should be mentioned that the viscosity in these samples depended not only on the activity of amylases, but also on the state of starch, its quality characteristics, and the presence of peripheral parts containing non-starch polysaccharides. The amylase index indirectly characterizes the amylolytic activity of the flour. A high amylase index indicates a weak activity of α-amylase in all the flour flows. The starch retrogradation index is connected with the ability of the finished product to resist staling. A high value of this indicator characterizes a faster staling rate.

At the third stage of the research, we studied the samples of Ramzes and Saur Triticale flour from different flows to determine the baking properties. To form a Triticale flour variety, three flows had to be formed on the basis of cumulative ash curves. These flows were three components of different anatomical parts of the kernels (Z – ash content, Y – yield). The first flow was Triticale flour from the central part of the endosperm, the second flow contained the peripheral part of the endosperm and the subaleurone layer, and the third flow was the flour from endosperm fragments and well-grin-ded shells. Below one can see the algorithm for the formation of three flows that form the Triticale flour varieties.

Flow formation for the Ramzes Triticale flour:

Milling 1 (reduced scheme):

Flow A – break II + reduction system III + reduction system I.

Total: yield/ash content was 29.6/0.69; $Z_A = 0{,}686 + 0.302\times10^{-3} Y_A$; $R^2 = 0.82$.

Flow B – reduction system I + reduction system IV + break III + break II + reduction system V.

Total: yield/ash content 35.8/0.80; $Z_B = 0.615 + 0.211\times10^{-2} Y_B$; $R^2 = 0.98$.

Flow C – reduction system VI + break IV + break V.

Total: yield/ash content was 6.7/1.50; $Z_C = 0.080 + 0.011 Y_C$; $R^2 = 0.99$.

Milling 2 (advanced scheme):

Flow A + B – reduction system I + reduction system II + break II + sizing system I + break I + break III + sizing system II + reduction system III.

Total: yield/ash content was 57.9/0.66; $Z_A = 0.462 + 0.319\times10^{-2} Y_A$; $R^2 = 0.97$.

Flow C – break IV + reduction system IV + reduction system V + reduction system VI.

Total: yield/ash content was 17.6/1.43; $Z_C = 0.086 + 0.990\times10^{-2} Y_C$; $R^2 = 0.99$.

Flow formation for the Saur Triticale flour:

Milling 1 (reduced scheme):

Flow A – break III + break II + reduction system II + reduction system I.

Total: yield/ash content was 39.3/0.58; $Z_A = 0.558 + 0.544\times10^{-3} Y_A$; $R^2 = 0.98$.

Flow B – break I + reduction system III + reduction system IV + reduction system V.

Table 7. Quality indicators for samples of Triticale grain flour

№	№ of milling, Triticale variety	Flour variety	Moisture, %	Gluten, %	Gluten quality		Falling number, sec
					Gluten Deformation Measurement	group	
1	Ramzes, milling 1	T-80	11.0	20.6	49	II (strong enough)	336
2	Ramzes, milling 1	T-120	11.0	19.5	50	II (strong enough)	322
3	Ramzes, milling 1	T-70	10.6	26.2	52	II (strong enough)	168
4	Ramzes, milling 2	T-80	11.0	26.7	71	I (good)	178
5	Ramzes, milling 2	T-120	10.8	20.8	46	II (strong enough)	305
6	Ramzes, milling 2	T-70	11.0	25.9	76	I (good)	167
7	Saur, milling 1	T-70	10.6	27.2	67	I (good)	171
8	Saur, milling 1	T-120	11.6	20.9	38	II (strong enough)	353
9	Saur, milling 1	T-80	9.8	27.6	66	I (good)	167

Table 8. Results of trial laboratory baking

№	Volume yield, cm^3/100 g		Shape stability	Weight, g		Appearance		
	Tin formed bread	Oven-bottombread		Tin formed bread	Oven-bottom bread	Shape	Crust surface	Crust colour
1	350	400	0.48	135	132	regular, semi-oval	slightly nodular	pale
2	350	350	0.48	136	132	regular, semi-oval	cracked crust	pale
3	390	380	0.45	135	133	regular, semi-oval	smooth, level	brown
4	430	450	0.51	134	128	regular, semi-oval	smooth, level	brown
5	380	400	0.52	140	134	regular, semi-oval	smooth, level	pale
6	460	470	0.58	134	130	regular, oval	smooth, level torn from three sides	brown
7	470	470	0.57	135	131	regular, semi-oval	smooth, level	brown
8	340	370	0.67	134	129	regular, semi-oval	smooth, level	pale
9	420	450	0.50	133	127	regular, semi-oval	smooth, level	brown

Total: yield/ash content was 30.3/0.83; $Z_B = 0.396 + 0.410\times10^{-2} Y_B$; $R^2 = 0.97$.

Flow C – reduction system V + reduction system VI + break IV.

Total: yield/ash content –was 7.8/1.58; $Z_C = -0.111 + 0.011 Y_C$; $R^2 = 0.97$.

After that, the Triticale flour flows from different technological systems were mixed in order to obtain individual types of flour. As a result, three types of flour were obtained in accordance with the State Standard 34142-2017*** 'Triticale flour. Technical conditions': T-70, T-80, and T-120. The conventional name for the varieties includes the T index (Triticale), and a number that stands for the ash content × 100. Thus, T-60 flour was flow A with 0.60% ash content; flour T-70 was a mixture of A + B flows with 0.70% ash content; flour T-80 was a mixture of streams B + C with 0.80% ash content; flour T-120 was a mixture of flows A + B + C with 0.12% ash content.

All the formed triticale flour samples were analysed for such quality indicators as humidity, the quantity and quality of gluten, and the falling number (Table 7).

At the fourth stage of the research, we defined the baking properties of the nine samples of Triticale flour varieties, obtained according to different technological schemes (Table 8). The bread was baked from Triticale flour of various varieties according to the methodology of the State Committee on Agriculture. The volume yield for one tin formed bread was 340–470 cm^3/100g of flour and 350–470 cm^3/100g of flour for one oven-bottom loaf.

Tables 8 and 9 represent the results of the trial laboratory bread baking from nine samples of Triticale flour.

The tin formed bread baked from T-70 Saur flour had the largest volume yield, while the smallest volume yield belonged to the bread baked from T-120 Ramses flour (Fig. 5). The tin formed bread sample made from T-120 Ramses flour had the largest weight, whereas the lowest weight was registered for the sample made from T-80 Saur flour. Patterns with a smooth level surface had are regular semi-oval shape (samples 3–5, 7–9). Sample 1 had a slightly nodular surface. Sample 2 had a cracked crust; sample 6 was torn at three sides, respectively. The colour of the crust in samples 1, 2, 5, and 8 was pale due to the low activity of amylolytic enzymes. Samples 3, 4, 6, 7, and 9 had a brown crust. All the samples demonstrated a good crumb resilience and fine texture with uneven porosity. The thickness of the pore walls was found thick-walled and poorly developed for samples 1, 2, and 8. The taste was typical of Triticale flour bread. No stickiness, crunch, or crumbling were registered in any of the samples.

The best volume yield and total bakery assessment results belonged to the following samples: samples of Ramzes T-70 and T-80 (advanced scheme), Ramzes T-70 (reduced scheme), and Saur T-70 and T-80 (reduced scheme). These loafs also demonstrated the highest sensory assessment results (5 scores). The worst total bakery assessment belonged to the sample made from T-120 Ramzes Triticale flour (advanced scheme).

*** State Standard 34142-2017. Triticale flour. Specifications. Moscow: Standartinform Publ., 2010. 8 p.

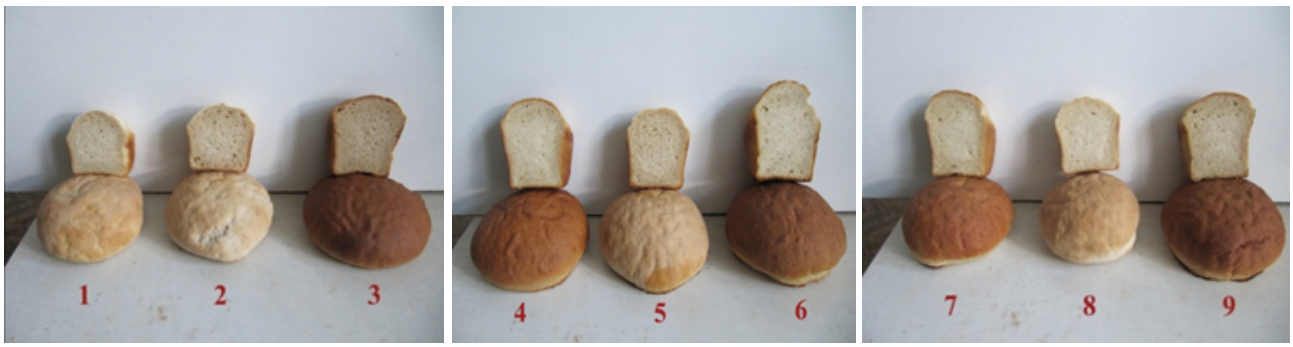

Fig. 5. Bread baked from the obtained varieties of Triticale flour

Table 9. Results of the trial laboratory baking

№	Porosity, %	Crumb condition		Sensory assessment	
		Elasticity, evenness, colour	Porosity	Appearance	Crumb
1	74	elastic, good, creamy-pale	fine, thick-walled, uneven, poorly developed	3	3
2	73	elastic, good, creamy	fine, poorly developed, thick-walled, uneven	2	3
3	78	elastic, good, dark	fine, thick-walled	5	5
4	81	elastic, good, creamy	fine, thick-walled	5	5
5	77	elastic, good, creamy-pale	fine, uneven	4	4
6	81	elastic, good, unevenly creamy-pale	fine, thin-walled	5	5
7	81	elastic, good, creamy-pale	fine, thin-walled	5	5
8	78	elastic, good, creamy-pale	poorly developed crumb	4	4
9	81	elastic, good, creamy-pale	fine, thin-walled, uneven	5	5

CONCLUSION

The research proved that, if processed according to the advanced technological scheme, Triticale grainincreases the total yield of flour by 0.6–3.4% compared to the reduced technological scheme. However, when Ramzes variety was processed according to the advanced scheme, the yield of T-60 flour with the lowest ash content (according to State Standard 34142-2017****) was 46%, and the reduced scheme failed to produce the T-60 flour at all. When Saur variety was processed according to the advanced scheme, it gave a 55% yield of T-60 Triticale flour, where the reduced scheme resulted in 48%.

The study also helped to establish the effect of the grain hardness on the grit formation and on the yield of graded flour.

Sieve purification of intermediate Triticale products proved to increase the yield of flour from the central part of endosperm and the total yield of graded flour.

The cumulative ash curve for Triticale flour processed according to the advanced technological scheme can be represented in the form of two, rather than three linear sections that are used to describe the reduced scheme.

The rheological properties of Triticale flour from various technological systems (flows) clearly demonstrated a regular increase in the baking absorption. Moreover, stability time during dough kneading decreased as the number of peripheral parts of the kernel. The state of protein-proteinase and carbohydrate-amylase complexes of individual flour flows had a more significant influence on the viscosity index. Other factors included non-starch polysaccharides from the peripheral parts of the grain. The index rose between the first and the third breaks and fell between the first to the sixth reduction systems.

T-70 and T-80 Triticale flour varieties, obtained from the central part of the endosperm, have excellent baking properties, both according to the reduced and the advanced schemes.

The advanced scheme with breaking, sieving, sizing, and reduction systems proved to be the most effective way to process Triticale grain into high-quality baker's grade flour.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this article.

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**** State Standard 34142-2017. Triticale flour. Specifications. Moscow: Standartinform Publ., 2010. 8 p.

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Detection of protein aggregation markers in raw meat and finished products

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Abstract: The effect of animal and plant proteases as well as starters, or starter cultures, on protein aggregates formation in raw pork and beef as well as meat products was studied. The proteomic analysis of raw meat revealed that animal proteases – pepsin and trypsin – caused the aggregation of isoform 2 of protein 1 containing 4.5 LIM domains. Vacuum packaged meat showed the same results during storage, while unpacking led to the acceleration of the aggregation process due to autolysis. In addition, mixed aggregated fragments, such as muscle creatine phosphokinase and glutathione-S-transferase, actin and perilipin, and type II keratin appeared in those samples. Starters with *Pediococcus pentosaceus* 31 from the Russian National Collection of Industrial Microorganisms (VKPM-8901) caused myoglobin and troponin I aggregation, while the formation of soy proteins aggregates (glycinin G1 and glycinin A3B4) was detected in meat products as a result of the autolysis process and the use of cholesterol-lowering starters. All in all, proteases which cause protein aggregation may be less effective for raw meat tenderization, whereas the proteins identified may be used as quality biomarkers.

Keywords: Proteins, meat, proteases, aggregates, biomarkers

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INTRODUCTION

Protein aggregation in this paper is defined as the interaction of denatured protein molecules resulting in the formation of weak or strong (for example, disulfide) molecular linkages. These linkages contribute to larger particles formation. Mostly, protein aggregation in meat occurs as a result of thermal denaturation when heating or autolysis in the rigor mortis stage.

Denatured and then aggregated proteins become less soluble and lose their biological properties. Rigor mortis also causes changes which lead to protein aggregation.

On the other hand, proteolytic enzymes are capable of weakening intermolecular interactions in proteins and provide proteolysis, which contributes to tissue tenderization [1]. Both proteolytic and meat enzymes act on protein substrate fragments and thereby influence protein components structures. The enzymes facilitate aggregates dissotiation, free SH-groups formation, and muscle tissue properties partial recovery after denaturation or rigor mortis. As a result of the enzyme impact, meat becomes tender, juicy, with pronounced taste and aroma.

The proteolytic activity of lactic-acid bacteria strains against sarcoplasmic and myofibrillar proteins was studied by different research groups [2-4].

In 2015, the hydrolysis of myofibrillar proteins during the fermentation of sausage models with starters or their mixture was studied by Lopez *et al.* [2]. The researchers used such starters as *Lactobacillus curvatus* CRL705 and *Staphylococcus vitulinus* GV318. The results had shown that the mixed culture accelerated proteolysis significantly. Thus, two-dimensional electrophoresis confirmed the hydrolysis of actin, myosin light chain 1/3, myosin light chain 2, and myosin heavy chain. 33 peptides from troponin T, myosin light chain 2, and, in particular, from actin were also identified by means of LC-MS/MS. In the matter of actin primary structure,

three of its regions were very sensitive to degradation. Although the inoculation of the mixed starter cultures accelerated proteolysis, peptides and amino-acids contents were reduced while *L. curvatus* CRL705 alone increased the abovementioned contents.

In 2018, De Almeida *et al.* [3] evaluated the ability of four species of lactic acid microorganisms to form low molecular weight peptides in a sausage model with a low sodium content. As a whole, 86 low molecular weight peptides obtained mainly from myofibrillar proteins were identified (56 of them – from actin). The inoculation of *L. curvatus* CRL705 and *Enterococcus mundtii* CRL35 led to the formation of 56 and 43 low molecular weight peptides, respectively. Larger quantity of peptides per unit time was released when using *Lactobacillus plantarum* and *L. sakei* in comparison to other models. Each strain in the models allowed creating the unique profile of small peptides and amino acids which might be used as a biochemical characteristic to differentiate certain fermented foods.

In the research conducted by Basso *et al.* [4], *Lactobacillus sakei* (DSM 6333), *Lactobacillus plantarum* (B21), and *L. farciminis* (DSM 20184) have demonstrated proteolytic activity to meat sarcoplasmic proteins. Thus, the 94kDa-band disappeared in samples with *L. farciminis* and *L. plantarum* and narrowed significantly in samples with *L. sakei.* Bands with a molecular mass from 94 kDa to 38 kDa decreased in all samples. According to capillary electrophoresis analysis, no signals corresponding to the retention time of 8.64 and 8.66 minutes were observed in all the samples. Moreover, MALDI-TOF MS method identified 94-kDa and 38-kDa bands as an isoform of muscle glycogen phosphorylase and glyceraldehyde-3-phosphate dehydrogenase.

In 2001, Italian researchers [5] obtained controversial results on the proteolytic activity of 27 *Staphylococcus xylosus* strains to sarcoplasmic and myofibrillar proteins. Neither electrophoretic nor spectrometric analysis confirmed the proteolytic activity of some strains. SDS-polyacrylamide gel electrophoresis revealed changes in pork protein profile for only 12 out of 24 strains which were able to hydrolyze muscle protein extracts on agar.

Montowska *et. al.* [6] has proved a favorable effect of trypsin along with super-high frequency treatment on rapid and efficient recovery of peptide markers in meat products. Meat was subjected to heating – considerable protein aggregation was observed – and then analyzed by means of tandem mass spectrometry to detect specific peptide markers. It was established that treatment of heat-treated product with trypsin for one hour contributes to the structure recovery of desirable peptide markers which could be easily identified using the known methods.

Ageing effects meat taste, tenderness, water-binding capacity (WBC), colour, and juiciness. The monitoring of biochemical processes occurring during meat ageing allows meat products quality biomarkers to be revealed [7]. Change in the proteome of muscle exudate from genetically similar pigs with the same meat characteristics within a regular ageing period, namely, 7 days, was studied [1]. It has been found that some quality characteristics of meat such as meat tenderness, cooking losses as well as the colour index CIE b* change considerably due to autolysis, notably, at the end of the ageing process. These data illustrated structural changes in the pork meat during the ageing process, which affected proteomic profiles. Three main groups of proteins which changed significantly during the ageing were detected: stress proteins, metabolic enzymes, and structural proteins. Undoubtedly, proteolysis plays a key role in protein fragmentation (for instance, enolase and titin). It has been also observed that the number of stress proteins increase. To monitor these changes, myofibrillar or sarcoplasmic proteomic fractions are usually used. However, using the muscle exudate as a more available substrate allowed previous research to be expanded. For example, it has been discovered that vinculin and peroxiredoxin-6 correlate to the WBC and the tenderness of meat, respectively. Such protein biomarkers are useful for monitoring the fresh meat quality and forecasting the autolysis course.

Oxygen, contained in meat tissues, also takes part in the formation of meat raw materials texture, namely, tenderness and juiciness: a high oxygen level contributes to protein intermolecular cross-linking and aggregates formation [9, 10].

The results of the research conducted by Moszkowska *et. al.* [11] have shown the formation of protein aggregates in the muscle tissue packaged in an oxygen-containing atmosphere. These aggregates, which consist of myosin heavy chains, are the result of cross-linking proteins and are able to affect the meat tenderness adversely. Aggregate formation depends not only on a type of packaging but also on a method of packaging. Thus, larger aggregates were observed in *biceps femoris* muscles in comparison to *longissimus lumborum* muscles and in meat packaged in the modified gas atmosphere (MGA) rather than in vacuum. It may be explained by the metabolism of muscles analyzed due to differences in postmortem glycolysis extent, the characteristics of muscle fibers and their glycolytic and oxidative properties.

Both SDS-gel electrophoresis and diagonal-PAGE, as well as immunoblotting, demonstrated the oxidative cross-linking of myosin heavy chains in meat packaged in MGA with high oxygen content. Moreover, aggregates of myosin heavy chains (MHC) were also detected in other packages containing high oxygen concentration (MGA and vacuum + MGA); apparently, protein oxidation occurred which led to myosin binding due to its polymerization [12]. Such an aggregation of MHC, occurred under severe temperature conditions (190°C), and took place in both raw meat and finished meat products [6].

The aggregate formation can be related not only to oxidation but to proteolysis as well. In this work, we identified a number of protein aggregates of different origin formed as a consequence of proteolytic and oxidative processes.

STUDY OBJECTS AND METHODS

In this research, we studied *m. longissimus dorsi* samples from Bos Taurus before and after treatment

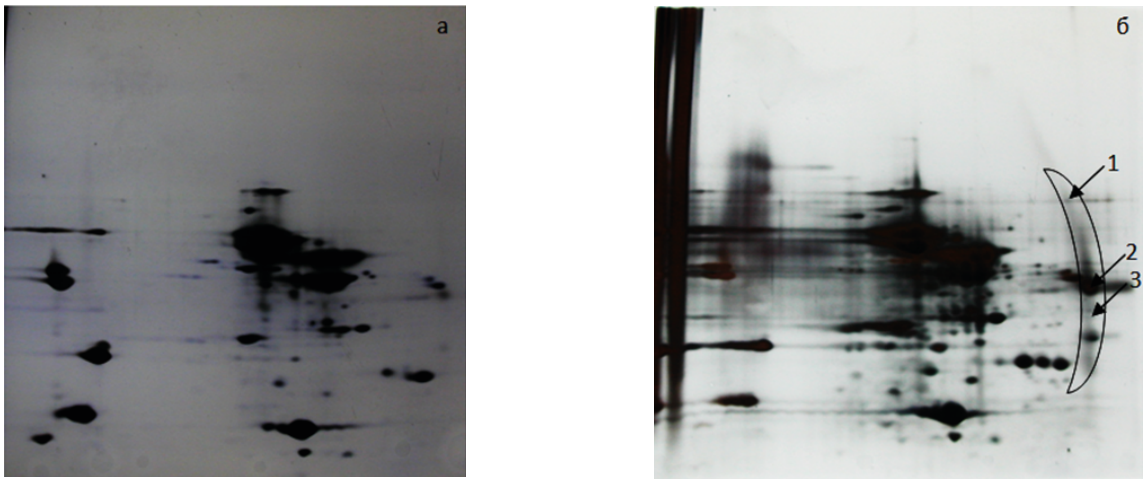

Fig. 1. Two-dimensional electrophoregrams of bovine skeletal muscle tissue samples: (a) control; (b) treated with trypsin. Silver nitrate staining. Arrows denote monomeric form, ellipse denotes track of protein 1, isoform 2 containing 4,5 LIM domains.

with proteolytic enzyme of animal and plant origin and a number of starters. Whole muscle of 500 g was injected with proteases solutions in the amount of 5 ml per 50 g raw meat and kept at 30°C for 40 min for trypsin and pepsin, and for 30 min for papain and bromeline treatment. In case of starter cultures, a sample was kept in vacuum at 11°C for 9 days.

We used 1.5% pig pepsin and bovine trypsin solutions (Himedia, India, 10, 000 NFU/mg and 2, 000 U/g, respectively) and 0.5% papain and bromeline solutions (Sigma, USA, 1.1 U/mg solid and 2, 370 U/g prot., respectively). The starter suspensions concentration was determined as 10^9 CFU/ml using McFarland standard. The following starters were used: *Pediococcus pentosaceus* 31 (VKPM-8901), *Pediococcus acidilactici* 38 (VKPM-8902), *Lactobacillus sakei* 105 (VKPM-8905), and *Lactobacillus curvatus* 2 (VKPM-8906).

Another set of experiments was carried out using uncooked smoked sausage samples. The control sample did not include starters. The experimental sample no. 1 had starters from the Microorganisms Collection of Moscow State University of Food Production. The experimental sample no. 2 was prepared using starters from Bactoferm SM 194 Chr. Hansen, Denmark, such as *Pediococcus pentosaceus*, *Staphylococcus carnosus*, *Staphylococcus xylosus*, *Lactobacillus sake*, and *Debaryomyce shansenii*. Uncooked smoked sausage for the experiment was manufactured by a halal meat production factory in the Moscow region. It included such ingredients as beef of best quality, beef and horse meat of good quality, soy granules, beef external fat, vegetable fiber with a coloring agent, nitrite salt, and spices.

In the third set of experiments, skeletal muscles of Bos Taurus (*m. longissimus dorsi*) were separated from a carcass in 30 min after slaughter and vacuum-packaged into individual packages. The samples were kept at ± 2°C for 4 and 7 days until analyzed.

Protein fractionation, colouring, computer densitometry, and mass-spectrometric identification were carried out as described in previous research [13, 14]. Protein identification was financially supported by the Ministry of Education and Science of the Russian Federation and performed in Shared Knowledge Center 'Industrial biotechniques' (RFMEFI62114X0002).

RESULTS AND DISCUSSION

The enzyme preparations listed are widely used in the meat industry. For instance, the American Food and Drug Administration of the Department of Health Protection and Human Services generally recognized five exogenous enzymes as safe (GRAS) to use in the meat industry. These are papain, bromeline, ficin, and *Aspergillus oryzae* and *Bacillus subtilis* proteases [15]. Therefore, a potential formation of complexes limiting the exoprotease usage due to their reduced effectiveness in meat is of great interest.

The silver nitrate staining of a bovine skeletal muscle treated with trypsin and pepsin revealed a protein track with molecular mass of 15–100 kDa in the alkaline zone of pH gradient (Fig. 1). The track was not detectable in staining with CBB R-250. The former staining technique is 100 times more sensitive than the latter one, which means a small quantity of the protein involved. The analysis of gel fragments from upper and lower zones of this track revealed isoform 2 of protein 1 containing 4.5 LIM domains, *FHLI* gene. The monomeric form of the protein corresponded completely to its physical and chemical parameters (molecular mass and pH) and contained more than 90% of protein substances in the track.

The amino acid sequence of the protein does not exceed 32 kDa in mass, whereas that in the track was considerably wider. It implies that the proteolytic treatment causes aggregates formation that gives an unusual electrophoretic track. The analysis of vacuum-packaged skeleton muscles of Bos Taurus also detected an identical track of the protein by day 4 of storage that decreased by day 7 as a consequence of the autolytic process. After unpacking, the trace formation process tended to accelerate during 3 days, which confirms that both proteolytic enzymes and free oxygen take part in this process.

The track formation can be explained by the fact that animal proteases act in injection sites, and the stage of

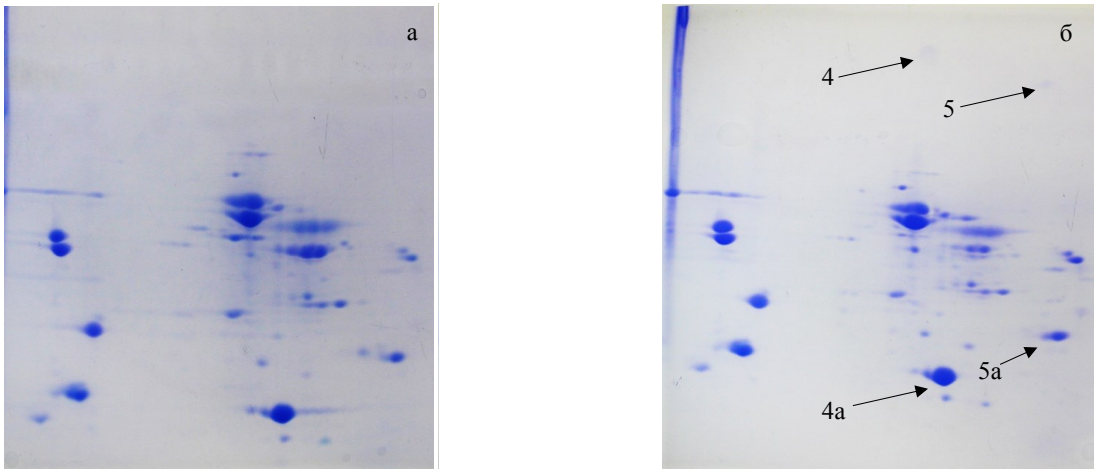

Fig. 2. Two-dimensional electrophoregrams of bovine skeletal muscle tissue samples: (*a*) control; (*b*) treated with *Pediococcus pentosaceus* 31 VKPM-8901. CBB R-250 staining. Arrows denote protein aggregates, arrows with letters denote their monomeric forms.

The track formation can be explained by the fact that animal proteases act in injection sites, and the stage of aggregates formation is impossible to record. However, one of the proteolysis stages is the removing of similar molecules. This implies that a track with reduced mass is formed which can be detected only at low protease concentrations, i.e. at some distance from injected sites. Plant proteases do not have such an effect, probably, because their pH optimums are more adapted to raw meat conditions.

Thus, bromeline resulted in the formation of five high molecular fractions of myosin heavy chains fragments with molecular weights of 170, 150, 60, and 44 kDa, respectively. Consequently, polypeptides with a high molecular weight, a few short peptides, and a small number of free amino acids are formed as a result of bromeline action. Bromeline has a low actomyosin affinity. The data resulted coincide with those of Istrati *et. al*. [8].

Another case of protein aggregates formation was detected when using *Pediococcus pentosaceus* 31 VKPM-8901as a starter. Fig. 2 demonstrates weight high molecular protein aggregates (160–200 kDa) that were identified as myoglobin and troponin (Table 1). Their subunits molecular weights did not exceed 21.5 kDa, and their monomers were represented in large quantities on the two-dimensional electrophoregram. In addition, fractions with much higher weight (up to 3% of monomers) were also recorded.

In vacuum-packaged skeletal muscle from Bos Taurus, aggregates of other proteins were also revealed (Fig. 3). Aggregates of mixed fragments, such as muscle creatine phosphokinase and glutathione-S-transferase, actin and perilipin, and type II keratin, appeared. A cause of their formation might be in a low oxygen concentration and naturally occurring autolysis. These factors slow down the aggregates formation process through the removal of structurally similar molecules complex from submolecular structures, which can be seen on the electrophoregrams.

Aggregation was also observed in uncooked smoked sausage samples with starters from the MSUFP collection (Fig. 4). Two of the samples contained soy protein aggregates (soy was used in the sausage formulation). The control sample (without starters) included two aggregates of glycin G1 fragments. The sample with starters had glycin G1 fragments and a glycinin A3B4 aggregate, and the third sample did not contain aggregates. These results demonstrate that the presence of

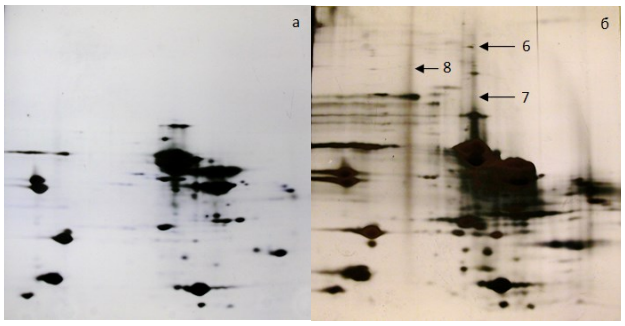

Fig. 3. Two-dimensional electrophoregrams of vacuum-packaged bovine skeleton muscle: (*a*) control; (*b*) day 7 of storage. Silver nitrate staining.

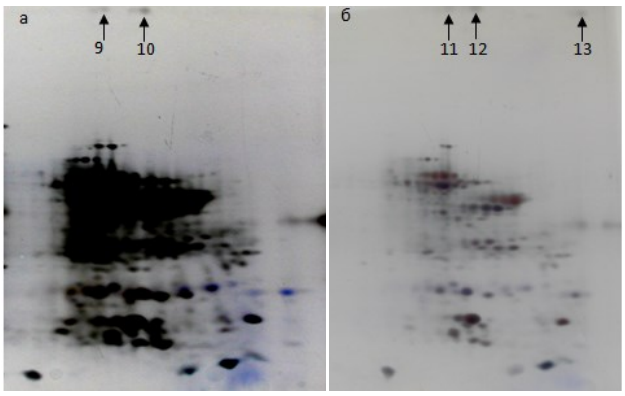

Fig. 4. Two-dimensional electrophoregrams of protein fragments of uncooked smoked sausages samples with aggregates detected: (*a*) control; (*b*) experiment. Silver nitrate staining.

Table 1. Results of mass-spectrometric identification (MALDI-TOF MS and MS/MS) of protein aggregates fractions

№	Protein name (gene symbol)	Numbers in Protein NCBI	S/M/C *	Мм/pI (exp.)**	Мм/pI (calc.)**
1	Aggregate of fragments (60–278 in amino acid sequence) of isoform 2 of protein 1 containing 4.5 LIM domains (FHL1)***(2)	NP 001106730.1	138 / 4 / 15	60.0 / 8.20	31.9 / 8.76
2	Isoform 2 of protein 1 containing 4.5 LIM domains (FHL1)	NP 001106730.1	120 / 26 / 63	31.0 / 40	31.9 / 8.76
3	Aggregate of fragments (60–278 in amino acid sequence) of isoform 2 of protein 1 containing 4.5 LIM domains (FHL1)***(1)	NP 001106730.1	95 / 5 / 20	24.0 / 8.50	31.9 / 8.76
4	Aggregate of myoglobin (MB)***(2)	NP 776306.1	159 / 14 / 75	200.0 / 7.30	17.1 / 6.90
5	Aggregate of fast skeletal muscle troponin I (TNNI2)	XP 00503574.1	129 / 17 / 48	160.0 / 8.20	21.4 / 8.88
6	Aggregate of muscle creatine phosphokinase and glutathione-S-transferase P (GSTP1)	AAD30974.1 NP 803482.1	150 /14 / 43 88 / 7 / 60	180.0 / 6.70	43.0 / 6.63 23.6 / 6.89
7	Aggregate of skeletal muscle alpha-actin (ACTA1) и S-terminal of perilipin 4 fragments (LOC510990)***(1)	AAI34666.1 XP 015327565.1	189 / 17 / 54 177 / 9 / 14	67.0 / 6.80	42.0 / 5.23 102.1 / 5.97
8	Aggregate of type II keratin fragments	OO220327.3	51 / 6 / 11	160.0 / 5.40	66.0 / 8.16
9	Aggregate of glycinin G1 fragment 324–493 [Glycine soy] (GY1)	KHN10744.1	159 / 14 / 37	300.0 / 6.70	55.8 / 5.95
10	Aggregate of glycinin G1 fragment 324–493 [Glycine soy] (GY1)	KHN10744.1	159 / 13 / 34	300.0 / 7.30	55.8 / 5.95
11	Aggregate of glycenin G1 fragment 322–492 [Glycine soy (GY1)	KHN10744.1	153 / 12 / 29	300.0 / 6.70	55.8 / 5.95
12	Aggregate of glycenin G1 fragment 324-436 [Glycine soy] (GY1)***(1)	KHN10744.1	129 / 5 / 15	300.0 / 7.30	55.8 / 5.95
13	Aggregate of glycenin G1 fragment 375–503 [Glycine max] (Gly A3B4)***(1)	BAA19059.1	135 / 6 / 17	280.0 / 9.20	58.2 / 5.46

*S/M/C is characteristics widely used for mass-spectrometric analysis: Score is suitability mark or 'score record'; Match peptides is number of coincided peptides; Coverage is percentage of amino acid sequence covered by identified peptides.
**Mm/pl (exp.) is experimental values obtained on the basis of electrophoretic mobility on DE, and Mm/pI (calc.) is values calculated from amino acid sequence data, taking into account the signal peptide removal but with no consideration for other postsynthetic modifications, using 'Compute pI/Mm tool' software (ExPASy).
***msms is the reference to identification confirmed by means of mass-spectrometry (brackets include number of sequenced tryptic peptides).

oxygen and the use of several types of proteases make aggregates formation with plant proteins possible.

CONCLUSION

According to the data obtained, only certain proteases provoked protein aggregation, and the presence of free oxygen accelerated the process. The proteases affected several types of raw meat proteins and also plant proteins in meat products (in particular, soy ones). Additionally, some proteins were also involved but one-dimensional electrophoresis was not able to detect them because of small quantities. Those proteins had alkali pI, therefore the use of immobilized pH gradients was useless to detect them as opposed to ampholines non-equilibrium pH gradient electrophoresis. The results obtained, along with those of previous research [12, 14], allowed us to assume that cross linkages formation and the aggregation of muscle proteins, primarily myosin, tended to reduce meat water-binding capacity, juiciness, and tenderness and affected meat flavor adversely. The negative impact of protein polymerization on the calpain activity also might retard the tenderization process in early proteolysis in the presence of free oxygen [12].

Apparently, those proteases which led to protein aggregate formation were less effective for meat tenderization and formation of consumer characteristics of finished meat products.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Using neural networks to identify the regional and varietal origin of Cabernet and Merlot dry red wines produced in Krasnodar region

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Abstract: This paper shows a possibility of establishing the authenticity and geographic origin of wines by neural networks based on multi-element analysis. The study used 144 samples of Cabernet and Merlot dry red wines produced in Krasnodar Region according to traditional technologies. The wines were provided by the producers or purchased in retail stores. The concentrations of 20 micro- and macroelements in red wines were determined by atomic emission spectroscopy with inductively coupled plasma. The analysis of average elemental contents showed a significant dependence of wine composition on the grape variety and place of origin, which enabled us to examine interrelations between the elements and think of a way to identify them by means of classification models. The software *STATISTICA Neural Networks* was used to assess a possibility of determining the grape variety and geographical origin. The neural networks constructed in the study contained five variables corresponding to the elements with statistically significant correlations between the names of the regions and the wine samples, namely Fe, Mg, Rb, Ti, and Na. These predictors were able to determine the grape variety and place of growth with a sufficiently high accuracy. In the test sample set, the accuracy reached 95.24% and 100% for variety and region identification, respectively. A software product was developed to automate the calculations based on the neural networks. The program can establish the grape variety from a minimal set of microelements, and then, based on the variety and the same set of microelements, determine its place of origin.

Keywords: Cabernet and Merlot red wines, regional and varietal origin of wine, multi-element analysis, neural network technologies, Neural Network

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INTRODUCTION

One of the most difficult tasks in analytical chemistry of wine is to identify its authenticity and geographical origin. Single quality assessment parameters are not sufficient to determine whether the product conforms to its labels. To establish the authenticity and geographical origin of wines, as well as changes occurring in case of their adulteration, analytical approaches are being developed that aim to determine the mineral and isotopic composition, study spectral characteristics, and identify phenolic and volatile compounds using various methods of analysis [1–2]. The identification of authenticity and origin criteria is based on obtaining a large amount of data and its processing by chemometric methods, which reveal hidden relations between the wine's components [1–9]. The combination of modern data analysis tools with the capabilities of chemometric methods ensures higher accuracy in identifying the geographical origin of wines. The information on the elemental composition of wines can be used to both control the technological process and, in combination with chemometric data processing methods, establish the origin of wines [10, 11]. For example, wines produced in various regions of Europe differ quite markedly in the metal content [12], which makes it a good criterion for identifying their geographical origin (Table 1).

Natural variability of wine quality is determined by the grapes growing conditions, such as the climate, the microelement composition of the soil, the technology of growing grapes, the period of grape harvest, etc. The mineral composition of wines can be influenced by various factors (soil, climate, relief, etc.); therefore, for

Table 1. The metal content of wines in different countries [12]

Element	Element content, mg/dm^3				
	Czech Republic	France	Germany	Italy	Spain
K	553–3056	265–426	480–1,860	–	338–2,032
Na	2.0–110	7.7–14.6	6–25	–	3.5–300
Ca	40–210	65–161	58–200	88–151	12–241
Mg	7.8–138	55–96	56–105	53–60	50–236
Al	–	0.56–1.27	–	–	0.57–14.3
Cu	–	n/d–0.48	0.02–0.71	–	n/d–3.1
Fe	0.9–5.2	0.81–2.51	0.4–4.2	–	0.4–17.4
Mn	0.28–3.26	0.63–0.96	0.5–1.3	–	0.1–5.5
Rb	0.56–1.20	0.64–0.72	0.2–2.9	0.50–9.90	0.1–5.3
Sr	0.34–0.53	0.22–0.47	0.12–1.28	0.40–1.16	0.28–1.50
Zn	–	0.44–0.74	0.3–1.5	–	n/d–4.63
Ba	0.09–0.12	0.025–0.24	0.04–0.26	0.07–0.14	0.01–0.35
Cd	–	n/d–0.0002	–	–	n/d–0.019
Co	n/d–0.018	0.004–0.011	0.004–0.005	0.003–0.006	n/d–0.040
Cr	0.032–0.037	0.030–0.057	0.022–0.078	0.023–0.034	0.025–0.029
Li	0.015–0.052	0.008–0.036	0.005–0.043	–	0.002–0.13
Ni	–	n/d–0.052	–	–	0.005–0.079
Pb	–	0.006–0.023	–	–	0.001–0.043
V	0.020–0.054	0.06–0.23	0.01–0.14	–	0.026–0.043

*n/d – not detected

identification purposes, many researchers study those elements which are least dependent on external factors in a given geographical area [3–6, 8, 9, 13, 14]. For example, some authors [13] use Sr, Mn, Mg, Li, Co, Rb, B, Cs, Zn, Al, Ba, Si, Pb, and Ca.

The content of metals in wines is widely different: 10–1000 of macroelements (Ca, K, Na, and Mg), 0.1–10 mg/dm^3 of minor elements (Al, Fe, Cu, Mn, Rb, Sr, and Zn), and 0.1–1000 μg/dm^3 of trace elements (Ba, Cd, Co, Cr, Li, Ni, Pb, V, etc.) [12]. Therefore, the problem of ascertaining the microelement "image" of grapes is of practical, as well as scientific, interest [14–18].

In cases when wines from different grape varieties have certain organoleptic similarities, for example, colour or astringent, sour taste, it is important to be able to identify the grape variety from the microelement composition of the wine [19]. In fact, the task comes down to establishing the grape variety and geographical origin based on the content of microelements in a sample of unblended wine.

The purpose of this work was to study a possibility of identifying the authenticity and geographical origin of red wines, namely Cabernet and Merlot varietal wines, based on multi-element analysis with *STATISTICA Neural Network*.

STUDY OBJECTS AND METHODS

The study used 144 samples of Cabernet (76) and Merlot (68) varietal dry wines produced from 2012 to 2015 by the main wineries in Krasnodar Region: ZAO Zaporozhskoye, OOO Kuban-Vino, OAO APF Fanagoria, OOO APK Millstream Black Sea Wines, ZAO AF Kavkaz, ZAO Abrau-Durso, ZAO APK Gelendzhik, ZAO AF Myskhako, OOO Firma Somelye, OOO AF Sauk-Dere, and OOO Soyuz-Vino (Table 2). Theses wineries are located in different geographic zones (subzones) of Krasnodar Region: the South-Piedmont zone, the Black Sea zone, the Anapa subzone, and the Taman subzone. The wines were provided by the manufacturers or purchased in retail stores.

The main vineyards of Krasnodar Region are located in five cultivation areas: Temryuk (the Taman Peninsula, the Taman subzone), Anapa (the Anapa subzone), the Black Sea zone (Gelendzhik and Novorossiysk), Krymsk (the South-Piedmont zone), and Novokubansk. The frequency distribution of Cabernet and Merlot samples by zone and variety is shown in Table 2

The elemental composition of the wine samples was established by atomic emission spectroscopy with inductively coupled plasma using iCAP-6000 (Thermo Scientific). The operating conditions of the spectrometer were optimised to detect 20 elements (Li, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Sr, Cd, Ba, and Pb). The most sensitive analytical lines were used for most of the metals, with the exception of Al, V, Ca, Mg, and Sr, for which alternative lines were chosen due to spectral overlays. For some macroelements, we needed to reduce the signal intensity. When optimising the conditions for element detection, we studied how the operating characteristics (generator power, argon flow rate) affected the analytical signal of elements in the model

Table 2. Wine sample frequencies by zone

Variety	Two-entry table of frequencies by zone				
	Taman subzone	Anapa subzone	South-Piedmont zone	Black Sea zone	Total
Cabernet	28	17	13	18	76
Merlot	33	23	12	0	68
Total	61	40	25	18	144

and sample solutions. We also investigated the mutual influence of micro- and macroelements, as well as background components, of the samples prepared for analysis in the model solutions containing variable amounts of the elements. The quantification of metals was carried out by diluting the wine samples, taking into account the data obtained [5, 14–18, 20, 21].

The following reference standards were used to study the test samples: GSO 7780-2000 (Li), GSO 8062-94 (Na), GSO 7767-2000 (Mg), GSO 7854-2000 (Al), GSO (K), GSO 7772-2000 (Ca), GSO 7205-95 (Ti), GSO (Cr), GSO 8056-94 (Mn), GSO 8032-94 (Fe), GSO 7784-2000 (Co), GSO 7785-2000 (Ni), GSO 7836-2000 (Cu), GSO 8053-94 (Zn), GSO 7035-93 (Rb), GSO 7783-2000 (Sr), GSO 7874-2000 (Cd), GSO 7760-2000 (Ba), and GSO 7778- 2000 (Pb). All the reagents used in the work were of chemically pure (C.P.) grade.

The chemometric analysis was performed using *STATISTICA Neural Networks* [22].

RESULTS AND DISCUSSION

The analysis of average element contents (Tables 3, and 4) showed a significant dependence of wine composition on the grape variety and place of origin. For example, the samples from the Anapa subzone had a high content of Fe, those from the South-Piedmont zone were rich in Ba, Ti, and V, whereas the Taman wines were abundant in Na, Mg, and Rb. The Cabernet wines had significantly different contents of many elements. For example, the Cabernet samples from the South-Piedmont zone contained the lowest concentrations of Li, Na, Al, Ca, Fe, and Sr, while the Merlot samples from the same zone had the lowest content of Al, Ca, Fe, and Li. As a rule, standard deviations did not exceed half of the average values. This suggests a small variation in the concentrations of elements, which means that an average value is a relevant characteristic of metal content in wine. The exceptions are Cu, Li, Ni, Na, Rb, and Ti; however, standard deviations exceeded the averages only in three cases: Cu (Cabernet, Anapa subzone) and Ni (Cabernet and Merlot, South-Piedmont zone).

Previously, we applied traditional statistical methods of discriminant analysis and classification trees to construct probabilistic-statistical models that allowed us to identify the varietal and regional origin of the same group of red wines using multi-element analysis data [23]. This study looked at a possibility of determi-

Table 3. Average elemental content and standard deviations (s.d.) in *Cabernet* samples from various geographical zones of Krasnodar Region, μg/dm^3

Element	Cabernet			
	Anapa	South-Piedmont	Black Sea	Taman
Al	761	668	1,074	777
s.d.	389	354	322	222
Ba	91	160	93	100
s.d.	32	69	46	29
Ca	60,042	54,707	59,864	65,516
s.d.	8,432	10,124	5,571	11,564
Cu	112	69	109	65
s.d.	128	36	43	31
Fe	8,098	3,398	4,188	3,657
s.d.	3,017	1,150	972	915
K	659,037	190,177	1064,056	983,958
s.d.	96,739	394,839	171,437	233,821
Li	19	13	25	28
s.d.	5	8	24	15
Mg	74,037	116,978	96,779	141,678
s.d.	12,846	21,053	27,030	59,802
Mn	956	1,585	1,096	1,338
s.d.	319	362	165	436
Na	21,434	31,760	35,268	33,699
s.d.	9,680	27,186	13,784	8,590
Ni	24	57	33	21
s.d.	10.3	65	31	9
Rb	514	977	817	1,515
s.d.	360	290	236	352
Sr	1,207	1,323	1,533	1,270
s.d.	207	504	720	284
Ti	7	31	16	8
s.d.	3	22	11	3
Zn	366	685	757	481
s.d.	140	222	151	144

Table 4. Average elemental content and standard deviations (s.d.) in *Merlot* samples from various geographical zones of Krasnodar Region, μg/dm^3

Element	Merlot		
	Anapa	South-Piedmont	Taman
Al	1,063	926	1,307
s.d.	381	178	537
Ba	106	148	163
s.d.	41	40	42
Ca	60,117	55,630	63,212
s.d.	5,280	582	8,701
Cu	53	62	47
s.d.	45	33	33
Fe	13,248	4,073	4,883
s.d.	4,214	606	1,661
K	728,426	1264,236	695,290
s.d.	74,086	369,588	106,987
Li	25	16	29
s.d.	14	7	11
Mg	71,221	114,459	158,520
s.d.	6,553	21,715	28,073
Mn	1,181	1,410	1,519
s.d.	401	499	398
Na	20,698	51,380	62,561
s.d.	10,063	28,799	30,852
Ni	29	92	112
s.d.	17	101	96
Rb	563	2,063	6,157
s.d.	531	1786	2,707
Sr	1,242	1,453	1,389
s.d.	172	376	292
Ti	11	33	28
s.d.	11	7	15
Zn	369	755	526
s.d.	83	138	140

Table 5. Average elemental contents and standard deviations (s.d.) in wine samples, μg/dm³

Wine		Cabernet				Merlot		
Zone/ subzone	Statistic	Anapa	South-Piedmont	Black Sea	Taman	Anapa	South-Piedmont	Taman
Fe	average	8,098	3,398	4,188	3,657	13,248	4,073	4,883
	s.d.	3,017	1,151	973	915	4,214	606	1,661
Mg	average	74,037	116,978	96,780	141,678	71,221	114,459	158,520
	s.d.	12,846	21,053	27,030	59,802	6,553	21,715	28,073
Na	average	21,434	31,760	35,268	33,699	20,698	51,380	62,561
	s.d.	9,680	27,186	13,784	8,590	10,064	28,798	30,852
Rb	average	514	977	817	1,515	563	2,063	6,157
	s.d.	360	290	236	352	531	1,786	2,707
Ti	average	7	31	16	8	11	33	28
	s.d.	3	22	11	3	11	7	15

ning the grape variety and geographical origin using *STATISTICA Neural Networks*, followed by a comparative analysis.

To select a number of elements as predictors of neural network classification models, we used a Spearman's nonparametric correlation coefficient that characterised the correlation between the names of wine samples, the region of grape origin, and the concentrations of trace elements in the samples. In particular, the elements with the largest statistically significant correlation links between the names of regions and wines (Fe, Mg, Rb, Ti, and Na) were selected as predictor variables.

In Table 5, which shows average elemental contents with standard deviations in both wine varieties from different regions, we can see some significant differences in the average values – the deciding factor for building classification models with neural networks. Most distinctly these differences are visualised by means of graphs. Fig. 1, for example, shows some box plots displaying Mg content in the Cabernet and Merlot wines from various regions. The box plots present ranges of values of a selected variable separately for groups of observations defined by the values of a categorical variable. The rectangles depicted around the midpoints (or squares) represent selected ranges of variation, for example, the standard error (the ratio of the standard deviation to the square root of the sample size). The segments with their ends outside the rectangles also reflect ranges of variation (average ± 1.96 × standard error). The diagram shows that the average values of Mg content, together with variation values, differ significantly between both the regions and the grape varieties.

As in [23], we were not able to build adequate neural networks that would allow us to identify the grape variety and region of origin from the concentrations of selected elements. Therefore, the problem was divided into two parts. First, networks were built to predict the grape variety from the concentrations of Fe, Mg, Rb, Ti, and Na. Then, based on the variety predicted (qualitative predictor) and the same set of elements (quantitative predictors), further networks were built to determine the place of grape origin. After assessing their predictive properties (productivity, number of classification errors, etc.), we selected the best network. Productivity is a percentage of correctly classified wine samples, with 100% taken as maximum. The higher the productivity, the more accurate the prediction. To improve predictive accuracy, the samples were divided into three groups: training, control, and validation sample. The most important were the values of adequacy criteria in the test set. By combining various network options, we tried to create a network with the best predictive capabilities; therefore, at each stage of the process, the number of networks was different.

Building a neural network to establish the varietal origin of wine. The program divided 144 wine samples into three groups: training set (102), control set (21), and test set (21). The productivity of the best network (MLP 5-5-2), selected out of 50, had high values of 99.02%, 90.48%, and 95.30% in the training, control, and test sets, respectively. MLP 5-5-2 is a combination of letters and numbers that represents a topology of a multilayer perceptron. The letters stand for the type of a neural network, a multilayer perceptron (MLP); the first numer-

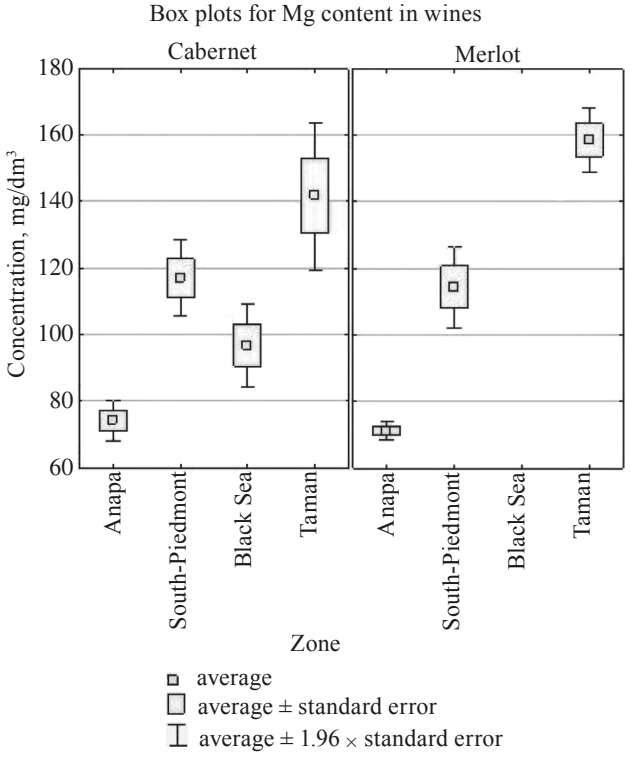

Fig. 1. Box plots displaying Mg content in Cabernet and Merlot wines.

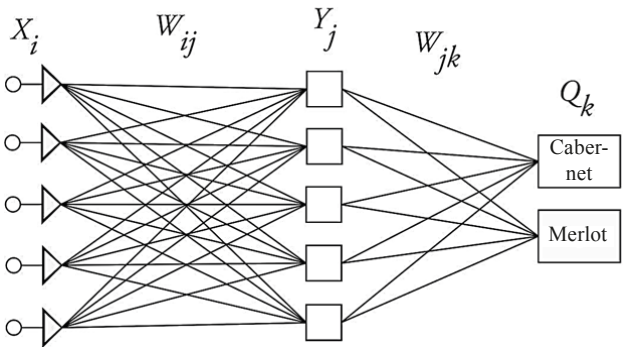

Fig. 2. Neural network to determine the variety of red wines.

al (5) refers to the number of predictor variables in the model, a sum of quantitative predictors and qualitative predictor values; the second (5) and the third (2) numerals refer to the numbers of hidden and output neurons, respectively.

The network topology is shown in schematic form in Fig. 2, where we can see five entries of predictor variables X_i; five hidden neurons Y_j; two output neurons representing objects of classification Q_k, the Cabernet and Merlot varieties, as well as connections between them in the form of weights W_{ij}, W_{jk}.

Table 6 shows the frequencies of correctly and incorrectly classified wines in the sample sets. As we can see, one Merlot sample from the training and the test sets and two Merlot samples from the control set were erroneously classified as Cabernet. All Cabernet samples were correctly identified in all the sets. The total number of erroneously classified samples was four out of 144 (app. 2.8%), i.e. the neural network identified 97.2% of the wine samples correctly. In [23], by comparison, the classification tree with seven terminal vertices only once misclassified a Merlot sample as a Cabernet, based on the concentration of seven microelements, i.e. 99.3% of the training sample was identified correctly.

Table 6. Wine classification results by variety

Sample set	Classification accuracy	Cabernet	Merlot	Total
Training	Total	57	45	102
	Correct	57	44	101
	Incorrect	0	1	1
	Correct, %	100	97.78	99.01
	Incorrect, %	0	2.22	0.99
Control	Total	12	9	21
	Correct	12	7	19
	Incorrect	0	2	2
	Correct, %	100	77.78	90.48
	Incorrect, %	0	22.22	9.52
Test	Total	7	14	21
	Correct	7	13	20
	Incorrect	0	1	1
	Correct, %	100	92.86	95.24
	Incorrect, %	0	7.14	4.76

Table 7. Network sensitivity analysis

MLP 5-5-2	Fe	Rb	Mg	Na	Ti
Test	141.09	52.18	40.62	42.80	17.12
Training	21.01	8.42	4.73	2.99	1.41
Control	6.86	3.58	5.63	3.12	2.64

The network sensitivity can be used to estimate a contribution of each predictor to its predictive properties: in our case, a contribution of the elements to the classification model. The sensitivity values (see Table 7) indicate a decreasing sequence of Fe, Rb, Mg, Na, and Ti, which represents their contributions to the predictive properties of the network.

Building a neural network to determine the regional origin. The possibility of predicting the wine variety based on five microelements made it realistic to create a neural network to identify the place of grape origin using the trace elements of Fe, Mg, Rb, Ti, and Na and the varieties of Cabernet and Merlot. In the same way, the program divided 144 wine samples into three groups: training set (102), control set (21), and test set (21). The best out of 18 networks (MLP 7-9-4) had productivity values of 100%, 80.95%, and 100% in the training, control, and test sets, respectively.

As can be seen in Table 8, all the wine samples (100%) from the Black Sea zone were classified by the network correctly. The next high accuracy area was the Taman subzone with 100%, 85.71%, and 100% of correctly classified samples in the training, control, and test sets, respectively. The lowest accuracy was observed in the Anapa subzone: 100%, 71.43%, and 100%, respectively. The total number of misclassified samples was four out of 144 (app. 2.8%), i.e. the neural network

Table 8. Wine classification results by region

Sample set	Classification accuracy	Anapa subzone	Taman subzone	Black Sea zone	South-Piedmont zone	Total
Training	Total	26	46	14	16	102
	Correct	26	46	14	16	102
	Incorrect	0	0	0	0	0
	Correct, %	100	100	100	100	100
	Incorrect, %	0	0	0	0	0
Control	Total	7	7	3	4	21
	Correct	5	6	3	3	17
	Incorrect	2	1	0	1	4
	Correct, %	71.43	85.71	100	75	80.95
	Incorrect, %	28.57	14.29	0	25	19.05
Test	Total	7	8	1	5	21
	Correct	7	8	1	5	21
	Incorrect	0	0	0	0	0
	Correct, %	100	100	100	100	100
	Incorrect, %	0	0	0	0	0

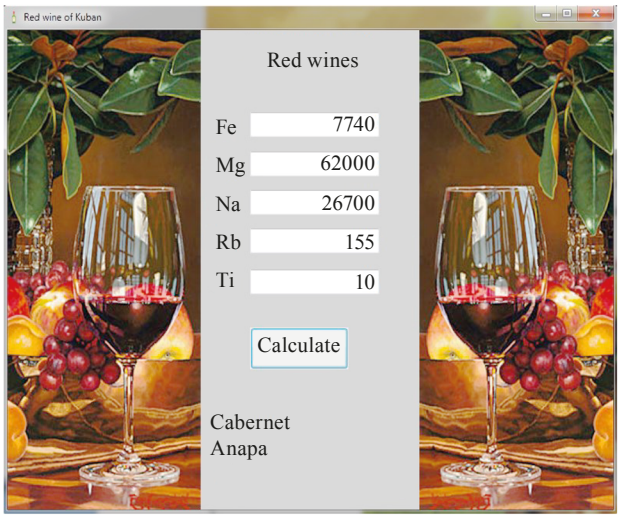

Fig. 3. The programme home screen

identified 97.2% of the wine samples correctly. It is noteworthy that all the samples in the test set were classified correctly, regardless of the place of origin.

The sensitivity analysis showed that the average predictor contributions to the network's predictive properties decreased in the following order: Variety, Rb, Ti, Mg, Fe, and Na. We can notice that this sequence is significantly different from the one for the variety identification network.

In [23], the problem of identifying the place of grape origin was solved separately for Cabernet and Merlot wines using two methods, discriminant analysis and classification trees. The discriminant analysis of Cabernet and Merlot wines involved 13 and 14 microelements, respectively, whereas only 7 and 3 microelements were used in the classification trees. However, both methods produced 100%-accurate classifications.

The above shows that the traditional methods of classification analysis, which used a larger number of elements, achieved a higher predictive accuracy. However, the neural networks also showed acceptable prediction accuracy with a significantly smaller number of predictors (5). The results were confirmed by the classification analysis in the test sample set, with a 100% accuracy of region identification and only one mistake in variety identification.

Thus, we managed to build adequate neural networks for two red wines, Merlot and Cabernet, with high predictive properties, able to determine the wine variety from a minimum set of elements, and then, identify the region of grape origin from the variety and the same set of elements.

To automate the process of identifying the varietal and geographical origin of red wines, we developed a program using *Visual C#* (C Sharp). The network parameters obtained during the training process, their topology and weights made it possible to create an autonomous software product that can function independently of *STATISTICA*. The home screen of the program is shown in Fig. 3. If you enter the concentration values of the trace elements Fe, Mg, Na, Rb, and Ti into the corresponding boxes on the interface and click 'Calculate', you will see the variety (Cabernet) and the place of the grape origin (Anapa) at the bottom of the screen.

CONCLUSION

Thus, the use of neural networks enabled us to successfully identify both the varietal and the regional origin of red wines. It is equally important that a certain set of elements in wine contains information not only about the grape variety, but also about the place of its growth. Traditional and heuristic methods of classification analysis used with modern data analysis tools allowed us to accurately determine the grape variety and region of origin from the "elemental" memory of the wine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Optimisation of important processing conditions for rice bran sourdough fermentation using *Lactobacillus plantarum*

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Abstract: The potentials of rice bran sourdough in bread making are recently gaining popularity. However, there is no information on the influence of processing conditions on the quality attributes of rice bran sourdough. To investigate the influence of fermentation time and temperature on the levels of acidity (pH and TTA) in rice bran sourdough fermented with *L. plantarum*, we applied response surface methodology (RSM). Furthermore, we studied the effect of different fermentation time and temperature on the total phenolic and volatile compounds in the sourdough. GC/MS measurements for the evolution of aroma volatile compounds (VOCs) in the rice bran sourdoughs were conducted. The higher and longer the fermentation temperature and time, the higher the acidity levels in the sourdoughs. Fermentation temperature and time do not have a significant effect on the total phenolic sourdough contents. Forty-seven VOCs were detected in the rice bran sourdoughs. The major VOCs were acetic acids, ethanol, 2-Methoxy-4-vinylphenol, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, acetoin, and 2-methoxy-Phenol. The sourdough fermented at 35°C for 13 ho contained the largest number (27) of aroma compounds and had the highest acidity. These fermentation conditions are close to the optimal parameters (temperature – 33°C, duration – 12.5 hours), obtained as a result of applying RSM for rice bran fermentation. Thus, high quality bran sourdough can be produced at the temperature of 33°C for 12.5 hours. The results of this study will be useful to produce a quality rice bran sourdough bread with appealing aroma and a long shelf-life.

Keywords: Rice bran, acidity, Response surface methodology, *Lactobacillus plantarum*, HS-SPME, GC/MS, volatile compounds

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INTRODUCTION

Rice bran is a by-product of rice milling industries, grossly underutilised in rice-producing countries. Although, rice bran is rich in dietary fibre, amino acids, minerals, and other bioactive compounds, which can have a beneficial effect on human health [1], it is commonly used as a livestock feed as opposed to human food formulation. This is because the use of bran in food industries, especially in baked goods, poses several technological problems, such as weakening of wheat dough structure and of baking quality, the decrease in bread volume and elasticity of bread crumb [2]. Bio-processing technologies, such as enzymatic and fermentation processes have received more attention recently due to their potential to improve nutritional and technological functionality of bran [3]. These processes have been employed for bran sourdough production [4–6].

Sourdough is used as a starter for bread making, because of its ability to improve bread quality [7, 8]. Traditionally, bread volume, crumb texture, flavour, and shelf-life of bread are enhanced using sourdough fermentation [2, 9, 10]. This is because baked products with sourdough are better protected from bacterial and mould spoilage, and have extended shelf-life [11, 12]. However, the preservative effect of sourdough depends on the bacteria used for the dough fermentation. Previous studies have reported an extended shelf life for sourdough bread produced with lactic acid bacteria (LAB), fermented rice bran sourdough [13, 14].

The flavour of baked foods, especially bread, is influenced by the recipe, processing condition, and vola-

tile organic compounds produced by a starter culture. The generation of odorants in sourdough occurs due to enzymatic and microbial processes during sourdough fermentation [15], for example, LAB is responsible for liberation of aroma precursors and acidification in sourdough [7].

Fermentation temperature and time are important processing factors to be considered in producing a quality sourdough. The extent of metabolic performances of microorganisms in sourdough depends directly on the fermentation time, while fermentation temperature plays an important role in the stability of sourdough ecosystem [16, 17]. There have been studies on the effect of processing conditions and starter cultures on the formation of volatile compounds in wheat sourdoughs [18]. Volatile compounds produced by different species of lactobacilli in rye sourdough have also been reported [7]. There is little information on the optimisation of fermentation conditions for rice bran sourdough production, and no information on volatile compounds in LAB fermented rice bran sourdough.

Response Surface Methodology (RSM) is a multivariate technique used for an analytical optimisation [19]. In recent years, RSM has been used to optimise fermentation conditions for cereal bran. Application of RSM for optimisation of rice bran fermentation conditions for protein concentrate extraction, using baker's yeast, has been studied [20]. RSM was also applied to study fermentation effects on the levels of phytochemicals in rye bran [5].

The use of rice bran sourdough is gaining popularity in bread making [13, 14, 21]. It is therefore important to establish optimum fermentation temperature and time for quality rice bran sourdough preparation. Furthermore, bread quality mostly depends on taste and aroma. Apart from aroma development during baking, aroma precursors are also produced during fermentation process. Volatile organic compounds (VOCs) in fermented sourdough contribute to bread flavour [22] and are produced from enzymatic, or autoxidation of flour lipids, microbial, yeast metabolism, and Maillard reaction [15]. However, publications about VOCs in sourdough are mostly about wheat, wheat germ, buckwheat, rye, rice, maize, Kefir or chestnut-flour sourdough using either GC-MS, or HRGC-MS [15].

Although, VOCs of rice bran oil have been characterised by GC-MS [23], to our knowledge, there has been no study on the characterisation of VOCs in rice bran sourdough using HS-SPME method. The objectives of this study were to optimise important fermentation factors (temperature and time) for rice bran sourdough production using RSM and to determine the effect of fermentation conditions on pH, titratable acidity (TTA), and total phenolics in L. *plantarum* fermented rice bran sourdough. For the first time, VOCs profile of rice bran sourdough samples fermented at different temperature and time was also identified and quantified by SPME-GC/MS analysis.

STUDY OBJECTS AND METHODS

Raw materials. Rice variety MR bran 219 was obtained from Padiberas Nasional Berhad (BERNAS) rice mill, Sekinchan, Selangor, Malaysia. The rice bran contained 7.2% of moisture, 11.7% protein, 18% fat, 8.6% fibre, 8.5% ash, and 45.9% carbohydrate, as determined in our previous study [21]. The bran was sieved using a Haver EML digital plus test sieve shaker (Harver and Boecker, 59302, OELDE, Germany) to particle size of < 300 μm.

Microbial strain. Lactobacillus plantarum. ATCC® 8014™ was purchased from the American Type Culture Collection (ATCC) (USA). SupelcoSPME fibre coated with 85-μm of Carboxen–polydimethylsiloxane (Carboxen/PDMS) was purchased from Bellefonte, PA (USA), SPME vials were purchased from LA-PHA-PACK (Germany). Distilled deionized water from Sartorius, Sdn, Bhd (Malaysia) was used for all sample preparations. *N*-alkanes (C_8–C_{20}), and 4-methyl-2-pentanol were purchased from Sigma-Aldrich (St Louis, USA). All other chemicals and reagents were of analytical grade.

Optimisation of rice bran fermentation conditions. We used RSM to investigate the combined effects of significant variables **(**fermentation temperature and time) in the production of rice bran sourdough. The adopted experimental design was a central composite design (CCD). The minimum and maximum independent variable levels were selected on the basis of previous sourdough studies [18, 5]. The CCD was divided into three blocks to calculate the reproducibility of the method. The CCD's use required 13 runs for each block and the total of 39 runs for the whole design. Table 1 shows the experimental runs in the order they were performed. The dependent/response variables for the rice bran sourdough quality were pH and total titratable acidity (TTA). The independent variable range was 20–35°C of fermentation temperature and 6–20 hours of fermentation time. For reproducibility, the experiments were carried out in triplicate. For each of the response variable, a model summary, and a lack of fit test were constructed for linear, square, interaction and quadratic models. We investigated the behaviour of the response surface for the response function (Y_p, predicted response) using a regression polynomial equation. The generalised polynomial mode to predict the response variable was:

$$Y_p = \beta_0 + \beta_1 T_e + \beta_2 T_i + \beta_{11} T_e^2 + \beta_{22} T_i^2 + \beta_{12} T_e T_i, \quad (1)$$

where β_0 was intercept, β_1 and β_2 were coefficients. The most accurate model was chosen based on a number of significant terms ($p < 0.05$), percentage of regression, the lack of fit test, and P-value for a regression model. A three-dimensional response surface plot was generated for each response variable. The reliability of the model was evaluated from the regression of the model (R^2) and predictive power of the model (Q^2). Generally, for a good fit model R^2 should be ≥ 0.8 [24], however, the models with R^2 and Q^2 values of ≥ 0.9 are considered excellent.

Optimal processing conditions to determine rice bran sourdough production were calculated by performing a multiple response method called desirability. The desires and priorities for each of the variables were incorporated into the optimisation method. pH and

Table 1. Composition of various runs of central composite design (CCD)

Runs	Block	Temperature,°C	Time, h
1	1	22.2	8.05
2	1	32.8	8.05
3	1	22.2	17.95
4	1	32.8	17.95
5	1	20.0	13.00
6	1	35.0	13.00
7	1	27.5	6.00
8	1	27.5	20.00
9[a]	1	27.5	13.00
10[a]	1	27.5	13.00
11[a]	1	27.5	13.00
12[a]	1	27.5	13.00
13[a]	1	27.5	13.00
14	2	22.2	8.05
15	2	32.8	8.05
16	2	22.2	17.95
17	2	32.8	17.95
18	2	20.0	13.00
19	2	35.0	13.00
20	2	27.5	6.00
21	2	27.5	20.00
22[a]	2	27.5	13.00
23[a]	2	27.5	13.00
24[a]	2	27.5	13.00
25[a]	2	27.5	13.00
26[a]	2	27.5	13.00
27	3	22.2	8.05
28	3	32.8	8.05
29	3	22.2	17.95
30	3	32.8	17.95
31	3	20.0	13.00
32	3	35.0	13.00
33	3	27.5	6.00
34	3	27.5	20.00
35[a]	3	27.5	13.00
36[a]	3	27.5	13.00
37[a]	3	27.5	13.00
38[a]	3	27.5	13.00
39[a]	3	27.5	13.00

Note: [a] Denotes centre point runs

titratable acidity (TTA) were chosen as the two most important quality parameters in this study, because sourdough quality depended mainly on them [25]. Since a rice bran falls within a high extraction rate category with 95% extraction rate, the desired pH target was in the range of 3.5 to 4.5 [26], while the desired TTA was in the range of 16 to 22 ml NaOH [26, 27].

Microbial strain and preparation of sourdough culture. *Lactobacillus plantarum* media were prepared according to a manufacturer's instruction and sterilised by autoclaving at 121°C for 15 min. *L. plantarum* was the culture of De Man Rogosa and Sharpe (MRS) broth at 37°C for 72 hours at the atmosphere of 5% CO_2. 1 ml of cultured MRS broth was mixed with 9 ml of sterile peptone water 0.1% (v/v) and diluted in ten-folds using serial dilution (10^{-2} to 10^{-10}). A serial suspension (0.1 ml) was incubated on a plate count agar, and the colonies were counted. The bacteria suspension (10^9 CFU/ml) in peptone water (0.1%) was used for bran fermentation.

Preparation of rice bran sourdough. Rice bran sourdough was prepared according to [21]. 100g of rice bran were mixed with 130 g of distilled deionised water and 15 ml of *L. Plantarum s*uspension (10^9 CFU/ml) in a sterilised beaker. Then the mixture was covered with aluminium foil and allowed to ferment in a fermenting box (Model Fx-11, Good and Well, SDN BHD, Malaysia) to produce sourdough. Fermentation temperature and time were in accordance with the experimental design of Table 1. The sourdough samples were frozen at –20°C until further analysis. Rice bran that was allowed to ferment naturally was used as a control.

pH and total titratable acidity (TTA) of rice bran sourdough. pH and TTA were measured as described by [25]. Frozen rice bran sourdough samples were thawed overnight in a refrigerator at 4°C. 10 g of the thawed sourdough were mixed with 100 ml of distilled water to obtain a suspension. The pH value of the suspension was measured by using a pH meter. TTA was determined by titrating the suspension against 0.1M NaOH to a final pH value of 8.5. TTA was expressed as the amount of NaOH (ml) used for titration and as a mean value of three replicates.

Total phenolic compound (TPC) analysis. A modified method of [28] was used for a total phenolic compound determination. 200 mg of the rice bran sourdough fermented with *L. plantarum* at different temperatures and time were extracted with 4 ml of 70% aqueous acetone by shaking in a rotary shaker (260 rpm, 2 hours) at room temperature. The supernatant liquid was collected after centrifugation (Hereaus Instruments D867 bench top centrifuge, Germany) at 3,000 g for 10 min. 200 µl of the supernatant were added to 1.5 ml of freshly prepared Folin Ciocalteu reagent (1:10 v/v with water). After 5 min equilibration, 1.5 ml of the sodium carbonate (60 g/l) solution was added to the mixture. The mixture was incubated at room temperature for 90 min. The absorbance of the mixture was read at 725 nm using 70% aqueous acetone as blank. The gallic acid calibration curve (0 to 1,000 µg/ml) was plotted, and TPC was expressed as micrograms (µg) of gallic acid equivalents (GAE) per gram of rice bran sourdough.

Characterisation of volatile compounds (VOCs) in rice bran sourdough. Volatile acids are generally produced, when cereals are fermented by lactic acid bacteria, such as *L. plantarum*. Volatile acids of cereal dough, such as rye dough and baked product (bread), are reported to be acetic and lactic acids. These acids are responsible for an acidic bread flavor [29].

Headspace solid phase microextraction (HS-SPME) analysis. The volatile fraction of rice bran sourdough samples was analysed by headspace sampling using solid phase microextraction (SPME) method described by [30], with some modifications. The VOCs of rice bran sourdoughs fermented with *L. plantarum* at different temperature and time (Table 2) were identified and quantified. 2 g of a sourdough sample fermented at

different temperature and time (Table 2, sample A–J) were placed in a 20 ml headspace vial. 2 ml of a distilled deionised water and 5 µl of 4-methyl-2-pentanol (internal standard, 100 mg/l) were added to the headspace vial. The vial was placed in a thermostatic block on a stirrer at 60°C, then the fibre was inserted and maintained in the sample head space for 30 min, after which it was removed and immediately inserted into the GC/MS injector port for the desorption of compounds. A silica fibre coated with 85-µm of Carboxen–polydimethylsiloxane (Carboxen/PDMS) was used for the analyses. The fibre was conditioned prior to its first use. The internal standard (4-methyl-2-pentanol) was used for all analyses to monitor the SPME extraction and fibre performance during the analysis, as well as for semi-quantitative analysis of VOCs.

Gas chromatography–mass spectrometry (GC/MS) analysis and VOCs identification. The method described by [31] and [30] was adopted for the GC/MS analysis. The VOCs of the sourdough samples were determined with a gas chromatograph and a mass spectrometer equipped with a 30 m, 0.25 mm ID, film thickness of 0.25 µm, and capillary column (TG-WAXMS, Thermo Scientific, USA). The gas carrier was helium with a flow of 1.5 ml/min. SPME injections were splitless (straight glass line, 0.75 mm ID) at 240°C for 20 min (during which time thermal desorption of analytes from the fibre occurred). The oven parameters were as follows: the initial temperature was 40°C held for 3 min, followed by an increase to 240°C at a rate of 5°C /min, then held for 10 min. A mass spectrometer was operated in a scan mode from m/z 33–300 (2 s/scan) at an ionisation potential of 70 eV.

Identification of volatile compounds was achieved by comparing their mass spectra with the reference mass spectra library (NIST, version 2.0) and by matching the retention indices (RI) calculated according to the equation of [32], based on a series of alkanes, ranging from n-octane to *n*-eicosane (extrapolation using C_{18}–C_{20} was used for compounds eluting after C_{20}). A semi-quantitative analysis was also obtained by comparison of the VOCs peak areas with that of the internal standard obtained from the total ion chromatograms, using a response factor. Blank of the fibre and blank of the empty vial were conducted after every 10 analyses. All the analyses were performed in replicates.

Statistical analysis. The experimental design and optimisation procedure were performed using Minitab® version 16.0 software (Pennsylvania, USA). The data obtained were subjected to one way analysis of variance (ANOVA) to determine regression coefficients and a statistical significance of model terms to fit the mathematical models to the experimental data. Significant differences in the total phenolic compounds were compared by means of Duncan's multiple comparison tests at 95% confidence level ($p \leq 0.05$). The data were reported as mean values of replicate analyses.

Table 2. Fermentation conditions for *L. plantarum* fermented rice bran sourdough

Sample code	Fermentation temperature, °C	Fermentation time, h
A	22.2	8.05
B	22.2	17.95
C	20.0	13.00
D	27.5	6.00
E	27.5	13.00
F	27.5	20.00
G	32.8	17.95
H	32.8	8.05
I	35.0	13.00
J*	35.0	13.00

Note: * Control sample (sourdough sample fermented without the addition of *L. Plantarum*)

RESULTS AND DISCUSSION

Influence of fermentation conditions on pH, and titratable acidity (TTA). The pH and TTA of the rice bran before fermentation (control) was 6.11 and 11.89, respectively (the results are not shown). The acidity levels (pH and TTA) of the rice bran sourdough fermented at different times and temperatures are presented in Fig. 1. The pH of the sourdough decreases with increasing fermentation time and temperature, whereas the TTA values increase.

The pH of the rice bran sourdough ranged from 4.0 to 4.6, while the TTA ranged from 13.9 to 23.4 ml of 0.1M NaOH/10 g sourdough (Fig. 1). Fermentation time was reported to be the most important parameter of wheat sourdough acidity, fermented with a single or combined starter of lactobacilli or yeast [18]. In contrary, the findings of this study showed that fermentation temperature and time were the most influential parameters of *L. plantarum* fermented rice bran sourdough acidity. The most acidic rice bran sourdough was the sample, fermented at 35°C for 13 hours, while the least acidic sourdough was the sample, fermented at 20°C for 13 hours (Fig. 1). This result indicates that there was a strong interaction between fermentation time and temperature during sourdough fermentation. The maximum TTA and the minimum pH values were obtained in the

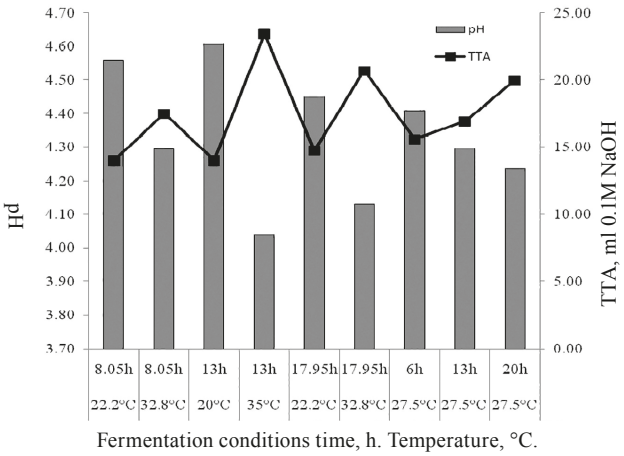

Fig. 1. pH and total titratable acidity (TTA) profiles of rice bran sourdoughs fermented with *L. plantarum* at different fermentation time and temperature.

sourdoughs, fermented at 35°C for 13 hours. This is probably because at the optimum proliferation temperature (37°C, which is close to 35°C) of *L. Plantarum*, and the optimum time for LAB activity (> 12 hours), *L. plantarum* would produce more lactic acid, which will lead to a reduction in pH, increased TTA, and thus, higher acidity in the sourdough. Similar observations were also reported in previous studies [18, 22, 15].

Optimisation and validation process. RSM was used to develop a prediction model for optimising *L. plantarum* fermentation conditions for rice bran sourdough production. The independent and dependent variables were analysed to obtain a regression equation, that could predict response (pH and TTA) within the given range. The developed mathematical models for the rice bran sourdough are presented in Table 3. High values of R^2 (the fit of the model to the experimental data) and Q^2 (predictive power of the model) were obtained in the acidity model. The obtained R^2 values (0.88) indicate that 88% of the variation can be explained by the fitted model (Table 3). Large Q^2 values (> 0.9) imply that the model has a good predictive ability with a small prediction error. A significant linear effect of time and temperature was observed in pH values of the rice bran sourdoughs. The latter indicates that the influence of fermentation time on pH values was dependent on fermentation temperature. And both independent factors had quadratic effect on TTA values (Table 3). p-values for both models significantly affected the acidity levels with the p-value lower than 0.05, however, the p-value (0.000) for the reduced quadratic model of TTA was more significant than that of the pH linear model (p-value = 0.017). These results agreed with the results of the previous studies of *L. plantarum* fermented wheat sourdoughs [18, 33].

As shown in Fig. 2 and 3, the modelling of pH values and TTA produced different surface response plots for fermentation of rice bran sourdough at different fermentation time and temperature. In Fig.2 fermentation temperature and time displayed a linear effect on the pH, where the sourdough pH decreased with the increase of fermentation temperature and time. This was evident in the regression equation (Table 3), where fermentation temperature and time had a negative coefficient, which indicated a decrease in the response. In Fig. 3 fermentation temperature showed a significant effect on the TTA. The TTA of the sourdough increased with the increase of fermentation temperature, as evidenced in the regression equation (Table 3), where the multiplying effect of fermentation temperature (T_e^2) had a positive coefficient. This is also confirmed by the experimental analysis, where sourdough sample fermented at the highest temperature (35°C) had the highest TTA value (Fig. 1).

Table 3. Effect of fermentation factors, expressed as their corresponding coefficients obtained in the model of rice bran sourdough acidity fermented with *L. Plantarum*

Acidity attributes	R^2	Q^2	p-value
pH	0.880	0.991	0.017
TTA	0.887	1.000	0.000

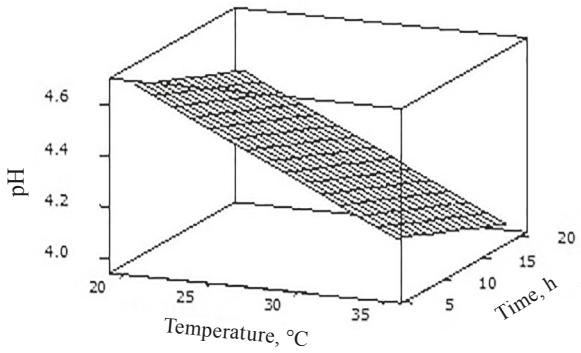

Fig. 2. Surface plot of effects of fermentation temperature, and fermentation time, on pH levels in *L. Plantarum* fermented rice bran sourdough.

The levels of the independent variables generated a targeted range of pH (4.0 to 4.6) and TTA (14–23 ml of 0.1M NaOH/10g) for the rice bran sourdough (Fig. 1). The optimum pH for the sourdough was obtained in 19.5 hours at 34.8°C while, the optimum TTA value was obtained in 10.65 hours at 32.6°C. Based on the multiple optimisation of the fermentation of rice bran sourdough, the optimum pH value of 4.3 and TTA value of 19.5 ml of 0.1M NaOH were obtained, when the sourdough was fermented at 33°C for 12.5 hours.

A two-sample t-test was conducted to test the adequacy of the final reduced models by comparing the experimental values with predicted values. The data obtained showed that there were no significant differences ($p > 0.05$) between the experimental and predicted values (Table 4). This observation showed the agreement between the two values, and thus, the adequacy of the response surface equations fitted by RSM was verified.

The influence of fermentation parameters on total phenolic compounds. Since sourdough can improve phytochemicals in baked goods, we investigated the effect of fermentation conditions on the level of total phenolic compounds (TPC) in rice bran sourdough. The result is presented in Table 5. The highest amount of TPC (484.9 µg GAE/g) was detected in sourdough fermented at the high temperature (32.8°C) for a long time (17.95 hours). The least TPC (410.2 µg GAE/g) was found in rice bran sourdough that was fermented at a low temperature (22.2°C) for 8.05 hours. Although, the TPC values of the sourdoughs increased with the rise

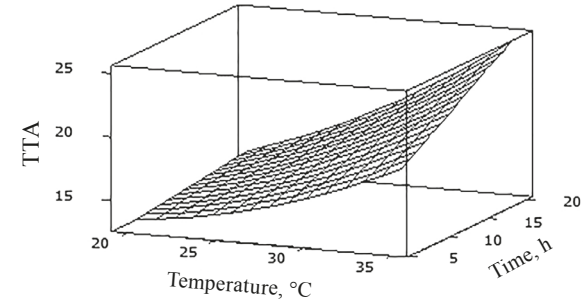

Fig. 3. Surface plot of effects of fermentation temperature and fermentation time on titratable acidity (TTA) levels in *L. Plantarum* fermented rice bran sourdough.

Table 4. Predicted and experimental values of responses at the optimum pH and titratable acidity (TTA)

Response variables	Predicted value[a]	Experimental value[a]
pH	4.22	4.03 ± 0.14
TTA	19.10	23.32 ± 1.14

Note: [a] no significant difference ($p > 0.05$) between predicted and experimental values

of fermentation temperature and time, there were not significant differences ($p > 0.05$) in the TPC of all the sourdoughs. This indicates that a high TPC value will be obtained at the optimum fermentation temperature (33°C) and time (12.5 hours). The trend of the TPC contents of the rice bran sourdoughs is in congruent with the previous findings on rye bran [5]. A higher TPC value obtained at a higher fermentation temperature and time could be due to the fermentation in liberating and synthesising bioactive compounds, such as phenolic compounds in cereal brans [5, 34, 35].

Volatile organic compounds (VOCs) of rice bran sourdough. The list of the volatile compounds detected in rice bran sourdough fermented at different temperature and time is presented in Table 6. It includes only those detected in more than one sample. Volatile analysis of the ten (10) rice bran sourdough samples (Table 2, A-J) showed the presence of 47 compounds. They were grouped into different chemical classes as esters (15), alcohols (7), acids (6), alkanes (5), pyridine (1), siloxane derivatives (3), aldehydes (2), phenol (1), furan (1), aromatic hydrocarbon (1), ketose (1), ketone (1), nitriles (1), and others (2). The VOCs from the rice bran sourdough could be generated from enzymatic and microbial processes during sourdough fermentation (e.g., acids, alcohol, esters, ketones, and aldehydes), lipid oxidation (e.g., aldehydes, and ketones), and Maillard and caramelisation reactions (e.g., furans, aldehydes, ketones, and pyridines), or from other compounds present in the flour [15].

Rice bran sourdough fermented at a higher temperature (> 27°C) for a longer time (> 8 hours) produced the largest number of VOCs (Table 6). Furthermore, the amount of volatile compounds released from *L. plantarum* fermented rice bran sourdough (A-I) was more than the VOCs from naturally fermented rice bran sourdough (J). The most abundant alcohol among the VOCs was ethanol, a primary product of fermentation, followed by 2-Methoxy-4-vinylphenol. 2-Methoxy-4-vinylphenol (spicy) is an aromatic substance used as a flavouring agent [15, 36]. Ethanol production increased with the rise of fermentation temperature and time (Table 6). This could be because the longer fermentation time is enough for the microbial strain to degrade simple sugars in the sourdough into carbon-dioxide and ethanol [37]. Heterofermentative LAB has been reported to produce a high amount of ethanol and carbon-dioxide during rye sourdough fermentation [7], thus, *L. plantarum* (a facultatively heterofermentative LAB) [38] was probably responsible for increased ethanol and CO_2 production during rice bran sourdough fermentation. Ethanol has been cited as a specific volatile compound in a wheat sourdough bread [15]. The highest amount of ethanol and 2-Methoxy-4-vinylphenol was detected in sourdough fermented at 35°C for 13h (sample I). This is an indication that rice bran sourdough can be a good alternative to wheat sourdough for bread production.

Estra-1,3,5(10)-trien-17á-ol, 2,3-Butanediol, 3-Ethyl-2-pentanol, 2-methyl-1-Hexadecanol, and 1-Nitro-2-acetamido-1,2-dideoxy-d-glucitol were other alcohols detected in the rice bran sourdoughs. Estra-1,3,5(10)-trien-17á-ol was present in all the *L. plantarum* fermented sourdough, but not found in the naturally fermented rice-bran sourdough (Table 6). This indicates that Estra-1,3,5(10)-trien-17á-ol is one of the main volatile compounds specific in *L. plantarum* fermented rice bran sourdoughs. This compound was also reported to be one of the main volatile compounds in moringa leave extract [40].

The presence of esters in sourdough and bread is an indicator of pleasant fruity aromas [39]. Esters in rice bran sourdough may be derived from products of free fatty acids reaction with some alcohols [41]. Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, was the most abundant ester in rice bran sourdough, and its highest quantity was detected in the sample fermented at 35°C for 13 hours (I). Higher quantities of this ester were also found in sample F and G, which were fermented for more than 17 hours, indicating that the longer fermentation time favours the formation of the esters. According to the literature, the LAB fermentation requires more than 12 hours to produce a sufficient amount of volatiles [15]. The higher concentration of the esters in sample I, compared to sample F and G, could be, because sample I was fermented at 35°C for 13 hours, that was close to the optimum fermentation conditions (33°C for 12.5 hours) for rice bran sourdough production. Other esters found in the sourdough were 9-Octadecenoic acid, (2-phenyl-1,3-ioxolan-4-y l) methyl ester, Methoxyacetic acid, 3-tridecyl ester, and Pentanoic acid, 4-methyl-ethyl ester. These esters were also detected at the relatively high amount in the sourdoughs. 9-Octadecenoic acid, (2-phenyl-1,3-ioxolan-4-y l) methyl ester, was also reported in buckwheat sprout [42].

Seven (7) esters were detected in two (2) samples (H and I) fermented at a high temperature for a short

Table 5. Total phenolic compounds (TPC) in rice bran sourdoughs fermented with *L. plantarum* at different temperature and time

Fermentation temperature, °C	Fermentation time, h	TPC, μg GAE/g
22.2	8.05	410.15[a] ± 19.75
32.8	8.05	472.73[a] ± 79.54
22.2	17.95	434.00[a] ± 9.04
32.8	17.95	484.86[a] ± 34.96
20.0	13.00	419.31[a] ± 37.50
35.0	13.00	473.92[a] ± 57.22
27.5	6.00	440.98[a] ± 59.70
27.5	20.00	454.20[a] ± 32.80
27.5	13	445.01[a] ± 25.61

Note: Values are mean ± standard deviations of triplicate analyses. Values in the same column with the same superscript letter are not significantly different ($p>0.05$)

time (32.8°C, 8 hours; sample H) and a longer time (35°C, 13 hours; sample I). Although, rice bran sourdough fermented at the lowest temperature for a short time (20°C, 8 h; sample A) had the largest number of esters (8); the quantity of Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, was the least in this sample. This indicates that high fermentation temperature and a long time favour the production of a large amount of esters in sourdough. However, there was variation in the type and quantity of esters present in all the rice bran sourdough samples.

A long fermentation time and a high temperature also favour the production of acids in the rice bran sourdoughs (Table 6). This observation is in conformity with the result of the sourdough acidity (Fig. 1), where sourdough sample fermented at 35°C for 13 hours had the highest acidity level. Sourdough acids have been reported to serve as a bio-preservative [39]. This is probably why *L. plantarum* fermented rice bran sourdough taftoon bread had a higher anti-mould activity than the doughs fermented by *L. Casei*, and *L. acidophilus* [13]. Rice bran sourdough fermented with *L. plantarum* at a higher temperature (> 32°C) for a longer time (> 12 h) (G and I) had the largest number of volatile acids. Thus, these sourdoughs can extend a bread shelf-life and improve its flavour [43, 44].

Lactic and acetic acids are important for the flavour and taste of sourdough bread [45]. Acetic acid is the most common volatile acids reported to be present in sourdoughs [46, 47]. This acid was detected in a large quantity (143 μg/kg) in rice bran sourdough fermented at 35°C for 13 hours (I), but not detected in the sourdough samples fermented at < 27°C for a short or long time. Even though LAB was used for sourdough fermentation, lactic acid was not detected in any of the rice bran sourdough samples.

Low levels of ketone were detected in the naturally fermented sourdough, compared to the *L. plantarum* fermented sourdough. The presence of ketone (acetoin) in the sourdough could be due to a long fermentation period. As shown in Table 6, the longer the fermentation time, the higher the amount of acetoin in the sourdoughs. Mild heating of flour for a long period under oxidising conditions has been reported to induce lipid peroxidation, consequently resulting in a lipid degradation to aldehydes and ketones [31]. The sourdough sample fermented at 32.8°C for 17:95 hours (G) had the higher acetoin value (146 μg/kg), than the sourdough fermented at 35°C for 13 hours (I). Acetoin was also reported to be present in a whole wheat sourdough [39] and chestnut-flour-based-sourdoughs [30]. Since acetoin is characterised with a butter, or popcorn like aroma [46, 47], the presence of acetoin in rice bran sourdough produced by fermentation of rice bran at a high temperature for a long time is an indicator, that the sourdough will add a special good flavour to bread, and retain bread freshness for a long time. The obtained results confirm the facts already known in the scientific literature [39].

Aldehydes are related to oxidative status of foods, characterised as off-odours in bread [46, 47]. The presence of aldehydes (hexanal and 3-benzyloxy-2-fluoro-4-methoxy- Benzaldehyde) in the rice bran sourdoughs could be due to the activities of lipid hydrolysing enzymes, such as lipases, and peroxidases present in bran [48]. The detection of linear chain aldehyde (hexanal) could be due to degradative oxidation of unsaturated fatty acids, such as oleic, linoleic, and linolenic acids [31], in which rice bran is rich [1]. Although the concentration of aldehydes in all the samples was low, a relatively high concentration of hexanal in samples F, G, I, and J, compared to other samples, could be due to the susceptibility of the samples to oxidative rancidity. Non detection of hexanal in samples A, B, and C could probably be explained by the activities of lipase, reduced at the temperature of 20–22.2°C. The storage of bran at a low temperature has been reported to hinder the activity of lipases [49].

Phenol (2-methoxy-Phenol) is one of the compounds responsible for the aroma of buckwheat [50]. It was present in all the sourdoughs, except those fermented at a low temperature (20–27.5°C) for a short and a long time (6–13 h) (A, C, and D). 2-methoxy-Phenol is formed during the thermal degradation of lignin under the pyrolysis condition or thermal degradation of barks [51]. This cannot be the case of this study, since the rice bran, or sourdough was not subjected to a high temperature. However, the contamination of rice bran during drying or dehusking of rice paddy could be responsible for the presence of the compound in the sourdoughs.

Furan (2-pentyl- Furan), characterised with a fruity odour [15], was detected only in the samples fermented at a low temperature for a long time (B and C). Although formation of heterocyclic compounds, such as furans were attributed to the thermal degradation and rearrangement of carbohydrate through Maillard reaction [31, 52], this may not be the case of this study. Since the headspace incubation temperature was 60°C, and Maillard reaction products are produced at a temperature between 80 to 120°C [42, 53]. The presence of 2-pentyl-Furan in rye sourdough and in wheat bread without sourdough has been reported [7, 47, 54]. 6-methyl-Octadecane was the only alkane found in all the sourdough samples, even though in a varying concentration. All other alkanes vary from one sample to another in terms of their detection and quantity. Aromatic hydrocarbon (toluene), ketose (l-Gala-l-ido-octose), and nitrile (Phenyl-α-D-glucoside) were not detected in a naturally fermented sample, but were detected at low concentrations in less than six (6) *L. plantarum* fermented sourdoughs. Nitrile was found in low amount in samples A, B, F, and H, but not detected in other sourdough samples, whereas pyridine (piperazine) was not found in samples A, B, C, and E, but was detected in other sourdoughs. Most of the siloxane derivatives found in the sourdough were detected in the samples fermented at a low temperature (A, B, C, and D), except for Cycloheptasiloxanetetradecamethyl, which was found in a low concentration in sample I. The 3 siloxane derivatives found in the rice bran sourdoughs are among the 4 main volatile compounds found in jasmine flower [55]. l-Gala-l-ido-octose was found only in two samples (F and G), while 2-pro-

Table 6. Volatile compounds detected in rice bran sourdoughs fermented at different temperature and time

Compunds	RI	A	B	C	D	E	F	G	H	I	J
Ethanol	447	82.9 ± 2.4	159.3 ± 9.1	71.4 ± 3.2	89.7 ± 1.2	150 ± 9.2	175.5 ± 3.2	213.7 ± 11.1	136.2 ± 11.1	539.2 ± 18.1	181.7 ± 9.3
2,3-Butanediol	827	nd	54.8 ± 1.5	nd	nd	168.1 ± 4.8	251.7 ± 11.6	82.5 ± 1.6	nd	20.7 ± 1.2	79.4 ± 3.3
3-Ethyl-2-pentanol	815	nd	nd	25.9 ± 1.0	5.4 ± 0.1	nd	nd	nd	nd	nd	nd
Estra-1,3,5(10)-trien-17á-ol	1,946	84.7 ± 0.9	52.5 ± 1.9	22.2 ± 0.6	5.2 ± 0.4	4.1 ± 0.2	42.1 ± 1.2	31.8 ± 0.4	26.2 ± 0.5	31.7 ± 0.9	nd
2-methyl-1-Hexadecanol	1,893	11.32 ± 0.1	7.3 ± 0.4	11.2 ± 0.6	nd	nd	10.4 ± 0.9	11.2 ± 0.6	27.1 ± 1.1	32.1 ± 0.9	12.2 ± 2.6
1-Nitro-2-acetamido-1,2-d ideoxy-d-glucitol	2,268	0.9 ± 0.1	nd	4.3 ± 0.5	7.0 ± 0.2	4.1 ± 0.1	nd	14.5 ± 8.1	3.9 ± 0.1	16.3 ± 0.8	17.3 ± 1.3
4-vinyl-2-Methoxyphenol	1,299	109.9 ± 4.5	340.3 ± 0.8	324.6 ± 2.1	117.6 ± 1.2	124.2 ± 5.4	213.3 ± 6.5	338.7 ± 7.5	333.6 ± 12.1	463.7 ± 8.9	410.9 ± 13.7
7-Methyl-Z-tetradecen-1-ol acetate	1,825	9.0 ± 1.1	nd	nd	nd	nd	nd	nd	37.7 ± 3.2	24.3 ± 7.4	nd
[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-methyl ester	2,206	13.7 ± 0.5	6.09 ± 0.1	nd	10.0 ± 0.4	nd	nd	nd	nd	nd	6.7 ± 0.2
Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	5,435	135.4 ± 3.2	188.6 ± 17.3	139.4 ± 7.8	191.0 ± 1.1	196.8 ± 15.1	204.6 ± 8.2	218.8 ± 13.0	192.7 ± 11.4	229.7 ± 13.1	170.6 ± 15.1
N-Benzyl-2-aminocinnam ate, methyl ester	2,256	16.61 ± 0.8	nd	5.7 ± 0.1	nd	nd	nd	12.9 ± 0.6	nd	nd	nd
N,N'-Bis(Carbobenzyloxy)-lysine methyl(ester)	3,427	nd	nd	nd	nd	nd	7.5 ± 0.3	8.3 ± 0.5	nd	nd	nd
Phthalic acid, ethyl pentadecyl ester	2,932	nd	nd	29.4 ± 1.1	nd	nd	20.2 ± 1.4	nd	14.6 ± 0.8	nd	nd
Methoxyacetic acid,2-tetradecyl ester	1,893	10.1 ± 0.7	nd	nd	nd	nd	nd	29.1 ± 0.9	nd	8.9 ± 0.7	nd
Methoxyacetic acid,3-tridecyl ester	1,786	39.79 ± 1.3	24.9 ± 0.4	nd	nd	nd	17.5 ± 1.4	nd	23.9 ± 1.7	12.2 ± 1.3	36.2 ± 3.1
Methoxyacetic acid,2-tridecyl ester	1,798	nd	nd	nd	nd	nd	23.4 ± 0.3	nd	10.9 ± 1.2	11.3 ± 1.0	nd
Cyclopropane, tetradecanoic acid, 2-octyl-methyl ester	2,735	14.5 ± 0.2	nd	nd	nd	nd	9.9 ± 0.6	nd	3.9 ± 0.4	nd	nd
9-Octadecenoic acid, (2-phenyl-1,3-ioxolan-4-y l)methyl ester	3,316	25.45 ± 1.4	6.3 ± 0.7	9.4 ± 0.1	nd	15.3 ± 0.7	8.2 ± 0.5	6.4 ± 0.1	nd	13.3 ± 0.6	9.6 ± 0.7
9-Octadecenoic acid (Z)-hexyl ester	2,580	nd	nd	nd	nd	4.7 ± 0.1	nd	nd	nd	19.1 ± 0.8	7.5 ± 0.6
Pentanoic acid, 4-methyl-ethyl ester	945	nd	nd	nd	nd	13.7 ± 0.3	41.7 ± 1.1	35.5 ± 4.5	nd	nd	30.2 ± 3.8
Geranyl isovalerate	1,579	nd	nd	11.2 ± 0.9	nd	nd	8.8 ± 0.2	nd	6.7 ± 0.9	nd	nd
Ethyl acetoacetate	905	nd	nd	nd	3.2 ± 0.3	4.2 ± 0.1	nd	nd	nd	nd	nd
Acetic acid	581	nd	nd	nd	nd	113.1 ± 7.7	129.1 ± 8.9	138.1 ± 9.1	122.8 ± 1.5	143.1 ± 9.1	33.3 ± 12.5
(Z)-2-Hydroxyimino-3-oxo butyric acid	1,363	nd	nd	26.4 ± 0.1	nd	nd	nd	nd	486.7 ± 9.5	nd	nd
Phosphonic acid, (p-hydroxyphenyl)	1,592*	nd	nd	nd	nd	15.1 ± 0.4	22.8 ± 0.7	40.3 ± 3.7	28.9 ± 0.5	26.9 ± 1.7	40.5 ± 1.2
Ammonium acetate	636	43.46 ± 0.5	nd	nd	nd	175.8 ± 3.8	256.2 ± 5.1	nd	25.9 ± 0.7	174.1 ± 14.3	223.37 ± 11.2
3-hydroxy-Dodecanoic acid	1,731	nd	nd	nd	nd	nd	nd	nd	9.7 ± 0.5	8.4 ± 0.7	nd
Ethyl acetoacetate	925	nd	nd	nd	3.2 ± 0.3	4.2 ± 0.1	nd	nd	nd	nd	nd
Acetoin	664	nd	27.9 ± 1.6	nd	nd	40.3 ± 3.2	71.9 ± 11.0	146.4 ± 11.5	104.9 ± 10.4	140.0 ± 4.6	20.8 ± 1.4
Hexanal	769	nd	nd	nd	6.9 ± 0.4	8.7 ± 0.5	12.5 ± 1.0	18.6 ± 1.7	6.8 ± 0.5	10.1 ± 0.9	13.0 ± 0.9
3-benzyloxy-2-fluoro-4-methoxy- Benzaldehyde	2,010	nd	nd	nd	2.2 ± 0.0	nd	nd	nd	2.9 ± 0.3	nd	nd
2-methoxy-Phenol	1,056	nd	17.21 ± 0.6	nd	nd	13.2 ± 1.3	33.5 ± 0.3	88.2 ± 3.1	33.3 ± 0.1	111.5 ± 7.7	107.8 ± 14.0
2-pentyl- Furan	981	nd	12.3 ± 0.3	11.9 ± 0.8	nd	nd	nd	nd	nd	nd	nd
Octadecane,1,1'-[1,3-propanediylbis(oxy)]bis	4,047	nd	nd	nd	nd	nd	5.1 ± 0.3	7.1 ± 0.4	nd	nd	nd
6-methyl- Octadecane	1,840	11.5 ± 0.3	9.6 ± 0.5	14.9 ± 0.2	3.9 ± 0.8	7.5 ± 0.3	7.9 ± 0.8	28.2 ± 0.7	19.9 ± 1.2	30.2 ± 1.1	27.9 ± 1.1
2,6,10-trimethyl-tetradecane	1,559	6.9 ± 0.1	nd	10.32 ± 0.8	nd	nd	nd	26.1 ± 1.3	nd	nd	nd
2-trifluoroacetoxytridecane	1,545	nd	18.5 ± 2.1	nd	nd	nd	nd	nd	5.8 ± 0.3	6.9 ± 0.2	8.7 ± 0.2
3-trifluoroacetoxypentadecane	1,648	nd	12.6 ± 1.9	7.6 ± 0.6	3.1 ± 0.5	nd	nd	8.6 ± 0.3	nd	7.0 ± 0.3	8.1 ± 0.1
Toluene	754	nd	73.5 ± 9.4	42.6 ± 2.8	5.9 ± 0.1	nd	nd	nd	115.0 ± 13.1	15 ± 0.6	nd
l-Gala-l-ido-octose	2,223	nd	nd	nd	nd	nd	5.7 ± 0.2	8.8 ± 0.3	nd	nd	nd
Phenyl-α-D-glucoside	2,286*	15.3 ± 0.6	19.7 ± 1.3	nd	nd	nd	26.4 ± 1.1	nd	9.8±0.4	nd	nd
Piperazine	855	nd	nd	nd	15.3 ± 0.8	nd	142.6 ± 2.3	281.2 ± 11.8	61.7 ± 0.9	437.7 ± 12.7	304.7 ± 12.3
Cyclohexasiloxane, dodecamethyl	1,348	10.1 ± 0.5	6.9 ± 1.1	6.6 ± 0.9	4.0 ± 0.2	nd	nd	nd	nd	nd	nd
Cycloheptasiloxane,tetradecamethyl	1,442	35.8 ± 1.7	12.6 ± 0.3	35.1 ± 1.5	11.2 ± 0.9	nd	nd	nd	nd	6.9 ± 0.7	nd
Cyclooctasiloxane,hexadecamethyl	1,657	16.46 ± 0.8	12.1 ± 0.7	15.0 ± 0.9	8.9 ± 0.3	nd	nd	nd	nd	nd	nd
2-propenylidene- Cyclobutene	737	26.2 ± 0.1	43.2 ± 0.	33.3 ± 0.7	21.96 ± 0.0	48.3 ± 0.7	170.8 ± 11.9	147.6 ± 5.1	20.1 ± 1.4	57.0 ± 6.8	29.1 ± 0.8
Hydrazinecarboxamide	795	257.5 ± 4.8	400.8 ± 4.2	105.3 ± 7.2	134.3 ± 9.2	170.8 ± 12.2	301.3 ± 10.5	467.3 ± 11.4	164.7 ± 1.5	586.0 ± 15.2	329.1 ± 10.8

Note: Values are mean ± standard deviation (μg/kg) (n = 3). RI (Retention index): Identification by comparison with RI database. A–J: are as defined in Table 2. * indicates RI values not reported in the library for matching. nd: not detected

penylidene-Cyclobutene was detected in all the sourdough samples, except sample B. Hydrazinecarboxamide was found in a high concentration in all the sourdoughs.

Generally, it was observed that rice bran sourdough samples fermentation at the higher temperature and time had the higher concentration of 4-vinyl-2-Methoxyphenol, ethanol, 2-methoxy- Phenol, acetoin, and piperazine. However, an opposite trend was observed in the case of siloxane derivatives and aldehydes, where there was low or no detection of the compounds, as fermentation temperature and time increased (Table 6). Meanwhile, fluctuating levels of alkanes, aromatic hydrocarbon, nitrile, 2-propenylidene-cyclobutene, esters, and alcohols (apart from 4-vinyl-2-Methoxyphenol, and ethanol) were detected in the rice bran sourdoughs. These observations were in agreement with the findings of [39], who reported the reduction in percent peak area of aldehydes and fluctuating levels of alcohols, esters, and acids in a whole wheat sourdough fermented for a long time.

According to the results of this study, sourdough fermented at 35°C for 13 hours (sample I) contained the largest amount (27) of aroma compounds, compared to other *L. plantarum* fermented sourdough, and eight (8) compounds more than the naturally fermented sourdough (sample J). Although sample I and sample J were fermented at the same temperature and time (35°C for 13 hours), the following VOCs were found in sample I: estra-1,3,5(10)-trien-17á-ol, 2-methyl-1-Hexadecanol, 3-hydroxy-dodecanoic acid, 7-methyl-z-tetradecen-1-ol acetate, methoxyaceticacid 2-tetradecyl ester, methoxyacetic acid 2-tridecyl ester, cycloheptasiloxanetetradecamethyl, and toluene. However, these VOCs were not detected in the naturally fermented sourdough (J).

Acetic acid and ethanol are among the most cited volatile organic compounds found in bread crust [47], crumb [46], and wheat bread without sourdough [15] and with sourdough [39]. Sample I had the highest concentration of acetic acids (143 µg/kg), ethanol (539 µg/kg), 2-Methoxy-4-vinylphenol (463 µg/kg), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (230 µg/kg), and 2-methoxy- Phenol (112 µg/kg) compared to all other sourdough samples. Sample I is also high in the aroma compound acetoin (140 µg/kg). The higher VOCs in sample I could be due to the fermentation conditions (33°C, 13 h), which are close to the optimum fermentation conditions for rice bran sourdough production.These characteristics of sample I indicate that the sourdough will produce bread with high consumer acceptability, extended freshness, and shelf-life.

CONCLUSION

The results of this study demonstrate that fermentation temperature and time have a significant effect on the quality parameters of rice bran sourdough. Acidity, total phenolic, and volatile compounds of rice bran sourdough increased, as fermentation temperature and time increased. The optimum fermentation conditions for *L. plantarum* fermented rice bran sourdough were achieved at 33°C for 12.5 hours. These conditions resulted in pH value of 4.3 and TTA value of 19.5 ml of 0.1M NaOH, which was not significantly different from the RSM predicted pH (4.2), and TTA (19.1ml of 0.1M NaOH) values. Fermentation temperature and time do not have a significant effect on the total phenolic content of the sourdough. Forty-seven (47) VOCs were detected in the rice bran sourdoughs. As fermentation temperature and time increased, the concentration of ethanol, phenols, ketone, pyridine, and acids increased in the rice bran sourdoughs, while aldehydes and siloxane derivatives levels decreased. The major volatile compounds in the sourdough were acetic acids, ethanol, 2-Methoxy-4-vinylphenol, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, acetoin, and 2-methoxy-Phenol. There were striking differences between the volatile composition of *L. plantarum* fermented rice bransourdoughsandthenaturallyfermentedsourdough.Differences also existed in the sourdough fermented with *L. plantarum* at a high temperature and time and those fermented at a low temperature and time. Former had the higher concentration of volatile aroma compounds than the latter. The information provided in this study can be used in bakery industry for producing quality shelf-stable rice bran sourdough bread with attractive aromas.

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Phytochemical screening and nutraceutical potential of sandbox tree (*Hura crepitans* L.) seed oil

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Abstract: The aim of this research was to assess the nutraceutical potential of *Hura crepitans* seed oil in food chemistry. For that, we determined the phytochemical composition, physicochemical parameters, mineral composition, as well as proximates of the oil under study. The yield of the oil obtained by the Soxhlet method was 47.8 ± 0.2%. According to the phytochemical screening result, the oil contained saponin, alkaloid, terpenoids, steroids, and cardenolides. Quantitative analysis of proximates for the *Hura crepitans* seed oil displayed 10.1 ± 0.4% of protein, 19.4 ± 0.1% of crude fibres, 14.5 ± 0.5% of carbohydrates, 5.3 ± 0.2% of water, and 2.9 ± 0.1% of ash. Some selected physicochemical parameters such as refractive index, pH, and specific gravity were 1.47; 5.2; and 0.80, respectively. Cloud point was 6.20°C. Metal content determination revealed the presence of mineral substances such as magnesium (119.51 ± 0.25 mg/kg), potassium (4.25 ± 0.04 ppm), iron (4.01 ± 0.03 mg/kg), manganese (3.66 ± 0.02 ppm), sodium (2.18 ± 0.02 ppm), calcium (0.11 ± 0.001 ppm), zinc (0.04 ± 0.001 µg/g), and cadmium (0.0028 ± 0.0004 mg/kg). However, such minerals as nickel and lead were not detected. The structural analyse was carried out based on physicochemical properties and spectroscopic data of the *Hura crepitans* seed oil. The results of the research proved the nutraceutical potential of this oil to use as raw materials in various areas of industry.

Keywords: Fatty acid, secondary metabolites, Soxhlet, *Hura crepitans*, proximate test

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INTRODUCTION

Recently, the use of plant oils in various industries has been consistently grown [1]. *Hura crepitans* seed oil is derived from a class of tree commonly known as the sandbox tree [2]. It originates from the tropical regions of North and South America and maintains its foliage throughout the year. It is also referred to as possumwood and jabillo aside sandbox tree in the earlier identified nomenclature [3].

Hura crepitans has can grow up to 30 m (100 ft) tall. It has large ovate leaves which can growth up to 2 ft wide in nature [4]. It is a typical rain forest tree with seed rich in oil [5, 6]. It is a prominent and salient medicinal plant widely used in sub-Saharan Africa [7]. *H. crepitans* seed oil has been reported to be essential raw material in biodiesel production [8]. It is also commonly called 'Odan Mecca' in Kabba part of the Kogi State and 'Aroyin' among Ilesha indigenes of the Osun State, both in the western region of Nigeria [9].

The woody fruits have resemblance with small pumpkin pods, with about thirteen seeds in each pod. This plant is used for the treatment of dermatitis, gastrointestinal disorders, and respiratory infections [10]. Oil obtained has been recognized to play the purgatory function. 'Hurin' is a non-color secretion from *Hura crepitans* which has been established by medicinal plant scientists as panacea for elephantiasis and leprosy [11].

Physiological and phenological reports [12] discovered this plant as edible food with diverse nutraceutical potentials aside utilization in biodiesel manufacturing [13]. Thus, this study deals with the evaluation of the seed oil of *H. crepitans* grown in the premises of the Covenant University, Nigeria. We analysed phytochemical compounds, physicochemical properties, as well as proximate and metal content compositions of the oil.

STUDY OBJECTS AND METHODS

Materials and measurements. The chemicals used

were manufactured and supplied by Sigma-Aldrich Chemicals, USA. They included acetone, bromine water, chloroform, ethanol, conc. HCl, conc. sulfuric acid, *n*-hexane, magnesium ribbon, $CuSO_4$, and NaOH. The IR and UV analyses were carried out with a PMA-50 Bruker FT-IR spectrophotometer, manufactured by Bruker Optik GmbH, Germany, and a 840-208200 Genesys 10s uv-visible spectrophotometer, manufactured by Thermofisher Scientific, USA. A LI-AREF-186 refractometer, manufactured by LABARD Instruchem Private Limited, India, was applied to determine refractive index. All temperature measurements were carried out with Stuart SMP10 melting point apparatus supplied by Cole Parmer Company, Staffordshire, UK. A Buck scientific 210VGP atomic absorption spectrophotometer was utilized to quantitatively analyse the digested sample for metal content determination. A Perkin-Elmer 241 polarimeter (PerkinElmer Incorporation, California, USA) was used for optical rotation determination. Moisture content was determined by a RS232 DSH moisture analyser (W&J Instrument Company, China). The ^1H (400 MHz) and ^{13}C (100 MHz) NMR analysis were determined in $CDCl_3$ with the help of a JEOL Delta NMR ECX 400 spectrometer (California, USA). The chemical shift values (δ_H and δ_c) were measured in part per million. When the oil extraction procedure finished, the *n*-hexane was evaporated with the help of a 0010004799 IKA® RV 10 Rotary evaporator (IKA-Werke GmbH and Co KG, Germany) to obtain the desired oil for further analysis.

Seed sample collection. Seeds of *Hura crepitans* were harvested in the early hours (from 7 a.m. to 9 a.m.) of the 15th May, 2017 on Covenant University campus, Nigeria, at 27 ± 2°C. From sandbox trees which were about eight years old, the matured and healthy fruits were randomly picked and thoroughly mixed together. These were then kept in a dark polyethylene bag and transported immediately to the laboratory for further processing. The plant species were identified and authenticated at the Department of Biological Sciences of the Covenant University.

Extraction and isolation. Extraction of the oil from the seed of *Hura crepitans* was done at Covenant University, Ota, Ogun State, Nigeria using the standard method [14, 15]. The dried seeds of *Hura crepitans* were thoroughly ground into powder to increase the extraction efficiency. 2 g of milled sample was weighed and carefully packed in filter paper and mounted on Soxhlet set-up and extracted with *n*-hexane for 6 hours. After that, we removed *n*-hexane from the mixture to ensure yellow oil, with yield of 47.79%.

Spectral data for the seed oil sample

IR (KBr): 2,974; 2,822; 2,485; 1,750; 1,600; and 1,038 cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ: 5.35–5.30 (m, 5H), 4.31–4.26 (dd, J_1 = 4.28 Hz, J_2 = 8.00 Hz, 1H), 4.16–4.11 (dd, J_1 = 5.96 Hz, J_2 = 8.00 Hz, 1H), 2.79–2.74 (t, J = 12.72 Hz, 2H), 2.34–2.31 (m, 4H, 2·CH_2), 2.03–1.99 (m, 6H), 1.60 (s, 3H), 1.29–1.24 (d, J = 12.72 Hz, 32H), 0.88 (m, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ: 173.30; 172.81; 130.32; 130.11; 127.81; 68.80; 62.20; 34.11; 32.01; 31.62 (3·CH_2); 29.80 (2·CH_2); 29.40 (2·CH_2); 29.20 (2·CH_2); 27.30 (3·CH_2); 25.70 (3·CH_2); 24.90 (CH_2); 22.70 (CH_3); 14.21 (CH_3); 13.50 (CH_3) ppm;

MS: in m/z (rel. %): 508.97 (M^+, 34.5%), 466.95 (100%), 354.40 (10.1%), 275.00 (22.5%), and 270.03 (35.2%).

Procedure for phytochemical test. Phytochemical screening was conducted using the standard procedure that we described earlier to reveal the secondary metabolites in *Hura crepitans* sample [16]. According to the result, the phytoconstituents in the sample were saponins, steroids, alkaloids, terpenoids, and cardenolides.

Test for terpenoids. 2 ml of chloroform was added to 5 ml the oil aliquot and stirred thoroughly. It was evaporated on the water bath and then heated up in the presence of 3 ml of concentrated H_2SO_4. Grey colour confirmed the presence of terpenoids.

Test for steroids. 2 ml of chloroform and 3 ml of concentrated H_2SO_4 were added to 5 ml of the oil. The appearance of red coloration within the lower chloroform portion depicted the presence of steroids.

Tannins testing. 10.00 ml of bromine water was cautiously added into 0.5 g of the oil. Decolorization of Br/H_2O was a clear indication of the availability of tannins.

Saponins testing. 5.00 ml of distilled water and the oil aliquot were mixed vigorously in a test tube. The frothing was mixed with few drops of olive oil and mixed vigorously. Foam formation indicated the presence of saponins.

Flavonoids testing. Shinoda test was used to determine flavonoids. Several drops of concentrated HCl were added to the aliquot sample. Then magnesium ribbon turnings were slipped into the solution. The observation of pink coloration implied the presence of flavonoids. This was also confirmed by alkaline reagent test.

Test for alkaloids. An aliquot of the sample was spotted on a precoated thin layer chromatographic plate. The plate was sprayed with the Dragendorf reagent. The appearance of orange spot depicted the presence of alkaloids.

Test for steroid. 2 mg of ground seed sample was agitated in $CHCl_3$. A solution of H_2SO_4 was carefully added through the test-tube wall to the $CHCl_3$ layer; then sulphuric acid was added slowly by the sides of test tube. The appearance of red colour implied the presence of steroids.

Proximate analysis procedure. The nitrogen content was determined by the Micro-Kjeldahl technique, while multiplication of N (%) by conversion factor (6.25) afforded crude protein [16]. Moisture content and crude fibres were determined using standard methods [16, 17].

Determination of total carbohydrate. The determination of carbohydrate percentage of the oil sample was ascertained by another approach. The technique involved the taking of sum-total of crude protein, lipid, crude fibres, moisture, and ash from 100 [16].

Qualitative analysis. We used the Biuret test to determine protein content. 6% NaOH solution and few drops of 1% $CuSO_4$ were added to the test solution in a sequential manner. Appearance of violet color in inference indicated the presence of protein is present [18].

In order to determine carbohydrate content, the Molisch's test was used**.** Several drops of α-naphthol in alcohol were added into the extracted oil sample. This was

followed by the addition of concentrated H_2SO_4 (1 ml) in a slowly and drop wisely manner through the side wall of the test-tube. Appearance of purple coloration that turned to violet in form of a ring at the junction of the resulting mixture depicted the presence of carbohydrates [19].

Determination of Total Ash. 5 g of the sample was weighed into a crucible which was previously dried to constant weight prior to use. The content was first heated gently over a low flame until charred, and transferred into a muffle furnace at 550°C until a white ash was formed. A standard procedure was adopted for both the ashing and calculation [11].

Mineral Content Analysis. Acid digestion method was used to prepare a solution of the sample in an acceptable format for the minerals content analysis according to a known procedure [15]. About 2 g of the seed samples were weighed and digested using double acid digestion with perchloric acid/nitric acid in ratio 1:1 using hot plate in the fume hood. When fume is invisible, the filtrate obtained was transferred into a 50 ml volumetric flask and prepared with distilled water prior to use. The solution was then analyzed for metals/elements such as Ca, Mg, Zn, Fe, Cd, Co, Pb, Ni, and Mn using AAS. Selective determination of K and Na was carried out with a PFP7 flame photometer (Jenway, UK).

RESULTS AND DISCUSSION

Spectroscopic characterization. Since seed oils find a wide application to different industries, seeds *Hura crepitans* are of great interest in present research. In the course of the study into the seed oil from African shore, we have herein evaluated the valuable parameters and salient compositions of *Hura crepitans* seed oil [15, 16].

Physiological and phenological analyses revealed that during germination of oil seeds, the utilization of the storage fat was initiated by the stepwise hydrolysis of triacylglycerol to free fatty acids and glycerol [12]. The structural characteristics of the extracted seed oil of *Hura crepitans* was validated using spectroscopic means. The FT-IR spectral data of the compound was run from 4,000 to 400 cm^{-1} but showed no noticeable band above 3,000 cm^{-1}. This implied that there was no N-H band and no OH band either free or hydrogen-bonded.

The highest absorption band was recorded at 2,974 cm^{-1} which was due to appearance of C-H of aliphatic followed by the second C-H aliphatic at the stretching vibrational frequency at 2,822 cm^{-1}. The C = O of ester was noticed at 1,750 cm^{-1} and it was doubly established with C-O of alkoxyl functional at 1038 cm^{-1}. The stretching vibration at 1,600 cm^{-1} was ascribed to the C = C functional moieties of alkene, and this value was in concordance with the literature value [14].

The ^1H NMR analysis of the compound was run in deuterated chloroform using TMS as internal standard. The most downfield signal away from TMS was recorded as five proton multiplet at δ_H 5.35–5.30, after which one proton dd at δ_H 4.31–4.26 ppm with *J* values of 4.28 Hz and 8.00 Hz was recorded. Another 1H doublets of doublet appeared at δ_H 4.16-4.11 with *J* values of 5.96 Hz and 8.00 Hz. The chemically equivalent sixteen methylene protons resonated upfield as 32 protons doublet at δ_H 1.29–1.24 ppm. The presence of three methyl groups was confirmed by two signals in the ^1H NMR spectrum as a 3H singlet (CH_3) at δ_H 1.60 ppm and as a highly shielded 6H ($2{\cdot}CH_3$) multiplet at δ_H 0.88 ppm, which agreed with the value for the highly shielded methyl group earlier reported [20].

Furthermore, the ^{13}C NMR analysis showed signals ranging from δ_C 173.3 ppm for C = O of ester to δ_C 13.5 ppm for CH_3 of alkyl group. In detail, the two C = O of ester appeared at δ_C 173.3 and 172.8 ppm while the carbon atoms of alkene resonated between 130.3 to 127.8 ppm. The two methine carbon atoms (CH) resonated at 68.8 ppm and 62.2 ppm while that of all the methylene carbon atoms (CH_2) appeared from 34.1 ppm to 24.9 ppm and three methyl carbon atoms (CH_3) were found at 22.7, 14.2 and 13.5 ppm.

The mass spectrometric analysis revealed the molecular ion and base peaks to be at m/z 508.97 and 466.95 with intensities of 34.5% and 100%, respectively. The molecular ion peak was in agreement with the theoretical value for molar mass of the compound, and the base peak had m/z ratio which resulted in loss of a propyl radical ($M - {\cdot}C_3H_7$). Other daughter peaks due to some fragmentation patterns were noticed at m/z of 354.4; 275.0; and 270.0 with relative peak heights of 10; 22.5; and 35.0%, respectively. The uv-visible spectrum of the targeted sample in *n*-hexane showed the presence of four peaks with the wavelength λ_{max} of 199 nm (log ε_{max} 5.2), 210 nm (log ε_{max} 4.9), 229 nm (log ε_{max} 4.8), and 277 nm (log ε_{max} 4.4) as shown in Table 1.

Phytochemical screening. In order to preview into potential bioactivity of the seed oil, the phytochemical screening was determined (Table 2). The phytochemical testing showed that cardenolides as well as saponins were present in low capacity, terpenoids and steroids in moderate capacity, and alkaloids and carbohydrates in high capacity. Saponins are glycosides with foaming ability [21]. Since saponins contain polycyclic aglycones which are attached to one or more sugar side chain, the presence of saponin in the *Hura crepitans* seed oil explained why it was rich in carbohydrates.

Table 1. UV-visible spectral data of *Hura crepitans* seed oil

	Peak 1	Peak 2	Peak 3	Peak 4
Wavelength λ_{max}, nm	199.0 ± 3.0	210.0 ± 5.0	229.0 ± 5.0	277.0 ± 4.0
Absorbance	1.70 ± 0.1	0.70 ± 0.1	0.58 ± 0.1	0.30 ± 0.1
Molar absorptivity ε_{max}, $M^{-1}.dm^{-1}$	167,000 ± 10	70,110 ± 7	58,000 ± 7	25,000 ± 4
Log of Molar absorptivity, log ε_{max}	5.2	4.9	4.8	4.4

Note: M = molar, nm = nanometer, dm = decimeter. Value is mean ± SD of triplicate measurements with n = 3

Table 2. Phytochemical compounds of *Hura crepitans*

Phyto-Constituent	Availability and intensity
Terpenoids	++
Tannins	–
Flavonoids	–
Phlabotannins	–
Phenols	–
Carbohydrates	+++
Steroids	++
Cardenolides	+
Oxalates	–
Alkaloids (Wagner technique)	+++
Alkaloids (Dragendorff technique)	+++
Saponins	+
Quinones	–

Note: +++ = high intensity; ++ = moderate intensity; + = low intensity; – = absent

Table 2 demonstrates phytochemical substances in the *Hura crepitans* seed oil. As one can see from Table 2, Wagner and Dragendorff methods revealed the presence of alkaloids in high intensity. Steroid hormones are responsible for many osmoregulatory functions of physiological factors in human. Hence, availability of these secondary metabolites in moderate intensity indicates that adequate intake of this oil will aid hormonal boosting in vertebrates. The result of the phytochemical analysis also showed the absence of secondary metabolites which include tannins, flavonoids, phenols, phlabotannins, oxalates, and quinones. Since oxalates and cyanogenic glycosides contribute immensely to the removal of important nutritional component, the oil has great nutraceutical potential due to the absence of oxalate and quinones [14].

Proximate determination. Having obtained the oil via Soxhlet extraction technique, proximate determination results showed that *Hura crepitans* is rich in oil with percentage yield of 47.79%, as shown in Table 3 [14]. Crude fat yield was higher than the value (36.4%) previously reported by Ezeh *et al.* [22], closely related to the value (47.80%) reported in [23], but lesser when compared with 53.81% yield in the previous work of Abdulkadir *et al.* [11]. Nevertheless, the oil yield of *Huran crepitans* in this study was much higher than the value documented for soybean oil (18%) and groundnut oil (43%) [24].

It is very crucial to have a deep understanding of the nature of constituents which furnish energy as well as enhance digestible conditioning in food prior to effective preparation of commercially quality diets [16]. Over three decades now, curative medicine and therapeutic prevention against cardiovascular disorder is linked with balance diet intake [25]. Low moisture content (5.3 ± 0.2%) was an affirmation of the tendency for long shelf-life [11]. The proximate determination results also revealed that *Hura crepitans* was rich in carbohydrates (14.5 ± 0.5%), crude fibres (19.4 ± 0.1%), and crude proteins (10.1 ± 0.4%). The ash content was 2.9 ± 0.1%, which contributed to the total organic matter content (91.8 ± 1.8%). This ash value was within the expected limit (various reports on the ash show it does not exceed 5% in fresh food [26, 27]).

Table 3. Proximate composition analysis of *Hura crepitans* seed oil

Proximate constituents	Value
Moisture content	5.3 ± 0.2%
Crude fibres	19.4 ± 0.1%
Carbohydrate	14.5 ± 0.5%
Crude fat	47.8 ± 0.2%
Crude protein	10.1 ± 0.4%
Ash content	2.9 ± 0.1%
Organic matter content	91.8 ± 1.8%

Note: values are mean ± SD for triplicate measurement

Njoku *et al*. investigated lipase properties of seed endosperm of *Hura crepitans* at the moment of germination. According to the results, its oil contained predominantly palmitic, stearic, oleic, and linoleic acids [12]. Idowu *et al*. reported that *Hura crepitans* contained oil and vitamins [28]. The high oil content in this research (47.8 ± 0.2%) implies the high level of essential vitamins.

The high carbohydrate content (14.5 ± 0.5%) ensured the valuable role of this substance for the enhancement of total well-being of man via the provision of energy for body metabolic processes [14]. The crude fibre content (19.4 ± 0.1%) will help in hasting digestion process [29]. Consistent and persistent intake of the recommended level of crude fibre helps the body to resist against numerous cardiovascular disorders [30].

The analysis also revealed that the crude protein was 10.1 ± 0.4% which implied that it was capable of ensuring the required cell division and growth to improve enzymatic action that is essential for hormonal balance in the body. We found that *Hura crepitans* seeds had remarkable efficiency as a means of boosting the energy load of the body. David *et al*. established that *Hura crepitans* seed oil possessed broad spectrum antimicrobial activity [30]. With MIC values of 1.25 mg/ml, the oil was effective against *Pseudomonas aeruginosa* (Z.O.I. = 5.5 mm), *Salmonella typhi* (Z.O.I. = 8.5 mm), *Escherichia coli* (Z.O.I. = 6.0 mm), and *Enterobacter sp.* (Z.O.I. = 6.0 mm) [30]. Thus, the high proximate profile together with the broad antimicrobial activities allowed us to conclude that *Hura crepitans* had great nutraceutical potential.

Physicochemical parameter determination. Table 4 shows the physicochemical properties of the *Hura crepitans* seed oil determined using standard methods. The parameters evaluated were refractive index (RI), specific gravity (SG), boiling point (BP), melting point (MP), cloud point (CP), etc.

The oil had low free fatty acid content (6.00) and acid value (3.00) which was an indicator that the oil was of good quality. The peroxide value was 4.80 (milliequivalent of O_2/kg) and was within the stipulated limit by ASTM [31]. Ramadan [32] found that free fatty acid might be less suitable parameter to assess degradability of oil. This is because FFA content is unstable and can change due to oxidation and hydrolysis. However, PV is a good quality control parameter in this study since

Table 4. Physicochemical parameters of *Hura crepitans* seed oil

Parameter	Value
Free fatty acid (FFA), mg KOH/g	6.0 ± 0.3
Acid value (AV)	3.0 ± 0.2
Peroxide value (PV), meq./ kg	4.8 ± 0.2
Saponification value (SV), mg KOH/g	147.7 ± 1.2
Iodine value (IV)	14.2 ± 0.8
Refractive index (RI)	1.4776 ± 0.1
Specific gravity, g/mL	0.8 ± 0.01
Boiling point (BP), °C	240 ± 0.9
Melting point (MP), °C	73–76
Pour point (CP), °C	–0.10
Cloud point, °C	6.20
Colour	Golden yellow
pH	5.2 ± 0.1

Table 5. Mineral content in *Hura crepitans* seed oil

Mineral	Value
Manganese (Mn), ppm	3.66 ± 0.02
Iron (Fe), mg/kg	4.01 ± 0.03
Magnesium (Mg), mg/kg	119.51 ± 0.25
Calcium (Ca), ppm	0.11 ± 0.001
Cadmium (Cd), mg/kg	0.0028 ± 0.0004
Zinc (Zn), µg/g	0.04 ± 0.001
Cobalt (Co), mg/kg	BDL
Lead (Pb), mg/kg	BDL
Nickel (Ni), mg/kg	BDL
Sodium (Na), ppm	2.18 ± 0.02
Potassium (K), ppm	4.25 ± 0.04

Note: values are mean ± SD of triplicate determinations with $n = 3$. BDL = Below Detectable Limit. ppm = parts per million

the lower PV value demonstrated that the oil had high quality, with no oxidative rancidity. The saponification value is 147.7 mg KOH/kg, which is an indicator of average molar mass of fatty acid. The acceptable range of SV for palm oil is 195–205 mg KOH/g according to Standard Organization of Nigeria [33]. Hence, the lower saponification value in this research, as compared with the standard value, clearly indicates that there are lesser ester bonds [34].

Moreover, iodine value (IV) for *Hura crepitans* was 14.23. It measures the degree of unsaturation in a fat or vegetable oil and determines how stable the oil is to oxidation [35]. A decrease in the total unsaturation of oils can indicate quality deterioration of stored oil [36]. Hence, the low IV in the oil under study might have contributed to a high stability of the oil during storage [34].

The refractive index of the oil was 1.478 and was in line with the American Standard of Test Material (ASTM) value (1.476–1.479). The boiling point (240°C), the pour point (–0.10°C), and cloud point (6.2°C) were in agreement with the values reported by O.O. Oniya *et al*. [13] who also investigated sandbox tree. Nonetheless, the cloud point, which had a value 6.2°C in this study, slightly varied from that obtained for peanut oil by Agarwal [37], which was 5°C. The specific gravity (0.88) at room temperature had the same value as in [38]. However, there was a slight variation in pH (5.2) from that in [22], where pH was 7.6. This might be due to different process condition.

Mineral content determination. The determination of mineral substances in seed oil is an important factor for nutritional assessment and human health benefits that sums up to provide the nutraceutical potential of the oil [39]. To determine the mineral and metal contents in the *Hura crepitans* seed oil, we used the AAS technique. The results are represented in Table 5.

Since mg/kg and µg/g are equivalent to part per million (ppm), the latter will be used in the discussion for uniformity. Of eleven minerals determined, eight were detected while three elements were below the detectable limit of the machine used (Buck Scientific 210VGP AAS).

The order of the detectable minerals based on their relative abundance was as follows: Mg (119.51 ± 0.25 ppm) > K (4.25 ± 0.04 ppm) > Fe (4.01 ± 0.03 ppm) > Mn (3.66 ± 0.02 ppm) > Na (2.18 ± 0.02 ppm) > Ca (0.11 ± 0.001 ppm) > Zn (0.04 ± 0.001 ppm) > Cd (0.0028 ± 0.0004 ppm). Among the detected minerals, magnesium (Mg) was present in the highest concentration (119.51 ± 0.25 ppm), while cadmium (Cd) displayed the lowest value (0.0028 ± 0.0004 ppm). Such metals as lead (Pb), nickel (Ni), and cobalt (Co) were not detected because of their insignificant amount. This gives a considerable advantage to the consumption of this oil as metal lead is harmful for the body.

As for the other mineral substances, the amount of iron (Fe) contained in the *Hura crepitans* oil (4.02 ± 0.02 mg/kg) might assist in replacing lost blood via enrichment of hemoglobin. In addition, regular consumption of the oil would prevent anaemia disease in humans [15]. The manganese (Mn) value (3.66 mg/kg) was within the acceptable standard, which was 2–4 mg per day. Magnesium is a valuable element in animal feed and the human diet.

Calcium (Ca) is responsible for the formation of strong bone and teeth. In this study, calcium was detected in trace quantity (0.11 ± 0.001 ppm). Nevertheless, regular consumption of the *Hura crepitans* oil could reduce hypocalcaemia and osteoporosis resulted from calcium deficiency in the human skeletal system [40]. Sodium (Na) and potassium (K) content determination is of great importance because they are both intracellular and extracellular cations. The sodium content indicated that it would be able to control plasma volume, neuron transmission, and muscular contraction [16]. The values for magnesium, sodium, potassium, zinc, and calcium are in good agreement with those in *Hura crepitans* oil documented by Abdulkadir *et al.* [11].

The heavy metals such as nickel (Ni) and cobalt (Co) were not detected in the oil sample. It is noticeable that lead was not detected in the *Hura crepitans* oil as well, since lead showed toxic effect on living organisms. This implied that the oil was medically safe for consumption and could be used in the human diet.

CONCLUSION

The seed of *Hura crepitans* has promising profile.

Solvent-extracted oil from *Hura crepitans* was analysed for physicochemical parameters, mineral content, and phytochemical composition in order to assess the nutraceutical potential. The results of the research allow us to conclude that this oil can be used as an alternative source of dietary supplement and raw materials in animal feed. Structural analyse of the *Hura crepitans* seed oil was carried out based on its physicochemical parameters and spectroscopic data. The data reported in this research were obtained with the help of diverse standard methods. The *Hura crepitans* seed oil has potential for further investigations of its mechanical properties for technical applications.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Pulsed infrared radiation for drying raw materials of plant and animal origin

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Abstract: The paper describes physical characteristics of drying animal- and plant-based raw materials with pulsed infrared emitters. Furthermore, it discusses how to select and use infrared emitters to produce high quality products with a long shelf-life. Using an experimental facility, we identified basic patterns of changes in the heat flux density. We also analysed the drying thermograms and assessed the influence of process factors on the removal of moisture from raw materials and the preservation of biologically active substances in dried and concentrated products. We determined specific kinetics of drying in different modes of power supply and selected the most efficient pulsed ceramic emitters. These emitters had a high rate of heat transfer and an ability to accurately target molecular bonds, thus reducing the drying time and energy costs. Mathematical modelling enabled us to obtain specific values of process parameters for pulsed infrared drying of plant materials. The heating time constant was calculated for root and tuber vegetables, depending on their moisture content and size. The study showed that root and tuber vegetables should not be heated to more than 60°C when irradiated with a 500 W medium-wave emitter at a working distance of 250 mm during a full 10-minute cycle. The optimal modes of drying liquid products with milk and plant proteins included a heating power of 400 W, a radiant heating temperature of 60°C, and a layer thickness of 10 mm. The selected modes of pulsed infrared drying of sugar-containing root and tuber vegetables reduced the duration of moisture removal by 16–20% and cut energy costs by 16.6%. This unconventional method of infrared drying of whole milk, whey, whey drinks, and milk mixture preserves beneficial microflora and increases the nutritional value and shelf-life, with a possible content of chemically bound water of polymolecular and monomolecular adsorption ranging from 10 to 15.58%.

Key words: Infrared, pulsed IR emitters, plant material, liquid raw milk, temperature, features, water

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INTRODUCTION

Animal- and plant-based foods and raw materials have a short shelf-life, tasking food technologists with finding ways to preserve their quality for consumers [1]. One of the oldest methods of food preservation is drying, ensuring microbiological safety by dehydration [2, 3, 33].

The current research aims to develop new technologies using unconventional drying methods to create biologically valuable long-life products of high quality [4, 5]. Dry products are widely used in various branches of industrial processing. Plant- and animal-based raw materials are commonly dried with facilities that use electrical energy converted to infrared radiation. Since electrically charged particles are stimulated by electric, magnetic, and electromagnetic fields, pulsed infrared treatment is considered one of the most effective methods of dehydrating raw materials and foods.

Infrared (IR) radiation is a transfer of energy from a radiation source to an object by means of electromagnetic oscillations at wavelengths between 0.78 and

750 microns through a medium that is transparent to thermal radiation. The technology of pulsed infrared drying of wet products can use almost 100% of delivered energy, with the drying process proceeding under gentle conditions [6, 7]. Infrared emitters used in this technology allow for drying at 40–60°C and thus preserving most of the original functional properties of food components. Biologically active substances and vitamins in the dried product account for about 90% of their content in the fresh product [8–11].

The physicochemical process can be described as follows: the product's water molecules absorb IR rays, which intensifies the thermal motion of atoms and molecules and causes them to heat. This way the energy is delivered directly to the product water, making thermal evaporation highly efficient. Such a direct heat supply allows for drying at relatively low temperatures (40–60°C). This method has two advantages. Firstly, it prevents cells and vitamins from being destroyed and sugar from caramelising, thus preserving the properties of raw materials and foods. Secondly, the walls of the drying equipment do not heat, thus reducing heat losses.

The following methods are currently used in Russia and abroad to dry raw materials of animal and plant origin: natural drying, conduction, convection, microwave drying, infrared drying, sublimation, and pseudo-boiling. Each of the methods has its advantages and disadvantages.

Infrared drying is comparable to other methods in many respects and even surpasses them in terms of energy consumption for the evaporation of moisture. Due to a high penetrating ability of infrared radiation, heat is released in the depth of the material, increasing the drying intensity 1.5–2 times and thus reducing energy consumption.

We established that the specific effect of IR radiation on food products is determined by the intensification of biochemical processes. This is due to the resonant effect of the absorbed energy on the bonds of atoms in molecules, whose oscillation frequencies are equal or multiple to the frequency of the incident radiation.

Of all existing drying facilities, infrared drying equipment is versatile: it can be used for drying both plant- and animal-based raw materials and products from them. It includes drying chambers and apparatuses with ceramic-coated heating elements emitting infrared rays. Pulsed infrared radiation is known to destroy microorganisms on the surface of a raw material, making it suitable for long-term storage [6, 12–17].

As a technological process, infrared drying is based on the fact that infrared rays of a certain wavelength are quickly absorbed by the water and not absorbed by the product microstructures. This allows for removing moisture at low temperatures (40–60°C) and thus preserving natural colour, vitamins, taste, aroma, and biologically active substances [6, 18–21].

Thus, infrared heating has the following advantages over other drying methods:

1. The amount of heat transferred, determined by the Stefan-Boltzmann law, is proportional to the difference in fourth powers of the temperatures of the emitter and the heated body. Infrared radiation is 4–5 times as intense as heat transfer by convection at the emitter temperature above 500°C.

2. The radiant flux is directionally focused on the heated object with the help of reflectors, ensuring minimal radiation in other directions. The temperature of the air through which the rays pass makes almost no difference for the heating or drying process.

3. This method allows for selective heating. Due to the low energy of quanta, infrared rays have a very limited ability to cause chemical reactions. Only the absorbed part of the radiant flux can accelerate chemical reactions in a substance or its heating. Substances vary in the ability to absorb infrared rays. Therefore, molecules of different compounds can have pronounced absorption maxima, and the emitters or heating temperatures need to be carefully selected to ensure intensive heating of surfaces or particles.

This method of dehydration may be used to produce various types of food concentrates such as cereals, soups, main courses, snacks, vegetable- and fruit-based baking powders, and components for dry infant formulas. Vegetables subjected to infrared drying, rather than traditional drying, retain their taste as close as possible to that of fresh vegetables. In addition, powders produced by infrared drying have antioxidant, anti-inflammatory, and detoxifying properties.

The use of ingredients produced by infrared drying in the dairy industry, confectionery or bakery allows them to expand the range of products with specific sensory properties. In addition, such products are environmentally friendly and free from exposure to harmful electromagnetic fields and radiation, as infrared radiation is harmless to humans and the environment.

It should be noted that dry products are easily stored and resistant to the development of microorganisms. They can be stored without special packaging (at low environmental humidity) for a year, with a 5–15% loss of vitamins. When sealed, dry products can be stored for up to two years. Drying reduces the volume of a product 3–4 times and its weight, 4–8 times.

This work aimed to study physicochemical patterns and develop a technology for thermoradiation drying of animal- and plant-based raw materials using pulsed infrared emitters to produce foods with a high biological value and an extended shelf-life.

STUDY OBJECTS AND METHODS

The experimental studies of the dehydration of animal- and plant-based raw materials and their physicochemical characteristics were conducted at the Kemerovo State University and the Ezhevsky Irkutsk Agrarian University, Russia.

The objects of study were root vegetables (carrot, beetroot, turnip, Jerusalem artichoke, and potato), liquid products with milk proteins (whole milk, whey, whey drinks, and fermented milk drinks), purchased in the retail chains of Kemerovo and Irkutsk, and oat protein extract [22].

The studies consisted of several stages followed by a substantiation of the technology for producing highly nutritional food concentrates containing sugar, milk, and plant proteins.

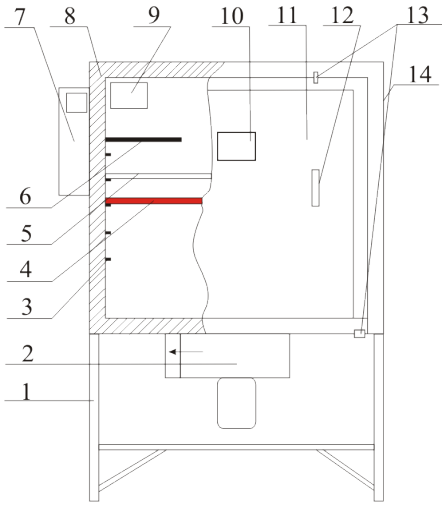


Fig. 1. The experimental production facility with controlled IR power supply: (1) drying chamber, (2) centrifugal fan, (3) cartridge holder, (4) removable panel IR irradiator, (5) raw material cartridge, (6) thermal sensor, (7) control panel, (8) heat-insulating material, (9) lamp, (10) viewing window, (11) sealed door, (12) handle, (13) door holders, and (14) metal coating.

An experimental production facility with IR power supply was designed to dry vegetables and fruits (Fig. 1). Liquid products with milk and plant proteins were dried under vacuum. The facility contained automatic circuit breakers, heating elements, and an electric motor with a fan, and was insulated with a thermal non-flammable material to reduce thermal losses.

Pulsed ceramic heating elements of ECS, ECP, ECH, ECX, and ECZ types with a capacity of 1 kW each were used as sources of infrared radiation. The control system maintained the required temperature and operating mode of IR emitters, thus reducing power consumption. Shredded fruits and vegetables were placed on trays in a layer of 1.5 cm, and liquid products in a layer of 10 cm, in the drying chamber. The choice of thermal emitters depended on the type of material to be dried. The temperature in the chamber was maintained by the control system and thermocouples. Evaporated moisture was removed by the fan. After a certain time, the product was taken out of the drying chamber.

The temperature regime, T (°C), of the elementary layer of biological objects exposed to IR treatment and drying with a given radiation power p (W) and duration τ (min) was represented by the following function:

$$T(p,\tau)=T_0+a\cdot\tau+b\cdot p^{u}\cdot\tau^{v}+\varepsilon(p,\tau), \qquad (1)$$

where T_0 is the initial temperature, °C;
a is the heating time constant, °C/min;
b is the structural coefficient of the energy level, °C/(W^u min^v); and
ε (p,τ) is the random deviation of experimental data from the calculated values.

Power consumption depends on radiation power and the ability of the material under treatment to absorb and accumulate energy:

$$p=\begin{cases}\alpha P, & radiation\\ \beta P, & pause\end{cases}, \qquad (2)$$

where P is IR radiation power, W;
α, β are the process efficiency values in the IR-treatment cycles.

Changes in the moisture content, W (%), in the elementary layer of objects exposed to IR treatment and drying with a given radiation power P (W) and duration τ (min) were represented by the following function:

$$W(P,\tau)=\left(b_{21}\cdot a_1^{\tau}+b_{22}\cdot a_2^{\tau}+b_{23}\cdot a_3^{\tau}\right)\cdot P^2+$$
$$+\left(b_{11}\cdot a_1^{\tau}+b_{12}\cdot a_2^{\tau}+b_{13}\cdot a_3^{\tau}\right)\cdot P+$$
$$+b_{01}\cdot a_1^{\tau}+b_{02}\cdot a_2^{\tau}+b_{03}\cdot a_3^{\tau}+W_0+\varepsilon(P,\tau), \qquad (3)$$

where a_j, b_{ij} are unknown process parameters determined by the material's properties;
W_0 is residual moisture, %; and
ε (P,τ) is the random deviation of experimental moisture data from the calculated values.

The characteristic numbers of the process operator representation $\lambda_1,\lambda_2,\lambda_3$ are related to the parameters a_j as follows:

$$e^{\lambda_j\tau}=a_j^{\tau},\ e^{\lambda_j}=a_j,\ \lambda_j=\ln a_j, \qquad (4)$$

where $j=1, 2, 3$.

Changes in the carbohydrate content, S (%), in the elementary layer of root vegetables exposed to IR treatment and drying with a given radiation power P (W) and duration τ (min) was represented by the following function:

$$S(P,\tau)=\left(b_{21}\cdot a_1^{\tau}+b_{22}\cdot a_2^{\tau}+b_{23}\cdot a_3^{\tau}\right)\cdot P^2+$$
$$+\left(b_{11}\cdot a_1^{\tau}+b_{12}\cdot a_2^{\tau}+b_{13}\cdot a_3^{\tau}\right)\cdot P+$$
$$+b_{01}\cdot a_1^{\tau}+b_{02}\cdot a_2^{\tau}+b_{03}\cdot a_3^{\tau}+S_0+\varepsilon(P,\tau), \qquad (5)$$

where a_j, b_{ij} are the process parameters under study, determined by the material's properties;
S_0 is the initial carbohydrate content, %;
ε (P,τ) is the random deviation of experimental sugar data from the calculated values.

Changes in the vitamin content, V (mg/100 g), in the elementary layer of vegetables exposed to IR treatment and drying with a given radiation power P (W) and duration τ (min) was represented by the following function:

$$V(P,\tau)=\left(b_{21}\cdot a_1^{\tau}+b_{22}\cdot a_2^{\tau}+b_{23}\cdot a_3^{\tau}\right)\cdot P^2+$$
$$+\left(b_{11}\cdot a_1^{\tau}+b_{12}\cdot a_2^{\tau}+b_{13}\cdot a_3^{\tau}\right)\cdot P+$$
$$+b_{01}\cdot a_1^{\tau}+b_{02}\cdot a_2^{\tau}+b_{03}\cdot a_3^{\tau}+V_0+\varepsilon(P,\tau), \qquad (6)$$

where a_j, b_{ij} are the process parameters under study, determined by the material's properties;
V_0 is the initial content of vitamins, mg/100 g; and
ε(P,τ) is the random deviation of experimental vitamin data from the calculated values.

The duration of radiation in the first cycle was determined by the formula given in [3, 23, 24]:

$$t_{\max}^{1}=t_{\max}\left(1-e^{-\tau_0/T_u}\right)+t_{\min}\cdot e^{-\tau_0/T_u}. \qquad (7)$$

The base was calculated by the formula:

$$t^1_{\max} = t_{\max} - t_{\max} \cdot e^{-\tau_0/T_н} + t_{\min} \cdot e^{-\tau_0/T_н}, \quad (8)$$

$$e^{-\tau_0/T_н}\left(t_{\max} - t_{\min}\right) = t_{\max} - t_{\min}, \quad (9)$$

$$e^{-\tau_0/T_н} = \frac{t_{\max} - t^1_{\max}}{t_{\max} - t_{\min}}. \quad (10)$$

After taking the logarithm of the above:

$$-\frac{a}{T_н} = \ln\frac{t_{\max} - t^1_{\max}}{t_{\max} - t_{\min}}, \quad (11)$$

$$\tau_o = T_н \ln\frac{t_{\max} - t_{\min}}{t_{\max} - t^1_{\max}}. \quad (12)$$

Since the duration of drying is significantly affected by the maximum rate of heating (V_{mh}), formula (12) takes the following form:

$$\tau_{01} = T_н \ln\frac{t_{\max} - t_{\min}}{t_{\max} - T_н \cdot V_{mh}}. \quad (13)$$

We identified some patterns in intermittent irradiation control, which could be described with the theory of power series. The duration of irradiation for the second and subsequent cycles was calculated by the formula:

$$\tau_{work} = exp(-T_{cycle}/T_H), \quad (14)$$

where T_{cycle}/T_H is the relation between the cycle (or its part) time and the heating time constant.

The analytical model that we developed, as well as the patterns of irradiation control, enabled us to justify the following effective discrete operation modes for an infrared emitter:

$$f(\tau) = \begin{cases} radiation, & \tau = 10k; \\ pause, & \tau = 2k; \end{cases} \quad k \in N. \quad (15)$$

The heating time constant is the time taken for the temperature rise to reach a steady-state value if there is no heat release to the environment. Since the drying process takes place in a closed chamber and there is practically no heat release to the environment, the heating time constant becomes a decisive factor for choosing the IR power feed mode. The heating time does not depend on the input power and is numerically equal to the ratio of the body heat capacity to its heat transfer [14, 21, 24]:

$$T_н = \frac{C}{Qpr}, \quad (16)$$

where C is the product's heat capacity, J/°C;
Q_{pr} is the product's heat transfer, J/°C·s.

In case of heat transfer during the time equal to the heating time constant, the product temperature rise reaches a value equal to 0.632 of the steady-state value. The heat capacity of the material depends on the heat capacity of dry matter and water. The specific heat capacity of dry matter in plant raw materials ranges from 0.733 to 1.550 J/(kg·°C). Since the heat capacity of water (C = 4.1868·10³ J/(kg·°C)) is much higher than that of dry matter, the heat capacity of plant raw materials decreases during drying.

The heat capacity of wet materials is determined by the formula:

$$C_M = \frac{C_{wm} \cdot (100 - \omega) + C_{H_2O} \cdot \omega}{100} = \frac{C_{wm} \cdot 100 + C_{H_2O} \cdot U}{100 + U}, \quad (17)$$

where C_{dm} and C_{H_2O} are the heat capacity of the product's dry matter and water, respectively, J/(kg·°C);
ω and U are the material's initial and ultimate moisture content, respectively, %.

Eq. (17) determines a linear dependence between the heat capacity and the initial and ultimate moisture content of the object of drying. The temperature rise is accompanied with an increase in the heat capacity.

The heating time constant was determined by the following equation:

$$T_н = \frac{C}{Q_{np}} = \frac{c \cdot M}{\alpha \cdot F}, \quad (18)$$

where C is the specific heat capacity of a root vegetable, J/kg·°C;
M is the mass of root and tuber vegetables, kg;
α is the heat transfer coefficient of root vegetables, J/m²·°C·s; and
F is the surface of the product, m²,
or as follows:

$$T_H = \frac{c \cdot \rho}{\alpha} \cdot \frac{V}{F}. \quad (19)$$

When V/F was expressed as σ, Eq. (19) for the heating time constant was as follows:

$$T_H = \frac{c \cdot \rho}{\alpha} \cdot \sigma. \quad (20)$$

Each experiment was repeated three times and the data were expressed as a mean ± standard deviation. The data were processed by standard methods of mathematical statistics. The homogeneity of the sampling effects was checked with Student's t-test. The differences between the means were considered significant when the confidence interval was less than 5% ($p \le 0.05$).

RESULTS AND DISCUSSION

Plant- and animal-based raw materials have certain electrophysical and thermal properties (heat capacity, thermal conductivity, electrical conductivity, dielectric and magnetic permeability, and optical properties) manifested at the exposure to electric, magnetic, and electromagnetic fields, as well as waves of different frequencies. The resulting changes in electrical charges lead to the release of heat and alter physicochemical characteristics of the materials.

Infrared treatment and drying work well for both vegetables (carrot, beetroot, turnip, celery, cabbage, spinach, tomato, parsley, pepper, potato, and Jerusalem artichoke) and fruits (apple, pear, plum, peach, apricot, grape, and banana). As objects of drying, they are characterised by a high water content and a relatively low dry matter content. About 5% of water is bound in cells, tissues, and colloids and is firmly held there, while the rest of it is in a relatively free mobile form.

Various modes of power supply and sources of pulsed infrared radiation were tried to establish effec-

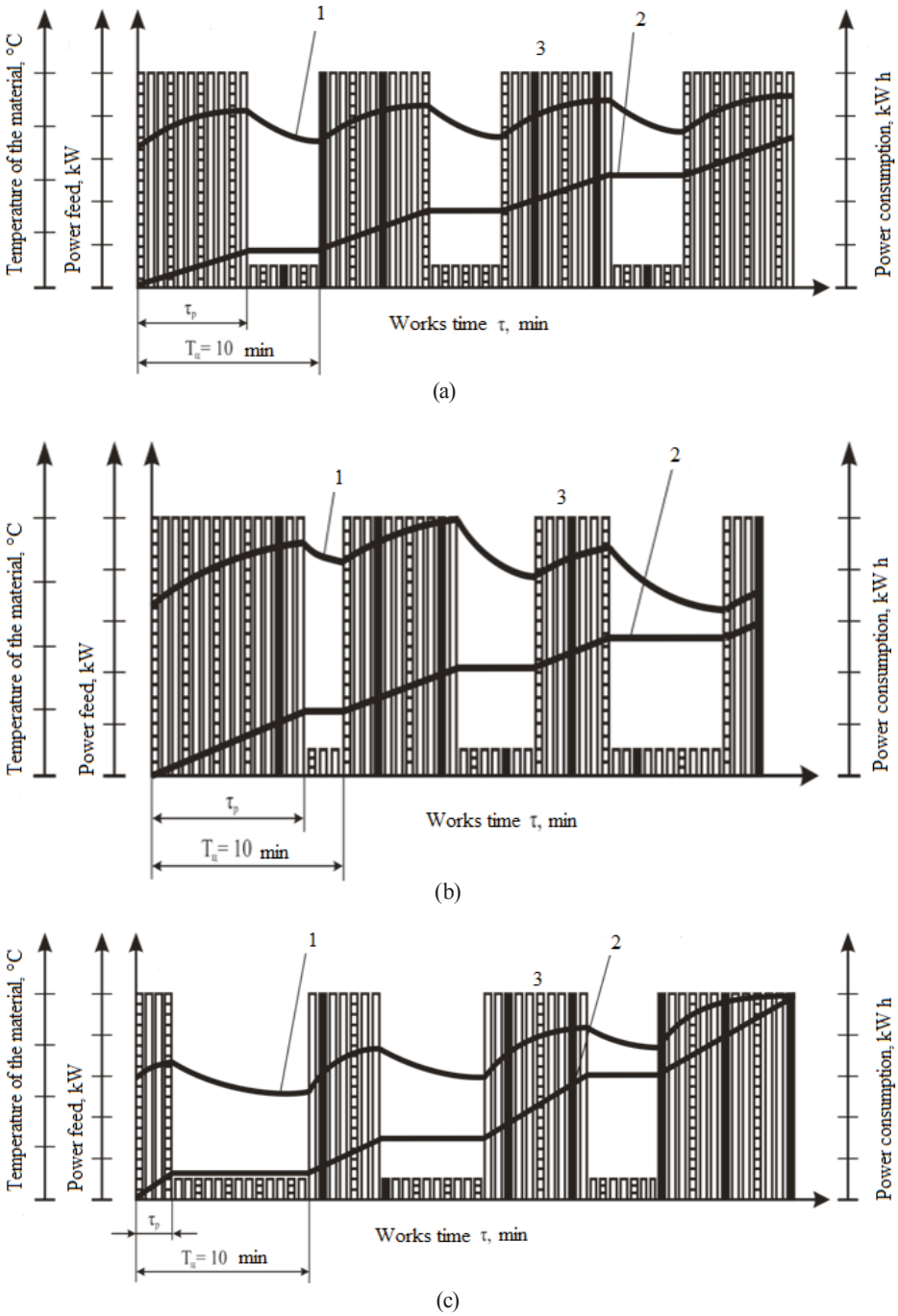


Fig. 2. Schematic representation of the IR irradiation process with: (a) increased power feed; (b) intermittent power feed; and (c) decreased power feed. (1) material heating curve, °C; (2) power consumption curve, kWh; (3) power consumption, kW.

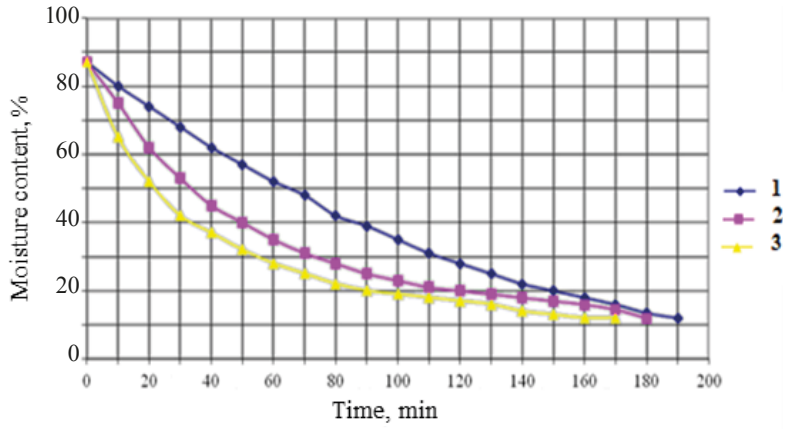
tive modes of treatment and drying. The optimum was achieved with 700 W emitters and decreased power supply. The preservation of nutrients was provided by the emitters of any capacity with decreased power supply.

Changing humidity and achieving the required moisture content in the raw material or product in a short time reduces the duration of drying and saves energy. The most efficient reduction in moisture content was provided by the mode of repeated short-term heating with decreased power supply. In addition, this mode of infrared treatment and drying ensured increased carbohydrate content and maximum vitamin content in sugar-containing root vegetables (beetroot).

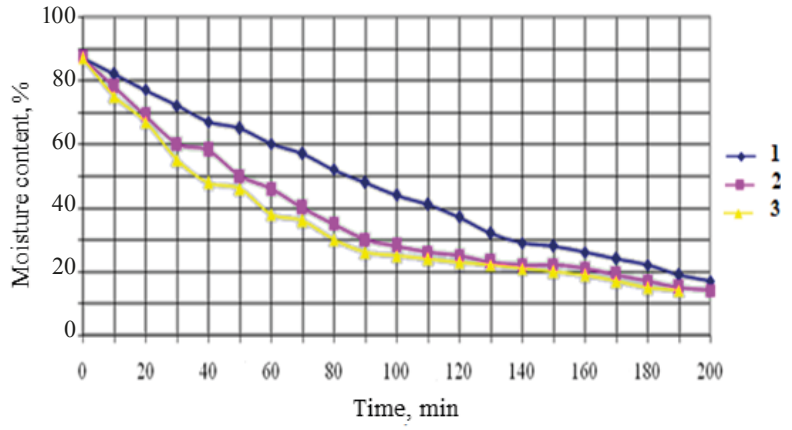
The above method enabled us to control electromagnetic radiation fluxes at the Planck constant level and produce foods with an optimal composition of active substances. Modern electrical facilities with advanced electronics make it possible to realise the main principles of quantum electrodynamics.

Pulsed ceramic IR emitters are efficient due to a high rate of heat transfer and a possibility of targeting molecular bonds, reducing the duration of the process, and thus saving energy. Their use in the infrared drying technology ensures high-quality products with a long shelf-life.

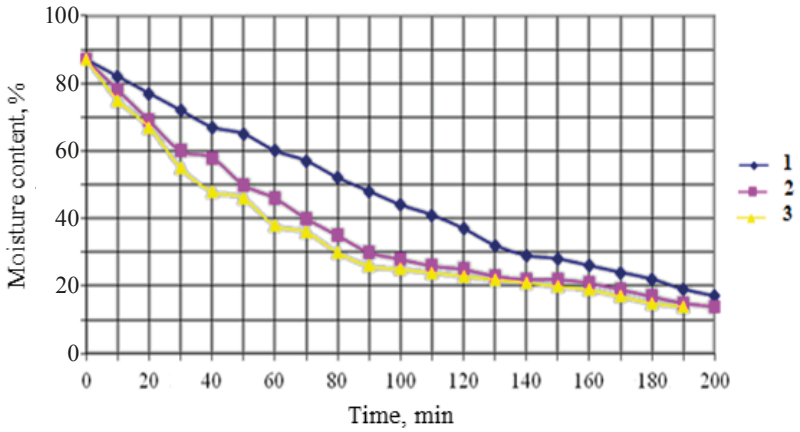
With pulsed IR emitters, there are three ways of controlling infrared power supply [26] with differing



(a)



(b)



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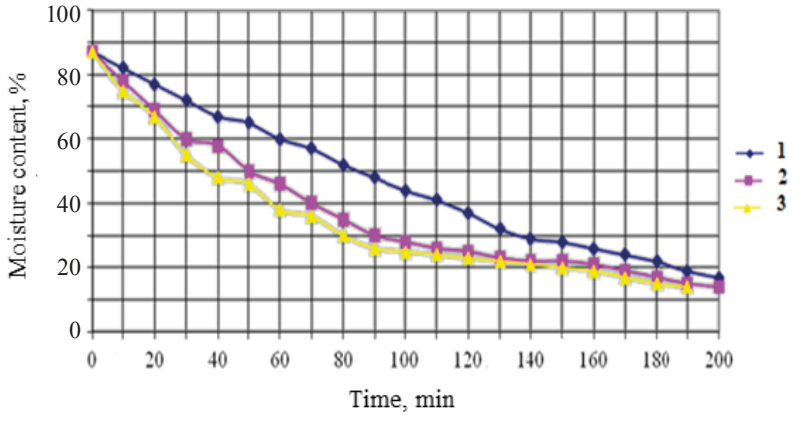

(d)

Fig. 3. Changes in moisture content of plant products during evaporation in the experimental facility with: (a) pulsed ceramic heaters; (b) tubular electric heaters; (c) a nichrome spiral; and (d) a mica-layer heater. (1) increased power feed; (2) intermittent power feed; (3) decreased power feed.

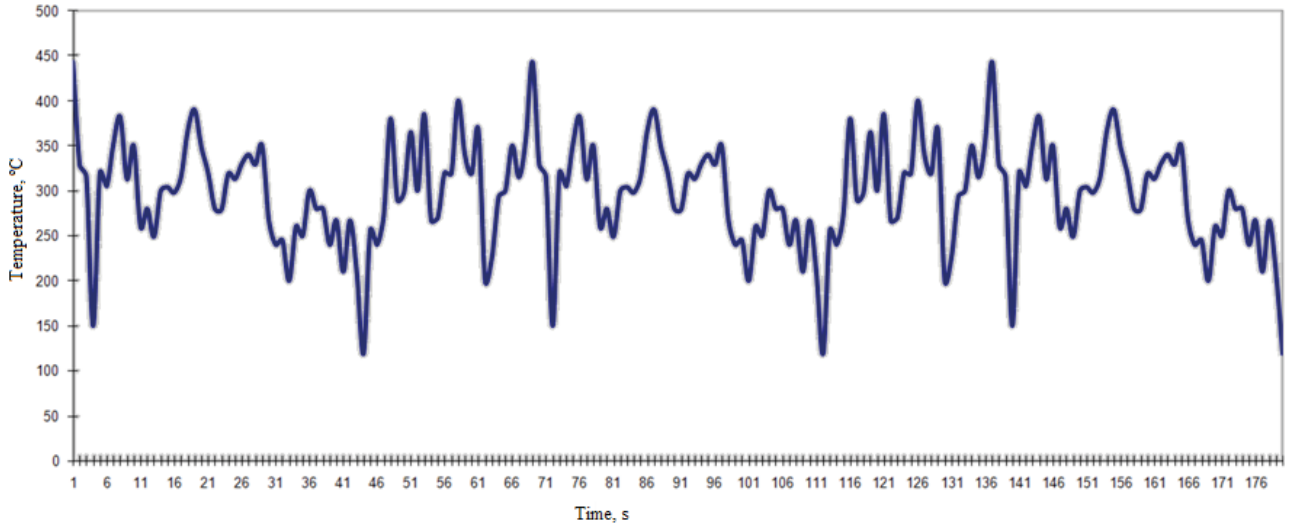

Fig. 4. Pulsed operation of the IR emitter.

relations between the heating temperature, irradiation power, and power consumption during the drying process (Fig. 2).

Using formulae (7)–(20), we calculated the heating time constant for root and tuber vegetables depending on their moisture and size. The experimental studies of pulsed infrared drying of sugar-containing roots and tubers identified effective modes for controlling IR power supply and their influence on the quality of carrot, beetroot, and Jerusalem artichoke. In particular, those modes included a decreased power feed with a power density of 0.8 kW/m^2 and a radiation surface temperature of 500°C at a 225–250 mm distance. Pulsed ceramic IR emitters operating in those modes created a uniform 40–60°C thermal field on the surface of root and tuber vegetables, thus maintaining the quality of the end product. Those effective modes of pulsed infrared drying of sugar-containing roots shortened the duration of moisture removal by 16–20% and reduced power consumption by 16.6%. The optimal modes for liquid products with milk and plant proteins included a heating power of 400 W, a radiant heating temperature of 60°C, and a product layer thickness of 10 mm.

Fig. 3 shows thermophysical features of thermal evaporation of water under infrared radiation. We monitored changes in the moisture content of plant raw materials (root vegetables) during evaporation in an experimental facility using IR power supply (Fig. 1) with various types of IR emitters. Different modes of power supply produced different drying kinetics. The analysis of the drying curves showed that pulsed ceramic heaters ensured a residual moisture of 12% with a product temperature of less than 60°C in less time. A higher temperature reduced the quality and the content of nutrients in root and tuber vegetables.

The most effective mode of drying plant raw materials is the one that ensures minimal duration and power consumption, thus leading to greater preservation of biologically active substances. Such a mode requires that the working temperature and wavelength correspond to the biotechnical conditions of heating. In addition, the spectral characteristics of an infrared emitter need to be consistent with the optical properties of the product. In this case, the energy slowly penetrates into its inner layers and displaces moisture from the depth to the surface.

The above mode can be established with pulsed ceramic emitters, when a product is exposed to high density pulses in a certain spectral range (the depth of penetration in the first approximation is proportional to the pulse density) [14]. The source of primary infrared radiation in pulsed ceramic emitters is a nichrome spiral located in a quartz glass tube with a multilayer ceramic coating. Due to this coating, the full spectrum of infrared radiation is converted from a heating element to a very narrow range of radiation emitted in a series of pulses that are 10–3,000 μs long (Fig. 4) and have a density of 120–350 W/cm^2 [14, 16, 21].

The effect of pulsed conversion is associated with cyclic energy transformations in the system. Ceramics accumulates thermal radiation, converts it and then 'shoots' impulses in a certain region of the spectrum. The wavelength of the radiation generated varies in the range of 1.7–5.8 microns. With pulsed infrared irradiation, the time required to heat plant raw materials to the ultimate temperature is significantly shorter than that with continuous irradiation. In addition, materials with a high specific thermal capacity heat up faster [14, 16, 27–29]. Furthermore, the high penetrating capacity of pulsed IR radiation leads to the dissociation of organic and bio-organic molecules and the destruction of microorganisms, spores, fungi, and viruses, thus increasing the product's shelf-life.

Fig. 5 shows the drying of whey and fermented milk drinks with pulsed ceramic IR emitters. We aimed for a dry matter concentration of 27% to 70%, depending on the further use of dried dairy materials [30–32].

The analysis showed that all the samples of concentrated dairy raw materials and semi-finished products containing animal or plant proteins had the following characteristics (Table 1).

Evaluated on a 10-point scale of organoleptic properties, the samples received an average of 9 points. The physicochemical indicators of concentrated dairy raw materials were as follows: 41% dry matter; 6.0–6.4%

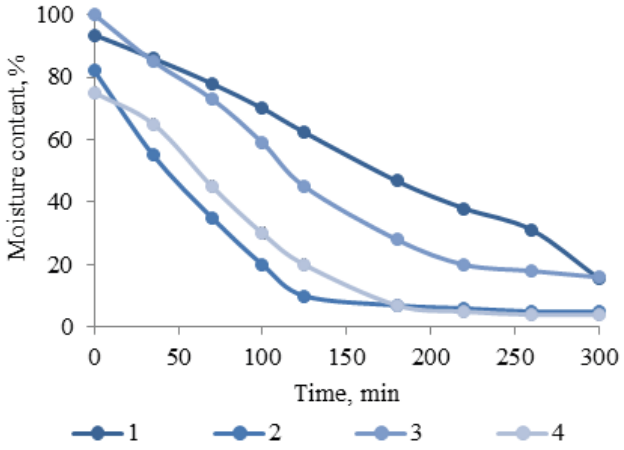

Fig. 5. Changes in moisture content of products containing milk or plant proteins during evaporation in an experimental facility with pulsed ceramic emitters. (1) whey; (2) whey product; (3) kefir; (4) oat protein extract.

fat, 7.6–7.8% protein (semi-finished products with plant proteins had 35% protein and 3% fat), and 1×10^9–1×10^{10} CFU/g of beneficial lactic acid microflora at a high functional level.

Some particular features of the process kinetics for various types of dairy raw materials and semi-finished products with plant proteins are determined by their chemical composition and moisture. The drying curves show the removal of the most energy-intensive adsorption moisture with an ordered structure. We established that the chemically bound moisture of polymolecular and monomolecular adsorption in liquid products with milk or plant proteins ranged from 4% to 15.58%, remaining so after drying.

CONCLUSION

The results of thermal evaporation showed a correlation between growing duration of infrared drying and increasing mass fraction of dry matter in the product.

Table 1. Sensory characteristics of concentrated dairy raw materials and semi-finished products containing animal or plant proteins

Characteristic	Assessment
Appearance	opaque, dense, and homogeneous mass
Colour	creamy or matching the filler (yellow, orange)
Flavour	pure, milky-sweet, with a pronounced taste of the filler or salty-sweet, without bitterness or foreign taste

The changes in the key quality indicators established 60°C as the optimal temperature of the heat flux in the drying chamber. In that case, the drying rate depended on the speed of moisture displacement inside the product, rather than on the rate of heat transfer.

The temperature graphs for infrared drying of whey and fermented milk drinks showed low temperatures on the surface of the samples (22–25°C), preserving their original natural properties. The analysis of experimental data on drying raw materials with milk and plant proteins established an optimal heating power of pulsed infrared lamps as 400–600 W. Concentrated dairy semi-finished products retained their properties after infrared drying at 22–25°C, with up to 90% of vitamins and other biologically active substances preserved.

The studies demonstrated that the temperature of heating root and tuber vegetables exposed to infrared radiation with a medium-wave 500 W emitter at a working distance of 250 mm during a full 10-minute cycle did not exceed 60°C. Since higher temperatures could lead to an irreversible loss of vitamins and mineral nutrients, those parameters of IR power supply were considered ‘effective’.

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Changes in physico-chemical properties of milk under ultraviolet radiation

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Abstract: The use of ultraviolet radiation in the treatment of milk and other liquid foods is a very promising field of study since it reduces their bacterial load. It is rarely used to increase the vitamin D_3 content and modify the protein and fatty acid composition of milk. The paper describes how different parameters of ultraviolet radiation influence such characteristics of raw and pasteurized milk as the mass fraction of total protein, nonprotein nitrogen content, active and titratable acidity, general bacterial load (QMA&OAMO), fatty acid composition, and vitamin D_3 content. Low-pressure gas-discharge lamps were used to treat a 400 µm moving layer of milk with ultraviolet radiation. The radiation time, its doses, and the milk flow rate changed in the ranges of 5–25 min, 5.1–102 mJ/cm^2, and 0.04453-0.13359 m^3/s, respectively. We identified optimal radiation ranges that lead to both a lower microorganism content and a higher vitamin D_3 content. Our study also determined specific correlations in the mutual changes of the given parameters. The treatment ranges did not produce any significant changes in other physico-chemical properties of milk. We also found that vitamin D_3 was synthesized in raw and pasteurized milk in a similar way. Moreover, there was an insignificant decrease in the vitamin D_3 content in milk treated with ultraviolet radiation during storage for up to 48 hours. On the whole, the results indicate that the treatment of milk with ultraviolet radiation in the dosage range from 5.1 to 102 mJ/cm^2 has a complex effect on the total bacterial load (QMA&OAMO) and vitamin D_3 content, whereas it has almost no effect on the protein and fatty acid composition.

Keywords: Ultraviolet radiation, milk, protein, fatty acids, vitamin D_3, (QMA&OAMO) CFU/cm^3

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INTRODUCTION

The assessment of the ultraviolet radiation (UVR) effect on the physico-chemical and vitamin composition, as well as the bacterial load of dairy and other food products, is a promising field of research as it permits directional regulation of their properties.

Numerous studies are currently conducted in this area. However, the data are still insufficient to accurately assess all the aspects and mechanisms of the ultraviolet radiation effect on food and dairy products during their processing. There is therefore a need for further research in this area.

Milk is an important source of nutrition. It contains proteins, milk fat, minerals, and different vitamins. The main components of milk vary according to the breed of cows, feeding, and livestock management. These changes mostly affect the content of fat-soluble vitamins, in particular vitamin D_3. In European countries, such as Denmark, the consumption of milk, cheese, and other dairy products accounts for about 12% of the total intake of vitamin D_3 [1, 2]. Dairy products with a low content of vitamin D_3 cannot serve as its natural source. Insufficient intake of vitamin D_3 increases the risk of developing hypertension, autoimmune diseases, diabetes, rickets, and cancer [1].

Lactating cows have two primary sources of increased vitamin D_3 content in milk. This vitamin can come with food, including vitamin-enriched supplements, or be produced endogenously under the impact of ultraviolet radiation on the cow's skin [3, 4]. Under ultraviolet light radiation, 7-dehydrocholesterol turns into precalciferols as a result of the prototropic rearrangement. Precalciferols serve to form the D vitamins (Fig. 1).

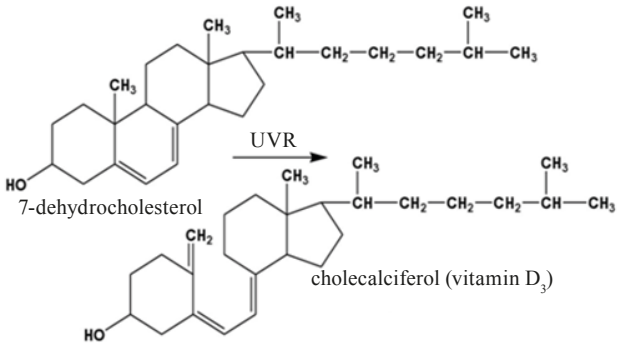

Fig. 1. Vitamin D_3 biosynthesis under ultraviolet radiation.

The vitamin D_3 content in milk and dairy products can be increased by its introduction at a particular stage of the technological process. This can also be achieved by ultraviolet treatment due to its directional effect on 7-dehydrocholesterol.

Various gas-discharge radiators are currently the most common sources of ultraviolet radiation. Mercury lamps are used because mercury in a gaseous state is activated at relatively low temperatures. Furthermore, the discharge in mercury vapor provides the largest number of intense lines in the ultraviolet spectrum. High- and low-pressure mercury lamps are used to ensure effective ultraviolet treatment of the material. They differ in their intensity depending on mercury vapor pressure. The advantage of low-pressure mercury lamps is that the largest share of radiation falls on the wavelength of $\lambda = 253.7$ nm, which has a maximum bactericidal effect. Therefore, lamps of this type are mostly used to reduce the bacterial load of the product. In high-pressure mercury lamps, the spectral area of impact has a higher wavelength range, which makes them less suitable for the bacterial treatment.

Flash xenon or argon high-pressure lamps became quite widespread, along with mercury gas-discharge lamps. Compared to argon lamps, flash xenon lamps have better bactericidal activity, shorter exposure time, and higher safety. Their disadvantages include a shorter period of guaranteed action, as well as increased operating costs.

We should note a shortage of comparative studies into the use of ultraviolet radiators in the field of food biotechnology.

Depending on the modes, the UV treatment can have a different effect on the composition and properties of milk. The most important components of milk – proteins, fats, and vitamins – can absorb UVR throughout its range. The energy absorbed by them can change the physico-chemical properties of these organic molecules. Furthermore, UVR produces active forms of oxygen that can change the chemical composition and properties of the main components of milk as a result of free radical reactions. The active forms of oxygen cause the DNA damage in microorganisms and oxidation of specific protein groups. Therefore, UVR is successfully used to reduce the bacterial load of dairy raw materials [5, 6].

The changes occurring in bacteria under UVR are multistage, with DNA molecules being the final stage of their action [7].

In addition to the direct influence on DNA, increased UVR intensity can cause cell mutations. This phenomenon is accompanied by the formation of free radicals and peroxides with mutagenic properties. For example, [8] reports an increased number of mutational changes in microorganisms cultivated in nutrient media.

UVR has the greatest oppressive influence on microorganisms in the wavelength range of 205–315 nm [9].

The first practical application of UVR as bactericidal treatment was for disinfection of water [10, 11]. Positive results were also achieved in the treatment of other highly transparent liquid foods, such as sugar solutions, their derivatives, and juices [12–17].

Numerous studies of UVR influence on milk and dairy products showed that the treatment is complicated by their low transparency due to the screening ability of protein and fat. Furthermore, dairy products have a complex composition, and their components are closely related to each other [18–23].

Nevertheless, those studies served as a basis for industrial facilities designed to reduce the quantitative content of bacteria in milk [6, 10, 24, 25].

Ultraviolet treatment has no direct effect on milk proteins in a certain range of parameters [26–28]. At the same time, we can use ultraviolet light to change the structure of proteins and give them new properties by changing treatment modes. This is confirmed by Cho *et al.* which showed that UV treatment could affect the molecular structure of β-lactoglobulin, the main allergen in milk [29]. Similar results were obtained in another study that recorded shifts in the molecular structure of β-lactoglobulin. These results also indicate a possibility of regulating the peptide profile of milk proteins and using UVR to reduce milk allergenicity [8].

Biotechnological methods used in the dairy industry can affect the secondary and tertiary structure of milk proteins [2, 9]. At the same time, the influence of physico-chemical factors can lead to the unfolding of the protein globule and increased proteolysis of denatured proteins.

This was confirmed by studying the proteolysis of milk proteins with pepsin and trypsin after UV treatment. The analysis of pepsin and trypsin hydrolysates showed that the number of cleaved protein substrates and peptide fractions was similar for all the milk samples subjected to ultraviolet radiation [13, 30]. Thus, the UV treatment of milk usually has no influence on the proteolysis of milk proteins with pepsin and trypsin and on its digestibility.

The UV treatment of milk can intensify the formation of vitamin D_3 [13, 24, 25]. However, this technology is not in wide industrial use yet due to a more common method of directly introducing vitamin supplements in dairy products. At the same time, a combination of the latter method with the benefits of UR remains highly relevant. Thus, the UV treatment of milk can be used to reduce its bacterial load, increase the vitamin D_3 content, and change certain components of milk.

Despite extensive studies in this area, the results are quite controversial. Moreover, most authors are mainly interested in studying the effectiveness of ultraviolet treatment in reducing the bacterial load or increasing the vitamin D_3 content.

This paper attempts to fully investigate the effect of certain ultraviolet radiation parameters on the above indicators and, at the same time, evaluate the changes in the protein and fatty acid composition of milk.

Our main objective was to find an optimal range of UVR which could reduce the bacterial load of milk and increase the vitamin D_3 content without having any significant effect on the protein and fatty acid composition of the treated product. Furthermore, our hypothesis was that the changes in the bacterial load and vitamin D_3 growth might be interrelated.

STUDY OBJECTS AND METHODS

Our objects of study were raw milk with a 3.8% mass fraction of fat and pasteurized milk with a 3.2% mass fraction of fat. The temperature of the product was 4 ± 2°C.

To conduct the experiment, we used a unit for treating milk in a 400 μm circular layer. Three symmetrically arranged Philips gas-discharge lamps (TUV 55W PL-L) were a source of UVR, with a wavelength of 253.7 nm. A thin layer of milk was passed through a gap between two cylinders. The outer cylinder was made of stainless steel, and the inner cylinder was made of quartz glass with gas-discharge lamps placed inside it. The outer diameter and the height of the cylinder were 120 and 600 mm, respectively.

The lamps were cooled with an electric fan that pumped air through the internal quartz glass cylinder.

Milk was supplied to the unit by a pump with adjustable capacity. Sampling for the study was carried out under aseptic conditions.

The study aimed to assess the UVR effect on the content of proteins, nonprotein nitrogen, fatty acids, and vitamin D_3, as well as the bacterial background of processed milk. The unit parameters included productivity (Q) 100–420 l/h; treatment time (τ) 5–25 minutes; the volumetric milk flow rate in the irradiated layer (V) 0.04453–0.13359 m^3/s; the surface bactericidal irradiation dose, i.e. the relation between the bactericidal irradiation energy and the irradiated surface area, (H_e) 5.1–102 mJ/cm^2.

Some samples were used to assess their protein and fatty acid composition, as well as vitamin D_3 content, both directly after the UV treatment and during storage, namely after 24, 36, and 48 hours, respectively. The assessment was carried out under standard conditions. We used the following State Standards to determine specific parameters, namely: State Standard 38892-2014 for active acidity (pH); State Standard R 54669-2011 for titratable acidity; State Standard 32901-2014 for the total number of microorganisms (QMA&OAMO); State Standard 23327-98 for the mass fraction of total protein; State Standard R 55246-2012 for nonprotein nitrogen content; State Standard 32915-2014 for the fatty acid composition; and State Standard R 54637-2011 for vitamin D_3 content. The analysis was conducted in triplicate. State Standard 26754-85 was used to regulate the milk temperature after the ultraviolet treatment.

The following equipment was used to measure the mass fraction of protein and nonprotein nitrogen:
– a SH220F digester (Hanon, China) with the maximum heating temperature of 450°C;
– a WD03 sulfuric acid vapour suction system; and
– a K9840 distillation system (Hanon, China) with an automatic supply of alkali, receiving solution, and distillate.

A 4000M Crystallux gas chromatograph (Russia) was used to separate and identify fatty acids in the samples. It was equipped with a Supelco-SP2560 capillary column (100 m×0.25 mm, df = 0.20 μm, Sigma-Aldrich, USA) and a flame ionization detector.

The following equipment was used to measure the vitamin D_3 content:
– a liquid chromatograph equipped with a spectrophotometer (Gilson, France);
– a Luna C18(2) column (5 μm, 250×4.6 mm, Phenomenex, USA); and
– a vacuum unit for Strata C18-E SPE with replaceable cartridges (Phenomenex, USA).

The analysis was performed in the following conditions:
– gradient mode of separation;
– mobile phase: acetonitrile (eluent A) – dichloromethane (eluent B);
– gradient elution programme: A/B = 100/0 at the beginning of the analysis; gradient A/B = 90/10 in 8 min; gradient A/B = 70/30 in 2 min; isocratic elution A/B = 70/30 in 10 min; gradient A/B = 100/0 in 3 min; isocratic elution A/B to 100/0;
– flow rate: 1.0 cm^3/ min;
– loop dispenser volume: 20 mcl;
– room temperature; and
– spectrophotometric detection with changing the wavelength of the light source during analysis: 0 min with a wavelength of 436 nm, 10 min with a wavelength of 280 nm, and 27 min with a wavelength of 436 nm.

RESULTS AND DISCUSSION

The experiments involved the evaluation of active (pH) and titratable (T°) acidity. We found that these indicators did not undergo any significant changes in the following range of treatment parameters: H_e = 5.1–102 mJ/cm^2, V = 0.04453–0.13359 m^3/s.

As can be seen in Table 1, the mass fraction of total protein remained unchanged, regardless of the irradiation time or type of milk.

Fatty acids, especially unsaturated, are an important component of the fat phase of milk. Tables 2 and 3 show the fatty acid composition of raw and pasteurized milk after different periods of the UV treatment.

The main fatty acids amounted to 95.78% in the control. Their composition and content hardly changed under the influence of UVR. Furthermore, the fat phase contained 23 minor fatty acids (4.22%). The UV treatment caused a slight increase in some of them and a slight decrease in others, with their total content remai-

Table 1. UVR effects on raw milk proteins

Indicators	Error	Actual values				
		Samples				
		Feedstock	Irradiation 5 min	Irradiation 10 min	Irradiation 15 min	Irradiation 25 min
Mass fraction of total protein in raw milk, %	± 0.06	3.28	3.30	3.24	3.21	3.30
Content of nonprotein nitrogen in raw milk, %	± 0.003	0.0279	0.0255	0.0271	0.0280	0.0279
Mass fraction of total protein in pasteurized milk, %	± 0.06	3.37	3.33	3.30	3.40	3.43
Content of nonprotein nitrogen in pasteurized milk, %	± 0.003	0.0276	0.0286	0.0242	0.0294	0.0280

Table 2. UVR effects on fatty acids in raw milk

Mass fraction of, %	Actual values				
	Samples				
	Feedstock	Irradiation			
		5 min	10 min	15 min	25 min
butyric acid	2.88	2.75	2.64	2.80	2.77
caproic acid	2.17	2.11	2.05	2.13	2.09
caprylic acid	1.35	1.32	1.28	1.34	1.36
capric acid	3.54	3.06	2.96	3.09	3.08
lauric acid	3.54	3.61	3.52	3.62	3.61
myristic acid	11.22	11.34	11.28	11.31	11.45
myristoleic acid	1.00	1.03	1.02	1.02	1.03
pentadecanoic acid	1.24	1.24	1.27	1.26	1.27
palmic acid	29.40	29.66	29.84	29.37	29.47
palmitoleic acid	1.62	1.68	1.67	1.67	1.70
stearic acid	10.85	10.98	11.00	10.98	10.98
elaidic acid	2.85	2.71	2.75	2.71	2.72
oleic acid	20.75	20.89	21.12	20.81	20.98
linoleic acid	3.37	3.24	3.23	3.46	3.47

Note: data are presented as mean value ± 3.0%

Table 3. UVR effects on fatty acids in pasteurized milk

Indicator: mass fraction, %	Actual values				
	Samples				
	Feedstock	Irradiation 5 min	Irradiation 10 min	Irradiation 15 min	Irradiation 25 min
Butyric acid	2.66	2.79	2.79	2.65	2.68
Caproic acid	1.91	2.20	1.95	1.99	1.98
Caprylic acid	1.20	1.23	1.24	1.24	1.28
Capric acid	2.68	2.76	2.78	2.79	2.79
Lauric acid	3.11	3.20	3.23	3.27	3.26
Myristic acid	10.52	10.61	10.69	10.73	10.76
Myristoleic acid	0.86	0.88	0.90	0.90	0.89
Pentadecanoic acid	1.19	1.21	1.21	1.22	1.23
Palmic acid	29.33	28.98	29.15	29.27	28.86
Palmitoleic acid	1.68	1.71	1.72	1.69	1.70
Stearic acid	12.81	12.61	12.35	12.39	12.09
Elaidic acid	2.74	2.73	2.70	2.64	2.73
Oleic acid	21.85	21.78	21.73	21.78	21.65
Linoleic acid	3.24	3.15	3.12	3.16	3.30

Note: data are presented as mean value ± 3.0% fractional

ning 4.02%. Similar results were obtained for pasteurized milk (Table 3). We should note that the analysis covered the whole spectrum of fatty acids (37 fatty acids). Those acids which are not included in Tables 2 and 3 showed no significant changes either.

Thus, the ultraviolet treatment of milk in the given range of exposure did not affect the physico-chemical properties of fatty acids and their composition.

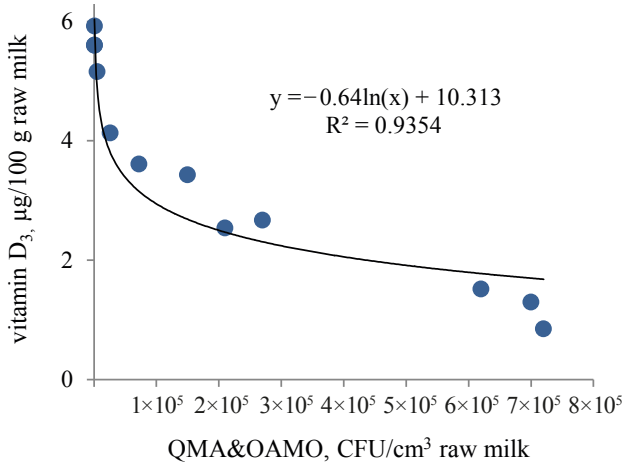

Fig. 2. Changes in vitamin D_3 content in raw milk depending on treatment time.

The study revealed a correlation between the changes in the vitamin D_3 content and the microbial load (CFU/cm^3) of milk (Figs. 2 and 3).

The data for both raw and pasteurized milk confirmed the interrelation between the vitamin D_3 content and QMA&OAMO and also indicated that an increase in the initial bacterial load negatively affected the vitamin D_3 growth.

Studying the influence of the volumetric milk flow rate (V = 0.04453–0.13359 m^3/s) and treatment duration (τ = 5–25 min) at different irradiation doses revealed a significant effect of UVR on the bacterial load in the given ranges (Table 4).

The feedstock in the experiments included raw milk with a bacterial load of 2.1×10^5 CFU/cm^3 and pasteurized milk with a bacterial load of 1×10^5 CFU/cm^3.

We found that the irradiation doses of over 30 mJ/cm^2 and the treatment duration of over 15 min allowed for a more intensive reduction of the bacterial load in raw milk, compared to pasteurized milk.

The experiments also showed an increase in the vitamin D_3 content within the UV treatment parameters specified in Table 4. This was confirmed by the results shown in Figs. 4 and 5.

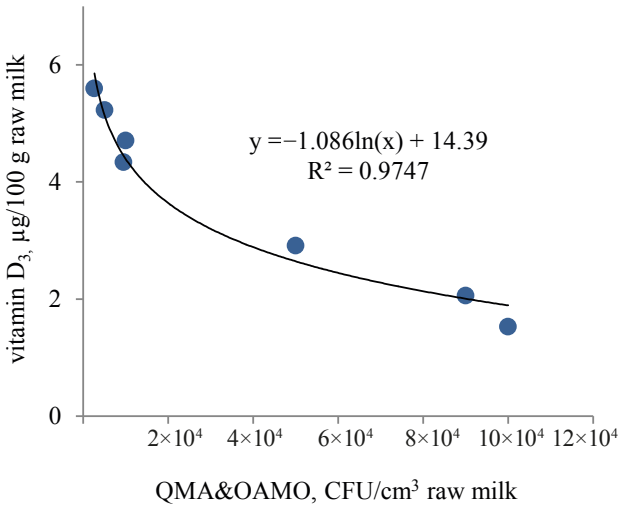

Fig. 3. Changes in vitamin D_3 content in pasteurized milk depending on treatment time.

Table 4. Effects of irradiation dose, duration, and volumetric flow rate on bacterial load

Treatment parameters			Number of microorganisms, CFU/cm³	
Duration, min	Volumetric flow rate, m³/min	Irradiation dose, mJ/cm²	Raw milk	Pasteurized milk
5	0.15585	17.7	2.6×10^4	–
15	0.15585	53.1	1.1×10^3	–
25	0.16476	82.5	8.0×10^2	–
5	0.17812	15.3	1.5×10^5	–
15	0.17812	30.6	5.1×10^3	–
25	0.17812	45.9	5.0×10^2	–
5	0.17812	15.3	–	9.0×10^4
15	0.17812	30.6	–	9.5×10^3
25	0.18702	58.8	–	5.0×10^3
5	0.16476	16.5	–	5.0×10^4
15	0.14249	57.6	–	1.0×10^4
25	0.14249	96.0	–	$2.6\cdot10^3$

The analysis showed that the irradiation doses below 5 mJ/cm² had an insignificant effect on the microbiological load and the vitamin D_3 content (Table 4; Figs. 4 and 5). Higher doses of irradiation, however, led to a rather intensive growth in vitamin D_3 and a decrease in the bacterial load.

On the whole, we found low-pressure gas-discharge irradiation sources effective in producing a considerable simultaneous effect on the vitamin D_3 content and the microbiological load of both raw and pasteurized milk within the treatment modes. At the same time, low irradiation doses up to H_e = 102 mJ/cm² did not have a significant effect on the protein and fatty acid composition of milk.

To assess changes in the vitamin D_3 content during storage, we treated raw and pasteurized milk with UVR at different durations in the range of 20–102 mJ/cm². After the treatment, the milk samples were stored at 4 ± 2°C. We found that the vitamin D_3 content in both raw and pasteurized milk remained almost unchanged

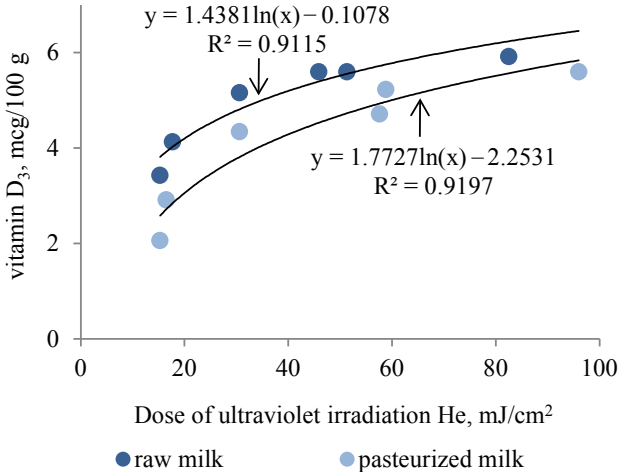

Fig. 4. Changes in vitamin D_3 content depending on ultraviolet irradiation dose.

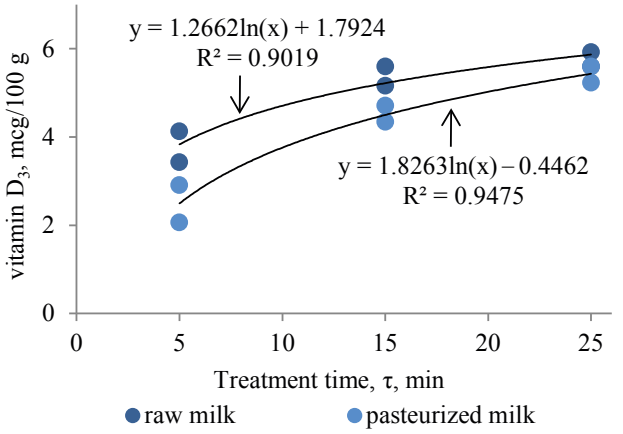

Fig. 5. Changes in vitamin D_3 content depending on treatment time.

Table 5. Changes in vitamin D_3 content during storage of raw and pasteurized milk

Sample number	Storage duration, h	Vitamin D_3 content, µg/100 g				
		Milk irradiation time, min				
		Feedstock	5	10	15	25
		Raw milk				
1	0	0.653	0.994	1.45	1.04	1.83
2	24	0.583	0.946	1.33	0.963	1.83
3	36	0.580	0.943	1.31	0.944	1.76
4	48	0.566	0.926	1.18	0.902	1.60
		Pasteurized milk				
5	0	0.357	0.562	2.01	1.93	3.15
6	24	0.313	0.525	1.83	1.84	2.95
7	36	0.310	0.526	1.82	1.81	2.90
8	48	0.286	0.480	1.82	1.55	2.40

during storage under standard conditions for two days (Table 5).

CONCLUSION

Thus, we found that the ultraviolet treatment of a 400 µm layer of milk with low-pressure gas-discharge

lamps at a wavelength of 253.7 nm in the dose range from 5 to 102 mJ/cm^2 makes it possible to simultaneously reduce the bacterial load and increase the vitamin D_3 content. The study proved that these processes are interrelated; furthermore, they do not cause any significant changes in the protein and fatty acid composition of milk, both after production and during storage. The patterns established are identical for both raw and pasteurized milk with slight changes during storage for 48 hours.

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Volatile aroma compounds in Moskovskaya cooked smoked sausage formed in different types of casings

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Abstract: The paper presents a study of Moskovskaya cooked smoked sausages formed in various artificial casings: fibrous (cellulose), collagen, and polyamide. An oxygen permeability oxygen permeability of the casings was above 40 cm^3 and below 30 $cm^3/m^2 \cdot 24$ h·bar. The study involved a sensory evaluation and instrumental tests using a VOCmeter multi-sensor system ('electronic nose') and a 7890A gas chromatograph with a 5975C VLMSD mass-selective detector (Agilent Technologies). We obtained original data on the qualitative composition and the quantitative content of substances that form the aroma of cooked smoked sausages in various types of casings. We found that the samples contained two groups of compounds with the chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. They had a ratio of (12–33):1 and were, apparently, the most significant aromatic substances. The main class of identified compounds was carboxylic acid esters, accounting for 76.61–81.60% of the total substances. We established a correlation between the aroma intensity and the concentration of chlorine-containing and nitrogen-containing compounds (except amines, amides, nitriles, and hydrazides) in the gas phase. The results did not confirm our hypothesis about the influence of the casing type and its permeability on the development of oxidative processes in the production of cooked smoked sausages. The practical significance of the study lies in creating a database of over 200 aromatic compounds that allows for a deeper understanding of aroma formation processes in cooked smoked sausages. The database can be used to exert a purposeful technological influence on the quality indicators and create various flavour compositions to adjust the sensory properties of the product.

Keywords: Flavours of meat products, sensory evaluation, 'electronic nose', gas chromatographic analysis, artificial casings

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INTRODUCTION

In recent decades, sausage producers have significantly expanded the range of casings for cooked, smoked, and cooked smoked sausages. The latter are highly popular, especially in the summer, due to their sensory characteristics, high nutritional value, long shelf-life, and a relatively low price compared to expensive dry sausages [1, 2]. Aroma is one of the key factors of consumer preference [3–9]. The classical technology of making cooked smoked sausages involves a fairly long cooking process that includes boiling, cooling, smoking (one or two stages), and drying. Such a process demands using only permeable casings [10–12]. Artificial casings made of collagen, cellulose, and polyamide are widely used by modern producers for various reasons. Some of them include standard characteristics of steam and gas permeability, as well as geometrical dimensions, which allow for automatic sausage forming [13]. Growing competition forces sausage producers to focus on technology, rather than the price or outcome, when choosing casings. In particular, they look at the effect that technology has on the product's sensory characteristics [14]. In this regard, of great scientific and practical interest is a study that aims to objectively assess the composition of volatile substances in the aroma of cooked smoked sausages formed in various types of artificial casings.

STUDY OBJECTS AND METHODS

Our objects of study were samples of Moskovskaya

cooked smoked sausage (whole sausages) produced by the same shift on the same day according to State Standard R 55455-2013. Boiled-smoked meat sausages. Specifications*. The sausages were formed in the following casings: sample no. 1 in a fibrous (cellulose) casing, sample no. 2 in a collagen casing, sample no. 3 in a highly permeable polyamide with an oxygen permeability above 40 $cm^3/m^2 \cdot 24$ h·bar, and sample 4 in a permeable polyamide casing with an oxygen permeability less than 30 $cm^3/m^2 \cdot 24$ h·bar.

All the samples were produced at a sausage factory. After cooling, they were packed in impermeable bags to preserve their aroma and sent to V.M. Gorbatov Federal Research Centre for Food Systems.

The sensory evaluation of sausages was carried out according to State Standard 9959-2015**. The taste panel consisted of 7 qualified experts. The results were confirmed by instrumental sensory data produced by the VOCmeter ('electronic nose'). The device is equipped with highly sensitive nanosensors capable of capturing volatile components released from the surface of the product. Prior to testing, the sausages were crushed and at least three 3 g samples were taken from each of them. The samples were placed in special vials and sealed. The vials were alternately placed into the chamber, where each sample was heated to 50°C. Then, the lid of the vial was punctured with a needle, and the gas phase was taken from near the sample surface. The gas phase entered the surface of the nanosensors sensitive to various classes of chemical compounds. Any physicochemical changes that occurred on the surface of the nanosensors were converted into an electronic signal, transmitted to a computer, and statistically processed by the software. We used four metal oxide nanosensors (M1–M4) sensitive to those aroma-producing volatile substances which are characteristic of meat products. They include products of protein breakdown, fat oxidation, ketones, aldehydes, volatile fatty acids, ammonia and other substances [15–16].

The composition of volatile aroma components was analysed by a 7890A gas chromatograph with a 5975C VLMSD mass-selective detector (Agilent Technologies, USA). For this, volatile substances were preliminarily extracted (1:1) with 40% aqueous ethanol and chloroform-methanol according to the Folch method, followed by methylation with a solution of acetyl chloride in methanol. The composition of aroma components was determined by a HP-5MS capillary column with a diameter of 0.25 mm, a length of 30 m, and a stationary phase layer thickness of 0.25 μm.

The chromatography was carried out under the following conditions:
– carrier gas: He;
– flow rate: 1 ml/min;
– injector temperature in a no-split mode: 250°C;
– initial temperature of the column thermostat: 100°C for 2 minutes;
– programmable heating from 100°C to 290°C at a rate of 20°C/min;
– an isotherm at 290°C: up to 25 min; and
– component analysis duration: 25 min.

The identification parameters were as follows:
– ion source temperature: 230°C;
– quadrupole temperature: 150°C;
– electron energy: 70 eV;
– scan mode: full; and
– mass range: 33–1050 amu.

The peaks were analysed using the NIST08 MS Library, an automated search and identification database, and the substances were named according to the IUPAC. The analysis covered those substances whose mass content in the mixture of volatile compounds exceeded 0.01%. The probability of peak correlation had to be at least 35% [17].

RESULTS AND DISCUSSION

The sensory evaluation of the Moskovskaya sausage samples in various casings did not reveal any significant differences in their consistency, colour, taste, or aroma. The tasters noted a more pronounced smoking aroma in samples no. 2 and 3, compared to samples no. 1 and 4, and a firmer surface layer in samples no. 1 and 2. They did not establish any differences in taste and aroma between samples no. 1, 2, and 3; however, they found them less pronounced in sample no. 4.

The 'electronic nose' was used to quantitatively identify the minimum differences in the gas phase aroma (Fig. 1).

The highly sensitive nanosensors revealed no significant differences in aroma between the samples. This was evidenced by the general nature of nanosensor responses, with the strongest signal coming from M4 and M2. Moreover, there was an image resembling a geometric figure and no intersection between the lines connecting the scale points that corresponded to the

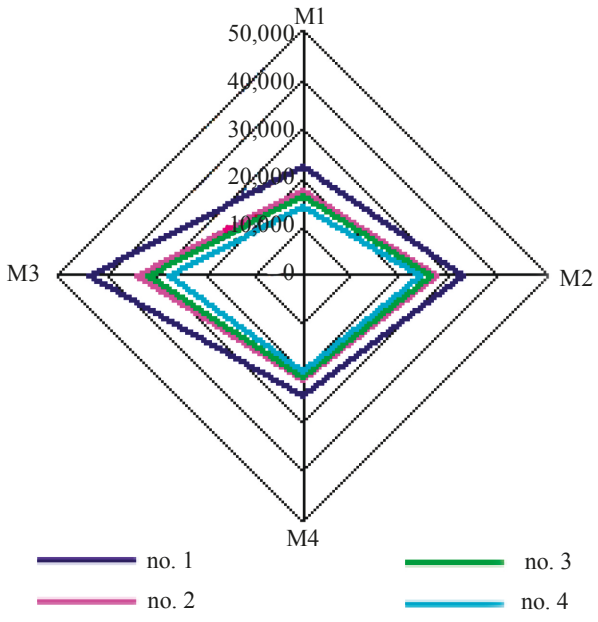

Fig. 1. Multisensory aroma profiles of Moskovskaya sausage samples produced by the 'electronic nose'.

*State Standard R 55455-2013. Boiled-smoked meat sausages. Specifications. Moscow: Standartinform Publ., 2014. 14 p.

**State Standard 9959-2015. Meat and meat products. General conditions of organoleptical assessment. Moscow: Standartinform Publ., 2016. 20 p.

signals of the four nanosensors. The multisensory profiles of samples no. 2 and 3 practically coincided, indeed.

The analysis of sample no. 1 showed stronger signals coming from M2 and M4. These nanosensors are sensitive to the presence of aldehydes, ketones, and heterocyclic aromatic compounds in the gas phase. This might result from more intensive oxidative processes and/or an increased concentration of volatile substances due to a rapid loss of moisture during heat treatment. Another reason might be a more intensive accumulation of substances that enter the product through the casing during smoking.

Table 1. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a fibrous casing (sample no. 1)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	8.196	862	869	880	1,582	2,171	0.20	0.10	4,6-Dimethyl-2-thioxo-1,2-dihydro-3-pyridinecarbonitrile	50
3	8.300	880	889	904	854	2,101	0.19	0.10	4-Acetamido-N,N-diisobutyl-3-nitrobenzamide	50
4	9.841	1,171	1,186	1,195	773	1,870	0.17	0.09	2-Pyrroline-3-carboxylic acid, 4-(4-chlorobenzylidene)-2-methyl-5-oxo-, methyl ester	46
5	10.884	1,375	1,387	1,399	3,141	4,046	0.37	0.19	2-Chloro-N-(1-m-tolyl-2,3-dihydro-1H-pyrrolo[2,3-b]quinolin-4-yl)-acetamide	38
6	12.160	1,621	1,633	1,642	2,296	3,677	0.34	0.17	3-Bromo-N'-(1-(2-thienyl)ethylidene)benzohydrazide	80
7	12.243	1,642	1,649	1,660	10,315	11,495	1.06	0.54	Methyltetradecanoate	87
8	12.383	1,660	1,676	1,687	33,372	33,679	3.10	1.57	2H-1-Benzopyran-2-one, 7-(4-methyl-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	64
9	13.519	1,879	1,895	1,909	14,416	21,256	1.96	0.99	9-Hexadecenoic acid, methyl ester, (Z)-	50
10	13.665	1,909	1,923	1,948	233,070	255,890	23.54	11.92	Pentadecanoic acid, 14-methyl-, methyl ester	95
11	13.960	1,963	1,980	1,996	7,701	9,879	0.91	0.46	Ether, methyl 1-tetradecenyl	50
12	14.163	2,005	2,019	2,035	1,730	3,353	0.31	0.16	10-Undecynoic acid, methyl ester	52
13	14.292	2,035	2,044	2,056	1,941	2,571	0.24	0.12	Heneicosanoicacid, methyl ester	50
14	14.443	2,056	2,073	2,089	1,233	3,928	0.36	0.18	Hydrazine, 1,1-diethyl-2-(1-methylethyl)-	47
15	14.817	2,119	2,145	2,158	605,268	1,087,059	100.00	50.65	9-Octadecenoic acid (Z)-, methyl ester	99
16	14.936	2,158	2,168	2,185	131,811	155,923	14.34	7.26	Octadecanoic acid, methyl ester	98
17	15.055	2,185	2,191	2,197	1,997	4,288	0.39	0.20	Ethanol, 2-[(2-ethylhexyl)oxy]-	91
18	15.112	2,197	2,202	2,212	2,377	4,235	0.39	0.20	Silane, triethyl-2-pentenyl-, (Z)-	38
19	15.200	2,212	2,219	2,233	6,390	9,057	0.83	0.42	Octadec-9-en-1-al dimethyl acetal	53
20	15.335	2,236	2,245	2,269	516	2,632	0.24	0.12	Acetamide, N-(4-hydroxycyclohexyl)-, trans-	37
22	15.834	2,335	2,341	2,347	4,034	7,132	0.66	0.33	1-Chlorosulfonyl-3-methyl-1-azaspiro[3.5]nonan-2-one	80
23	15.942	2,347	2,362	2,383	60,559	104,085	9.57	4.85	10-Undecenoyl chloride	43
24	16.083	2,383	2,389	2,407	2,145	7,197	0.66	0.34	Pentanoic acid, methylester	35
25	16.492	2,461	2,468	2,479	766	1,853	0.17	0.09	1,2-Ethanediamine, N,N,N'-trichloro-N',1,1,2,2-pentafluoro-	47
26	16.658	2,482	2,500	2,509	843	1,893	0.17	0.09	Cyclohexasiloxane, dodecamethyl-	37
28	17.042	2,569	2,574	2,617	36,177	71,967	6.62	3.35	2-Methyl-3,4,5,6-tetrahydropyrazin	84
30	18.692	2,884	2,892	2,911	825	2,221	0.20	0.10	Perhydro-htx-2-one, 2-depentyl-, acetate ester	38
32	19.383	3,016	3,025	3,034	699	1,891	0.17	0.09	5H-Cyclopropa[3,4]benz[1,2-e] azulen-5-one, 9,9a-bis(acetyloxy)-1,1a,1b,2,4a,7a,7b,8,9,9a-decahydro-2,4	43
39	22.631	3,640	3,651	3,670	4,500	16,866	1.55	0.79	Ledeneoxide-(II)	38
40	22.869	3,691	3,697	3,712	553	1,697	0.16	0.08	4-(3,4-Methylenedioxyphenyl)-2-butanone	46
41	23.035	3,718	3,729	3,736	757	1,644	0.15	0.08	Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy] phenyl]-1,1,1-trimethyl-	43
43	23.705	3,847	3,858	3,862	664	1,689	0.16	0.08	N-Methyl-1-adamantaneacetamide	35
44	23.954	3,898	3,906	3,922	583	2,230	0.21	0.10	2,4-Di-tert-butyl-6-(tert-butylamino)phenol	37
47	24.332	3,970	3,979	3,988	823	2,468	0.23	0.12	11H-Dibenzo[b,e][1,4]diazepin-11-one, 5,10-dihydro-5-[3-(methylamino)propyl]-	49

Table 2. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a collagen casing (sample no. 2)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	4.040	58	68	82	540	2,314	0.37	0.18	Butyric acid, 4-(4-chloro-5-methyl-3-nitro-pyrazol-1-yl)-	35
5	8.196	853	869	877	903	1,803	0.29	0.14	Indolizine, 6-ethyl-2-phenyl-	47
6	8.300	877	889	898	1,422	2,272	0.37	0.18	2,3,4-Trimethoxyphenylacetonitrile	64
7	9.841	1,168	1,186	1,195	885	2,408	0.39	0.19	[5-[(Furan-2-carbonyl)amino]-3-methylpy-razol-1-yl]acetic acid, ethyl ester	60
8	10.878	1,357	1,386	1,405	2,961	5,092	0.83	0.40	p-Pentyloxybenzylidene p-hexylaniline	53
9	12.155	1,603	1,632	1,642	2,568	4,091	0.66	0.32	Benzaldehyde, 2-(2-phenoxyethoxy)-, 1-cy-clohexylsemicarbazone	45
10	12.243	1,642	1,649	1,657	6,230	6,647	1.08	0.52	Pentanoic acid, 4-methyl-, methyl ester	72
11	12.388	1,663	1,677	1,687	64,541	61,950	10.04	4.81	2H-1-Benzopyran-2-one, 7-(4-methy-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	72
12	13.519	1,882	1,895	1,909	7,860	12,314	2.00	0.96	9-Octadecenoic acid (Z)-, methyl ester	53
13	13.659	1,909	1,922	1,936	125,948	139,968	22.68	10.87	Hexadecanoic acid, methylester	95
14	13.955	1,960	1,979	1,993	6,428	9,077	1.47	0.71	Butanoic acid, 2-hexenyl ester, (E)-	50
16	14.292	2,026	2,044	2,050	1,217	2,335	0.38	0.18	Undecanoic acid, methyl ester	64
17	14.443	2,065	2,073	2,086	1,732	3,725	0.60	0.29	Ethane, isothiocyanato-	43
19	14.801	2,116	2,142	2,158	367,029	617,113	100.00	47.91	9-Octadecenoic acid (Z)-, methyl ester	99
20	14.931	2,158	2,167	2,182	74,923	96,174	15.58	7.47	Octadecanoicacid, methyl ester	94
22	15.112	2,194	2,202	2,209	3,697	4,520	0.73	0.35	Silane, triethyl-2-pentenyl-, (Z)-	50
23	15.195	2,209	2,218	2,227	5,201	6,583	1.07	0.51	1-Hexadecen-3-ol, 3,5,11,15-tetramethyl-	43
24	15.740	2,314	2,323	2,335	5,643	8,862	1.44	0.69	4-Hexadecen-6-yne, (E)-	53
26	15.932	2,350	2,360	2,383	30,041	46,693	7.57	3.63	Hexadecanoic acid, 2-hydroxy-1-(hy-droxymethyl)ethyl ester	46
27	16.077	2,383	2,388	2,404	1,063	2,428	0.39	0.19	Dodecanoic acid, 2-methyl-	52
29	16.767	2,509	2,521	2,527	1,697	2,520	0.41	0.20	Silane, triethyl-2-pentenyl-, (Z)-	72
30	16.923	2,533	2,551	2,563	49,234	103,407	16.76	8.03	9-Oxabicyclo[6.1.0]nonane	86
31	17.032	2,563	2,572	2,611	14,819	44,786	7.26	3.48	Oxalic acid, isobutyl tridecyl ester	35
37	18.983	2,944	2,948	2,962	553	1,810	0.29	0.14	trans-2,3-Methylenedioxy-b-methyl-b-ni-trostyrene	53
38	19.180	2,977	2,986	3,013	1,353	6,405	1.04	0.50	4-Piperidineacetic acid, 1-acetyl-5-eth-yl-2-[3-(2-hydroxyethyl)-1H-indol-2-yl]-. alpha.-methyl-, methyl ester	38
39	19.585	3,055	3,064	3,082	669	2,209	0.36	0.17	4,6-Bis(diethylamino)-1,3,5-triazine-2-car-bonylhydrazide	43
44	21.515	3,421	3,436	3,442	978	3,758	0.61	0.29	N-Methyl-1-adamantaneacetamide	40

The statistical processing of the nanosensor signals showed the following multisensory profile areas that characterized the intensity of the samples' aroma ($S \cdot 10^7$, cu, $P > 0.95$): 179.06; 118.91; 106.51; and 84.87 for samples no. 1, 2, 3, and 4, respectively. Thus, if we take the aroma intensity of sample no. 4 (minimum value) as 100%, the intensity of samples no. 1, 2, and 3 was 211%, 140%, and 125%, respectively. These differences indicated a need for further analysis of volatile substances.

It is noteworthy that it was the first study into the composition of volatile components in cooked smoked sausages. The most studied aroma is that of fermented raw and dry sausages [3–6]. Moskovskaya cooked smoked sausage is only made of beef and fatback, as well as a nitrite-curing mixture, sugar, and spices (black pepper, cardamom or nutmeg). Therefore, it was an excellent model for studying aroma in this type of meat products.

Tables 1–4 present the identification and statistical processing results for volatile substances in the sausage samples obtained with the gas chromatograph software and the automated search and identification database [22].

We used the NIST08 MS Library automated database to identify volatile substances with a peak correlation probability of more than 35%. Of total volatile substances, we identified 85.9; 93.31; 94.43; and 93.72% of substances in samples no. 1, 2, 3, and 4, respectively. These amounts corresponded to the peaks presented in Tables 1–4.

The atomic composition of the identified volatile substances contained 10 elements from Mendeleev's Periodic Table, including hydrogen, carbon, oxygen, and nitrogen. These elements are the most typical in

Table 3. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a highly permeable polyamide casing (sample no. 3)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
2	4.128	76	85	103	410	1,452	0.30	0.15	Iron, (2-formyl norbornadiene)tricarbonyl	35
3	10.883	1,372	1,387	1,396	817	1,518	0.31	0.15	1,3-Dimethyl-7-O-tolyl-5,5-bis-trifluoro-methyl-5,8-dihydro-1H-pyrimido[4,5-d] pyrimidine-2,4-dione	45
5	12.238	1,642	1,648	1,660	3,520	4,767	0.97	0.48	Nonanoic acid, methyl ester	59
6	12.378	1,660	1,675	1,684	9204	11,033	2.24	1.12	1,2,3,4-Tetrahydroisoquinolin-6,7-diol, 1-phenylmethylene-, 2,6,7-triacetate	59
7	13.514	1,876	1,894	1,903	5,305	6,745	1.37	0.69	4-Nonenoic acid, methyl ester	42
8	13.654	1,909	1,921	1,948	111,673	120,593	24.53	12.25	Tridecanoic acid, methyl ester	97
12	14.796	2,119	2,141	2,158	300,232	491,592	100.00	49.92	9-Octadecenoic acid, methyl ester, (E)-	99
13	14.925	2,158	2,166	2,185	79,623	90,670	18.44	9.21	Octadecanoic acid, methyl ester	97
15	15.195	2,209	2,218	2,230	1,689	2,793	0.57	0.28	Pentanoic acid, 5,5-dimethoxy-, methyl ester	50
18	15.735	2,311	2,322	2,329	2,962	4,410	0,90	0.45	Methyl 3-hydroxyoctadec-9-enoate	74
20	15.927	2,350	2,359	2,383	25,335	41,069	8.35	4.17	15-Hydroxypentadecanoic acid	50
21	16.077	2,383	2,388	2,401	1,211	2,480	0.50	0.25	Methyl 18-methylnonadecanoate	43
24	16.923	2,530	2,551	2,566	43,792	92,020	18.72	9.34	9,17-Octadecadienal, (Z)-	42
25	17.032	2,566	2,572	2,632	12,672	32,128	6.54	3.26	Undecanoylchloride	35
27	18.371	2,827	2,830	2,851	749	3,101	0.63	0.32	Phenol, 4-[2-(5-nitro-2-benzoxazolyl) ethenyl]-	43
29	18.692	2,869	2,892	2,893	764	3,392	0.69	0.34	Alanine, 3,3,3-trifluo-ro-2-[(4-methoxybenzoyl) amino]-N-[3-(trifluoromethyl)-2-quinoxalinyl]-, ethyl ester	38
31	18.843	2,911	2,921	2,929	680	2,007	0.41	0.20	1,2-Benzenediol, O,O'-di(propargyloxycarbonyl)-	35
36	19.408	3,025	3,030	3,040	811	1,638	0.33	0.17	1,2,4-Benzenetricarboxylic acid, 1,2-dimethyl nonyl ester	35
42	20.84	3,298	3,306	3,313	556	1,507	0.31	0.15	1,2-Bis(trimethylsilyl)-3,6-dimethylcyclo-hexane-1,4-diene	47
43	21.224	3,373	3,380	3,388	703	1,999	0.41	0.20	2,6-Naphthalenediol, 1,5-bis[(piperonylimino)methyl]-	35
46	22.605	3,634	3,646	3,670	2,024	9,306	1.89	0.95	Benzamide, 3-me-thoxy-N-[4-(1-methylcyclopropyl) phenyl]-	43
48	23.29	3,769	3,778	3,790	558	1,665	0.34	0.17	Benzamide, N-(1,1-dimethylethyl)-4-me-thoxy-	47

products of animal and plant origin with a cellular structure. Also present were chlorine, sulphur, silicon, fluorine, bromine, and iron (Table 5).

The presence of organosilicon compounds was due to the use of a capillary column based on (5%-phenyl)-methylpolysiloxane. This group of compounds accounted for 0.36% to 0.64% of total volatile substances. Due to their origin and insignificant amount, they were excluded from further analysis.

As can be seen from Table 5, all the studied samples contained two groups of compounds with the general chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. Apparently, they were the most significant compounds in the aroma of Moskovskaya sausage. Their content was 33:1, 12:1, 32:1, and 25:1 in samples no. 1, 2, 3, and 4, respectively, which could be summarized as 12–33:1.

The greatest variety of compounds was found in sample no. 1 (fibrous casing) and sample no. 4 (permeable polyamide casing). The total amount of oxygen-containing compounds was slightly higher in sample no. 3 (highly permeable polyamide casing) than in sample no. 4 (polyamide casing with lower permeability). At the same time, the content of oxygen-containing compounds in sample 1 (fibrous casing) was 11.88% (absolute value) lower than in sample no. 3. Thus, the formation of a significant amount of oxygen-containing substances in the gas phase of a product could not be explained by the choice of casing or its degree of permeability.

Table 6 shows the content of volatile substances belonging to different classes of chemical compounds. As can be seen, carboxylic acid esters were the main

Table 4. Identification and analysis of major volatile substances in Moskovskaya sausage formed in a permeable polyamide casing (sample no. 4)

Peak no.	Time, min	Mass scanning			Peak height, cu	Peak area, cu	Content, % of max. peak	Content, % of amount	Substance	Probability of peak identification for standard mass spectrum, %
		Start	Max.	Finish						
1	2	3	4	5	6	7	8	9	10	11
1	3.868	28	35	61	1,153	3,970	0.28	0.16	Benzeneethanamine, N-[(pentafluorophenyl)methylene]-4-[(trimethylsilyl)oxy]-	35
3	8.196	859	869	877	1,998	2,381	0.17	0.10	4,6-Dimethyl-2-thioxo-1,2-dihydro-3-pyridinecarbonitrile	50
4	8.3	883	889	904	1,594	2,321	0.17	0.09	2-Methyl-7-phenylindole	47
5	8.907	985	1,006	1,021	851	2,086	0.15	0.08	10-Undecynoic acid, methyl ester	72
6	9.846	1,171	1,187	1,201	580	1,671	0.12	0.07	1,2-Dihydroindeno[1,2,3-cd]pyrene	37
7	10.884	1,378	1,387	1,402	3,894	4,724	0.34	0.19	(6-Phenylsulfanyl-5-trifluoromethylpyridin-3-yl)carbamic acid, prop-2-ynyl ester	50
8	11.408	1,474	1,488	1,501	624	1,748	0.12	0.07	2-p-Chlorophenyl-6,8-dimethyl-4-[1,2-epoxy-2-propyl]quinoline	35
10	12.248	1,639	1,650	1,663	12,357	14,835	1.06	0.59	Tridecanoic acid, 12-methyl-, methyl ester	83
11	12.388	1,663	1,677	1,690	90,045	84,605	6.04	3.37	2H-1-Benzopyran-2-one, 7-(4-methyl-5-phenyl-2H-1,2,3-triazol-2-yl)-3-phenyl-	59
12	12.959	1,768	1,787	1,795	807	2,070	0.15	0.08	Cyclopentanetridecanoicacid, methyl ester	53
13	13.519	1,876	1,895	1,903	18,196	25,427	1.82	1.01	9-Octadecenoic acid (Z)-, methyl ester	95
14	13.67	1,909	1,924	1,948	270,802	327,084	23.36	13.03	Pentadecanoic acid, 14-methyl-, methyl ester	97
15	13.96	1,963	1,980	1,990	6,884	10,126	0.72	0.40	trans-2-Decen-1-ol, methyl ether	50
16	14.158	2,005	2,018	2,026	1,961	3,616	0.26	0.14	2,4,3,5-Diethylidene-l-xylose	50
17	14.292	2,038	2,044	2,056	2,635	3,243	0.23	0.13	Dodecanoic acid, methylester	64
19	14.817	2,110	2,145	2,161	749,047	1,400,183	100.00	55.78	9-Octadecenoic acid (Z)-, methyl ester	99
20	14.936	2,161	2,168	2,182	137,725	172,630	12.33	6.88	Octadecanoicacid, methyl ester	97
21	15.05	2,182	2,190	2,194	1,853	4,677	0.33	0.19	Cyclopropanenonanoic acid, methyl ester	47
22	15.117	2,194	2,203	2,212	3,839	6,142	0.44	0.25	Silane, triethyl-2-pentenyl-, (Z)-	50
23	15.2	2,212	2,219	2,242	6,331	8,829	0.63	0.35	Phytol	38
24	15.745	2,317	2,324	2,335	6,674	10,928	0.78	0.44	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	64
25	15.828	2,335	2,340	2,350	3,107	6,316	0.45	0.25	4-(6-Methyl-4-methylene-3,4,5,6-tetrahydro-2H-pyran-2-yl)-1-butanol	38
27	16.083	2,383	2,389	2,401	1,515	2,373	0.17	0.10	Pentanoicacid, methyl ester	58
31	16.933	2,530	2,553	2,563	96,726	174,952	12.49	6.97	9-Oxabicyclo[6.1.0]nonane	86
32	17.037	2,563	2,573	2,605	26,231	67,728	4.84	2.70	Eicosanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	38
35	17,909	2,725	2,741	2,743	953	2,876	0.21	0.12	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	37
45	22,158	3,556	3,560	3,580	663	2,067	0.15	0.08	Benzamide, 4-methoxy-N-[4-(1-methylcyclopropyl)phenyl]-	35

class of identified compounds in all the samples. Their mass fraction in the total amount of identified substances ranged from 76.61% to 81.60%. Another 4 classes of compounds, present in all the samples, were represented less evenly. For example, the content of alcohols, oxygen-containing heterocycles (except ketones and aldehydes), and nitrogen-containing heterocycles (except heterocyclic amines, amides and hydrazides) ranged from 0.3% to 0.51%, 0.2% to 8.03%, and 0.26% to 5.02%, respectively.

A detailed analysis of the classes of substances present in the aroma of the samples, as well as their elemental analysis, did not reveal any relationship between the type and permeability of the casing and the characteristics of volatile substances.

Carboxylic acid esters were mainly represented by methyl esters and less frequently by ethyl esters. On the one hand, this could be explained by the sample preparation method using methylation. On the other hand, methyl and ethyl esters could already be present in the product during its manufacture. The esters identified in the samples differed in their molecular weight, chain length, and the presence of not only carbon, hydrogen, and oxygen, but also nitrogen, chlorine, and fluorine.

Table 5. Atomic composition of volatile compounds in Moskovskaya sausage aroma

Chemical formula of identified compounds	Content of identified compounds, % of total amount			
	Sample			
	no. 1	no. 2	no. 3	no. 4
1	2	3	4	5
C_iH_k	–	0.69	–	0.07
$C_iH_kN_l$	3.35	0.14	–	0.09
$C_iH_kN_lF_mCl_n$	0.09	–	–	–
$C_iH_kN_lS_m$	–	0.29	–	–
$C_iH_kN_lS_mSi_n$	0.10	–	–	0.10
$C_iH_kO_l$	74.12	84.46	87.41	89.12
$C_iH_kO_lCl_m$	4.85	–	3.26	–
$C_iH_kO_lN_m$	2.27	7.00	2.76	3.57
$C_iH_kO_lN_mBr_nS_pSi_q$	0.17	–	–	–
$C_iH_kO_lN_mCl_n$	0.28	0.18	–	0.07
$C_iH_kO_lN_mF_n$	–	–	0.49	–
$C_iH_kO_lN_mF_nSi_p$	–	–	–	0.16
$C_iH_kO_lN_mS_nCl_p$	0.33	–	–	–
$C_iH_kO_lN_mS_nF_p$	–	–	–	0.19
$C_iH_kO_lN_m Si_n$	0.08	–	–	–
$C_iH_kO_lS_m$	–	–		0.10
$C_iH_kO_lSi_m$	0.09	–	–	–
$C_iH_kO_lFe_m$	–	–	0.15	–
$C_iH_kSi_l$	0.20	0.55	0.36	0.25
Total identified compounds	85.93	93.31	94.43	93.72
Including compounds containing:				
- oxygen	82.19	91.64	94.07	93.21
- nitrogen	6.67	7.61	3.25	4.18
- chlorine	5.55	0.18	3.26	0.07
- sulphur	0.60	0.29	–	0.39
- silicon	0.64	0.55	0.36	0.51
- fluorine	0.09	–	0.49	0.35

In total, we found over 35 compounds with a number of carbon atoms from 6 to 23. The most represented in all the samples were the methyl esters of oleic acid with the number of carbon atoms C_{19} (Table 7). The predominance of this ester was due to the fatty acid composition of fatback: the content of this monounsaturated acid ranged from 30% to 45% of the total fatty acids.

We were mostly interested in those groups of substances which were found in all Moskovskaya sausage samples as a result of the sensory evaluation and the "electronic nose" tests. The data allowed us to check our hypothesis about a correlation between the aroma intensity established by the "electronic nose" and the total content of substances in the gas phase of the samples (Table 8).

The correlation analysis produced an unexpected result: an increase in the aroma intensity was proportional to the increase in the content of nitrogen-containing heterocycles and chlorine-containing substances. In that case, it was reasonable to consider only positive values of the correlation coefficients, since the hypothesis that the nanosensor signals increased as the concentration of certain substances decreased had no physical sense.

Table 6. Major classes of chemical compounds in Moskovskaya sausage aroma

Class	Content of compounds by class, % of total amount			
	Sample			
	no. 1	no. 2	no. 3	no. 4
1	2	3	4	5
Hydrocarbons				
alkanes	–	0.98	–	–
arenes	–	0.14	–	0.07
Oxygen-containing				
alcohols	0.3	0.51	0.32	0.45
aldehydes	–	–	9.34	–
carboxylic acid	–	0.18	4.17	–
esters	77.9	76.61	77.5	81.60
heterocyclic aldehydes (including nitrogen-containing)	-	0.32		0.14
heterocyclic ketones (including nitrogen-containing)	0.62		0.15	3.37
other oxygen-containing heterocycles	0.79	8.03	0.2	7.22
Nitrogen-containing				
amines	0.09	0.4	–	0.12
amides	0.08	0.29	–	–
hydrazines	0.18	–	–	–
nitriles	–	0.18	–	–
heterocyclic amines	–	–	–	0.16
heterocyclic amides	0.41	–	1.12	0.08
heterocyclic hydrazides	0.17	0.17	–	–
other nitrogen-containing heterocycles	5.02	4.95	1.12	0.26
Iron-containing				
heterocycles	–	–	0.15	–
Total (without silicon-containing compounds)	85.29	92.76	94.07	93.21

CONCLUSION

The study produced original data on the qualitative composition and the quantitative content of substances that form the aroma of Moskovskaya cooked smoked sausage. It involved a detailed comparative analysis of the main classes of compounds present in the gas phase of the samples formed in various types of casings. We found that all the samples contained two groups of compounds with the general chemical formulas of $C_iH_kO_l$ and $C_iH_kO_lN_m$. With a ratio of (12–33):1, they appeared to be the most significant in the formation of the Moskovskaya sausage aroma. Furthermore, we established that carboxylic acid esters were the main class of compounds identified in all the samples. Their mass fraction ranged from 76.61% to 81.60% of the total substances.

The data revealed no relationship between the oxidative processes and the degree of casing permeability. The correlation analysis identified the main chemical compounds that increase the intensity of cooked smoked sausages.

The practical significance of the study lies in creating a database of over 200 aromatic compounds.

Table 7. Elemental composition of esters identified in the gas phase of Moskovskaya sausage samples

Ci in the ester molecule	Chemical formula of identified esters	Total amount of esters with Ci in the gas phase of samples, % of total substances			
		Sample no. 1	Sample no. 2	Sample no. 3	Sample no. 4
1	2	3	4	5	1
C_6	$C_6H_{12}O_2$	0.34	–	–	0.1
C_7	$C_7H_{14}O_2$	–	0.52	–	–
C_8	$C_8H_{16}O_4$	–	–	0.28	–
C_{10}	$C_{10}H_{18}O_2$, $C_{10}H_{20}O_2$	–	0.71	1.17	–
C_{11}	$C_{11}H_{19}ClO$, $C_{11}H_{21}ClO$, $C_{11}H_{22}O$	4.85	–	3.26	0.4
C_{12}	$C_{12}H_{20}O_2$, $C_{12}H_{24}O_2$	0.16	0.18	–	0.08
C_{13}	$C_{13}H_{15}N_3O_4$, $C_{13}H_{24}O_2$, $C_{13}H_{26}O_2$	–	0.38	–	0.32
C_{14}	$C_{14}H_{10}O_6$, $C_{14}H_{12}ClNO_3$, $C_{14}H_{28}O_2$	0.09	–	12.45	–
C_{15}	$C_{15}H_{30}O$, $C_{15}H_{30}O_2$	1.00	–	–	0.59
C_{16}	$C_{16}H_{11}F_3N_2O_2S$, $C_{16}H_{27}NO_3$	0.10	–	–	0.19
C_{17}	$C_{17}H_{32}O_2$, $C_{17}H_{34}O_2$	12.91	10.87	–	13.03
C_{19}	$C_{19}H_{36}O_2$, $C_{19}H_{36}O_3$, $C_{19}H_{36}O_4$, $C_{19}H_{38}O_2$, $C_{19}H_{38}O_4$	57.91	63.45	59.58	63.75
C_{20}	$C_{20}H_{28}O_6$, $C_{20}H_{40}O_2$	0.42	–	0.17	–
C_{21}	$C_{21}H_{32}O_2$, $C_{21}H_{42}O_2$	–	–	0.25	0.44
C_{22}	$C_{22}H_{18}F_6N_4O_4$, $C_{22}H_{44}O_2$	0.12	–	0.34	–
C_{23}	$C_{23}H_{32}N_2O_4$, $C_{23}H_{46}O_4$	–	0.5	–	2.7

Table 8. Correlation coefficients between aroma intensity ('electronic nose') and groups of substances in the samples gas phase

Groups of substances in the product gas phase	Correlation coefficient between groups of substances and aroma intensity
1	2
Substances with the general formula $C_iH_kO_l$	–0.9932
Substances with the general formula $C_iH_kO_lN_m$	–0.2812
All oxygen-containing substances	–0.9540
incl. oxygen-containing heterocycles	–0.5121
All nitrogen-containing substances	0.5812
incl. nitrogen-containing heterocycles (except amines, amides, nitriles, and hydrazides)	0.7927
All chlorine-containing substances	0.8128
Alcohols	–0.5419
Esters	–0.4805
incl. esters with total carbons C19	–0.7561

This database allows for a deeper understanding of aroma formation processes in cooked smoked sausages under various technological conditions. As a result, we can exert a purposeful influence on the quality indicators and create various flavour compositions to adjust the sensory properties of the finished product.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Optimisation of functional sausage formulation with konjac and inulin: using D-Optimal mixture design

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Abstract: In this study, we applied the D-optimal mixture design method to optimise prebiotic sausage formulation with inulin, konjac (*Amorphophallus konjac* L.), and starch. Also, we investigated the effect of each component individually as well as their mixtures on cooking characteristics, texture, colour and sensory properties of prebiotic sausages. The results of this study revealed that the increase in inulin content in the formulations of sausages led to lower frying loss, and increased water holding capacity (WHC), lightness, and overall acceptability. The incorporation of konjac increased the cooking yield, hardness, cohesiveness, redness, and yellowness. On the other hand, konjac added into the sausage formulation decreased overall acceptability. The mixtures of inulin, konjac, and starch improved the cooking characteristics and overall acceptability of the sausages without significant negative effect on the color or sensory properties. The results of the study clarified that the optimum amounts of inulin, starch, and konjac were 2.09; 2.76; and 0.146 %, respectively. The obtained results make it possible to use the combination of these components to produce prebiotic sausage.

Keywords: Inulin, konjac, sausage, functional, formulation

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INTRODUCTION

In recent years, unhealthy food habits and stressful life style have significantly increased the risk of serious health disorders such as obesity, cancer, high blood cholesterol, and coronary heart diseases. This has created and increased demand for new health products with enhanced nutritional value. As a result, a number of research have been conducted in order to develop foods that are designed to improve digestive system health. One of these approaches is the development of functional foods using probiotics or prebiotics. Prebiotics can improve the host health by stimulating the growth of beneficial bacteria in gastrointestinal tract [12]. Along with the nutritional value of a functional product, its structural properties, such as water holding capacity (WHC) and sensory characteristics, and effective cost should be taken into consideration [23].

Inulin is a dietary fibre that has been approved by WHO as a safe prebiotic. It is a well-known and successful food ingredient in meat industry due to its unique ability to enhance both taste and texture in various processed meat products through binding water, forming gel and mimics the oral tactile sensation of fat. The effectiveness of inulin has been approved in many investigations in a wide range of processed meat products such as scalded sausages, canned meat products, meat balls, liver pâté, and fermented sausages [19].

Konjac glucomannan, a neutral polysaccharide made from the tuber *Amorphophallus konjac*, is another prebiotic that is known for its important technological properties and its ability to improve health. USDA recently accepted the use of konjac as a binder in meat and poultry products. Studies suggested konjac has the ability to lower serum cholesterol, serum triglyceride, glucose, bile acid levels and laxative effect as well (Yang *et al.*, 2017).

Some investigations reported that appropriate amounts of konjac in the diet could help prevent diabetes

Table 1. Sausage samples with konjac (K), inulin (I), and starch (S) in a three component constrained D-optimal mixture design

Samples	Ingredients, %		
	K	I	S
1	0.5	0	4.5
2	0.375	1.188	3.438
3	0	0	5
4	0	2.5	2.5
5	0.5	0	4.5
6	0.5	4.5	0
7	0	0	5
8	0	5	0
9	0.125	3.688	1.188
10	0.375	3.438	1.188
11	0.5	2.25	2.25
12	0.25	4.75	0
13	0.125	1.188	3.688

and aid gradual weight loss. Several studies used konjac as a fat substitute, emulsifier, and gelling and thickening agent in various meat products, such as low-fat frankfurter, bologna sausage, hot dogs, pepperoni, and summer sausage [10]. Usually, the use of konjac in large amounts decreases the firmness of meat products, and its combination with other ingredients such as inulin, starch or carrageenan, could moderate undesirable effect.

Mixture design methodology is a new method to determine an effect of each ingredient in the formulation of processed meat products and demonstrate the result of ingredient interactions by applying reduced numbers of experimental trials [1].

It should be noted that this is the first investigation on effects of inulin and konjac on the physical and sensory properties of functional sausage. Hence, the objective of this research is to determine the influence of adding inulin, konjac, starch, and their mixtures on properties of sausages using the D-optimal mixture design and develop the optimal formulation to produce a high quality sausage.

STUDY OBJECTS AND METHODS

1. Experimental design. To determine the optimum proportions of the prebiotic sausage formulation, we used Design-Expert (7.1.5) software. D-optimal design was used with three components: konjac (K), inulin (I) and starch (S). The experimental design and the amounts of the relevant ingredients used are shown in Table 1. The component ranges were as follows: $0 < K < 0.5$; $0 < I < 5$; and $0 < S < 5$. Design-Expert software designed 13 samples. Effects of inulin, starch, and konjac on properties of sausage were evaluated, and optimum combination was determined. For optimization, depending on the influence of each factor; the combination of factors that led to the best responses was determined.

2. Sausage preparation. We prepared minced meat for sausage according to a basic formulation. The minced meat consisted of 55% lean beef meat with fat content of about 12.8 ± 1%, 10% soybean oil, 2.2% wheat flour, 1.5% sodium chloride, 0.35% sodium polyphosphate, 0.012% sodium nitrate, 0.02% ascorbic acid, 0.2% red pepper, 0.2% ginger, 0.1% savory, 0.2% garlic powder, and 17.418% water. All the ingredients were mixed in a 3,000 RPM cutter (Talsa Bowel cutter 15, Spain). Then 13 sausage samples were produced (5 kg each). Each sample contained 4,750 g of the minced meat and various proportions of konjac, inulin, and starch treatment (Table 1). The sum of starch, inulin, and konjac in each sample was 5%. Sausages were stuffed into polyamide casings and cooked in a steam oven at 80°C for 60 min until reaching an internal temperature of 72 ± 3°C. Inulin (Inulin Frutafit TEX®) and konjac flour was obtained from Roosendaal (the Netherlands) and Shandong (China), respectively.

3. Physical properties

3.1. Water holding capacity (WHC). The WHC of the sausages was measured using the method described by Asgharzadeh *et al*. and Méndez-Zamora *et al.* [5, 18]. About 0.3 g of sausage was placed between two filtre papers and then placed between two 12×12 cm plates. Four kg force was applied for 20 min. The released liquids in the paper were considered as meat-free water. WHC was calculated using Eqs. (2) and (3). The experiment was performed in triplicate for each sample.

$$\% \text{ of free water} = [(Iw\text{-}Fw)/Iw]\times 100, \quad (2)$$

$$WHC = 100 - \% \text{ of free water}, \quad (3)$$

where Iw is the initial weight of the sample (0.3 g) and Fw is the final weight.

3.2. Cooking yield. A slice of raw sausage 3 mm in thickness was cooked on a hot plate at 160°C for 2 min according to the procedure described by Amini *et al.* [4]. Cooking yield was calculated using the initial and final weights and expressed in g/100 g the initial sample weight. Three replicates were carried out for each sample.

3.3. Frying loss. Frying loss was determined based on the procedure described by Bengtsson *et al*. with some modification [6]. Sliced cooked sausages, 1 cm in thickness, deep fat fried in a fryer (moulinex, DR5), maintained at 174°C, for 2 min until the center temperature reached 72–73°C and then left to cool at room temperature. The frying loss was calculated by weighing the samples before and after frying. The test was done in triplicate for each sample.

4. Texture profile analysis (TPA). Texture profile analysis (TPA) was evaluated using an Instron M350-10CT (500 N load cell, England, Rochdale). The textural parameters were determined according the Procedure described by Bourne [7]. Textural measurements included hardness and cohesiveness.

5. Colour. Four samples from each formulation were used to evaluate internal colour (cross-section) of the sausages. For that, we used 2 cm cross-sections of recently cut sausage. The colour values of the samples were determined using a Chromo meter (CR-400, Minolta Co, Konica, Japan) with D65, 2° observer to objectively measure CIE Lab values (L* relative lightness, a* relative redness and b* relative yellowness). Colorimeter calibrated with white standard plate (L* = 94, a* = 0.3158, b* = 0.3322). The calculated results were expressed with mean value of these measurements.

6. Sensory evaluation. Sensory analyse was performed according to the international standards (ISO, 1985) in the sensory laboratory at the National Nutrition and Food Technology Research Institute (NNFTRI). Private stands under white fluorescent lights were prepared for each panelist. Samples of each formulation were presented randomly for panelists. Tap water was available to clear the taste between samples. 15 panelists, 7 men and 8 women, comprising of postgraduate students of food science and technology were asked to evaluate characteristics using a 9-point hedonic scale. The age of the panelists ranged from 20 to 40 years old. The panelists were trained with two training sessions in the product and terminology. Overall acceptability of the samples was scored as follows: 1 (extremely dislike) to 9 (extremely like).

7. Statistical and data analysis. Three equation models were fitted to each of the responses (Y) with the independent variables:

$$\text{Linear model: } Y = b1X1 + b2X2 + b3X3;$$

$$\text{Quadratic model: } Y = b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3; \text{ and}$$

$$\text{Cubic model: } Y = b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b123X1X2X3,$$

where X1is konjac, X2 is inulin, X3 is starch, and b is the regression coefficients calculated from the experimental data by multiple regression.

All parametric tests were performed in triplicate for each experiment and all the data demonstrated the mean and SD (standard deviation). The physicochemical and textural properties were studied using one-way ANOVA independently, and Duncan test was employed to determine differences between the experimental groups ($p < 0.05$). Sensory evaluation was analyzed by the same software using Mann–Whitney U test. Correlation analyses were conducted by using the Pearson correlation model where $p < 0.05$ was taken as significance.

RESULTS AND DISCUSSION

Fitting for the optimal model. The optimal model was fitted according to low standard deviation, low predicted sum of squares and high R-squared. P-values of the acceptable model were lower than 0.05.

For frying loss, cooking yield, hardness and overall acceptability, linear was found the best model. For cohesiveness, a* and b* quadratic was adequately fitted. The model which best matched to water holding capacity and L* were modified special cubic and special cubic, respectively.

Water Holding Capacity (WHC). According to the regression coefficients in Table 3, all three components increased WHC, however konjac had the greatest effect. Interestingly, the mixtures of inulin, starch, and konjac showed a substantial effect on increasing the WHC of sausages. This result is well correlated with results illustrated in Table 2, where samples no. 2 and 11 demonstrated the highest WHC.

The results revealed that, although adding inulin to the formulation of sausage could enhance WHC, the higher levels of inulin (more than 2.5%) decreased the WHC significantly. Sample no. 8 (contained 5% inulin) demonstrated the least WHC. The synergetic effect of konjac and inulin in absorbing water is in agree with the study of Mendez-Zamora *et al*. He involved inulin and pectin in the formulation of frankfurter sausages and showed that the addition of 15% inulin and pectin improved WHC [18]. Studies performed by [9] showed that konjac blend usually had been used as multi-ingredient fat replacer in meat products. In addition, incorporation of konjac blend with carrageenan and starch in low fat bologna increased WHC, produced more stable gel matrix with higher cooking yield and more acceptable texture. López-López *et al.* (described the type of fibers and quantity of their polysaccharides are the factors that influence water holding capacity of product [17]. They mentioned large particles create open structures that enhance the properties of hydration. Álvarez and Barbut also investigated the effect of beta-glucan (BG), inulin, and their mixture on the emulsion stability, and concluded combination of BG and inulin compensa-

Table 2. Cooking and sensory characteristics of experimental sausage samples

RUN	WHC	Cooking yield	Frying loss	Overall acceptability	Hardness	Cohesiveness	L*	a*	b*
1	63.78 ± 0.19	95.09 ± 0.16	21.05 ± 0.21	5.18 ± 0.11	22.63 ± 0.24	0.69 ± 0.01	39.84 ± 0.08	10.98 ± 0.09	15.56 ± 0.16
2	73.08 ± 0.21	93.58 ± 0.14	19.44 ± 0.17	5.57 ± 0.10	23.47 ± 0.21	0.59 ± 0.00	37.56 ± 0.08	8.39 ± 0.11	13.24 ± 0.19
3	59.37 ± 0.14	90.38 ± 0.14	17.59 ± 0.14	5.80 ± 0.10	21.10 ± 0.23	0.66 ± 0.01	38.01 ± 0.07	11.64 ± 0.08	16.19 ± 0.17
4	51.5 ± 0.22	89.76 ± 0.23	16.04 ± 0.12	6.27 ± 0.07	23.71 ± 0.24	0.62 ± 0.00	38.31 ± 0.08	12.27 ± 0.13	16.62 ± 0.16
5	63.77 ± 0.16	95.09 ± 0.16	21.03 ± 0.18	5.16 ± 0.09	22.63 ± 0.24	0.68 ± 0.00	39.83 ± 0.07	10.96 ± 0.07	15.59 ± 0.19
6	68.36 ± 0.28	93.86 ± 0.15	18.29 ± 0.21	6.06 ± 0.10	27.31 ± 0.22	0.62 ± 0.01	40.56 ± 0.07	10.58 ± 0.12	16.56 ± 0.19
7	59.37 ± 0.14	90.4 ± 0.16	17.62 ± 0.18	5.78 ± 0.07	21.09 ± 0.21	0.65 ± 0.00	38.01 ± 0.07	11.64 ± 0.09	16.19 ± 0.16
8	36.01 ± 0.22	89.07 ± 0.22	14.52 ± 0.15	6.83 ± 0.15	26.29 ± 0.21	0.71 ± 0.01	40.58 ± 0.07	18.25 ± 0.07	21.28 ± 0.18
9	54.16 ± 0.23	90.57 ± 0.17	16.20 ± 0.19	6.38 ± 0.12	25.33 ± 0.24	0.60 ± 0.00	38.88 ± 0.07	11.88 ± 0.14	15.97 ± 0.19
10	72.10 ± 0.17	92.97 ± 0.15	18.07 ± 0.19	6.05 ± 0.15	25.84 ± 0.25	0.58 ± 0.02	38.11 ± 0.07	8.98 ± 0.12	14.18 ± 0.18
11	82.96 ± 0.20	94.48 ± 0.15	19.62 ± 0.14	5.64 ± 0.14	25.00 ± 0.25	0.60 ± 0.02	36.78 ± 0.08	8.61 ± 0.14	14.34 ± 0.15
12	52.84 ± 0.14	91.49 ± 0.22	16.42 ± 0.21	6.46 ± 0.15	26.82 ± 0.24	0.61 ± 0.00	40.66 ± 0.06	12.51 ± 0.13	16.64 ± 0.14
13	62.25 ± 0.12	91.23 ± 0.14	17.71 ± 0.14	5.91 ± 0.15	22.74 ± 0.26	0.6 ± 0.010	37.82 ± 0.07	9.44 ± 0.12	13.93 ± 0.19

Table 3. Regression coefficients and correlation for the adjusted model to experimental data in D-optimal mixtures design for physical properties, textural parameters, color parameters, and sensory analysis

Parameter	K	I	S	KI	KS	IS	KIS	Pred-R^2
WHC	103.49	36.00	59.37	284.30	–	15.27	682.63	0.9997
Cooking yield	137.18	89.06	90.40	–	–	–	–	0.9951
Frying loss	10.40	2.90	3.51	–	–	–	–	0.9939
Overall acceptability	–0.01	6.99	5.99	–	–	–	–	0.9579
Hardness	36.44	26.31	21.10	–	–	–	–	0.9908
Cohesiveness	16.15	0.70	0.65	–18.13	–16.89	–0.23	–	0.8950
L*	6.44	40.58	38.01	37.70	55.36	–3.95	–129.30	0.9961
a*	631.78	18.26	11.64	–767.03	–696.53	–10.72	–	0.9981
b*	783.11	21.27	16.20	–899.03	–859.00	–8.41	–	0.9923

ted undesirable effect of fat reduction by increasing WHC [3]. Liu *et al*. also prepared konjac-egg white protein gels and determined that konjac could significantly improve the water retention capacity [15].

Cooking yield and frying loss. The results in Table 3 revealed that konjac with its positive coefficient had significantly ($p < 0.05$) increased cooking yield, while inulin and starch with their negative coefficient decreased this parameter in the product. The samples no. 1 and 5, which contained highest amount of konjac (0.5% konjac, 4.5% starch, and 0% inulin), showed the highest cooking yield and frying loss. As one can see in Table 1, two pair sam-

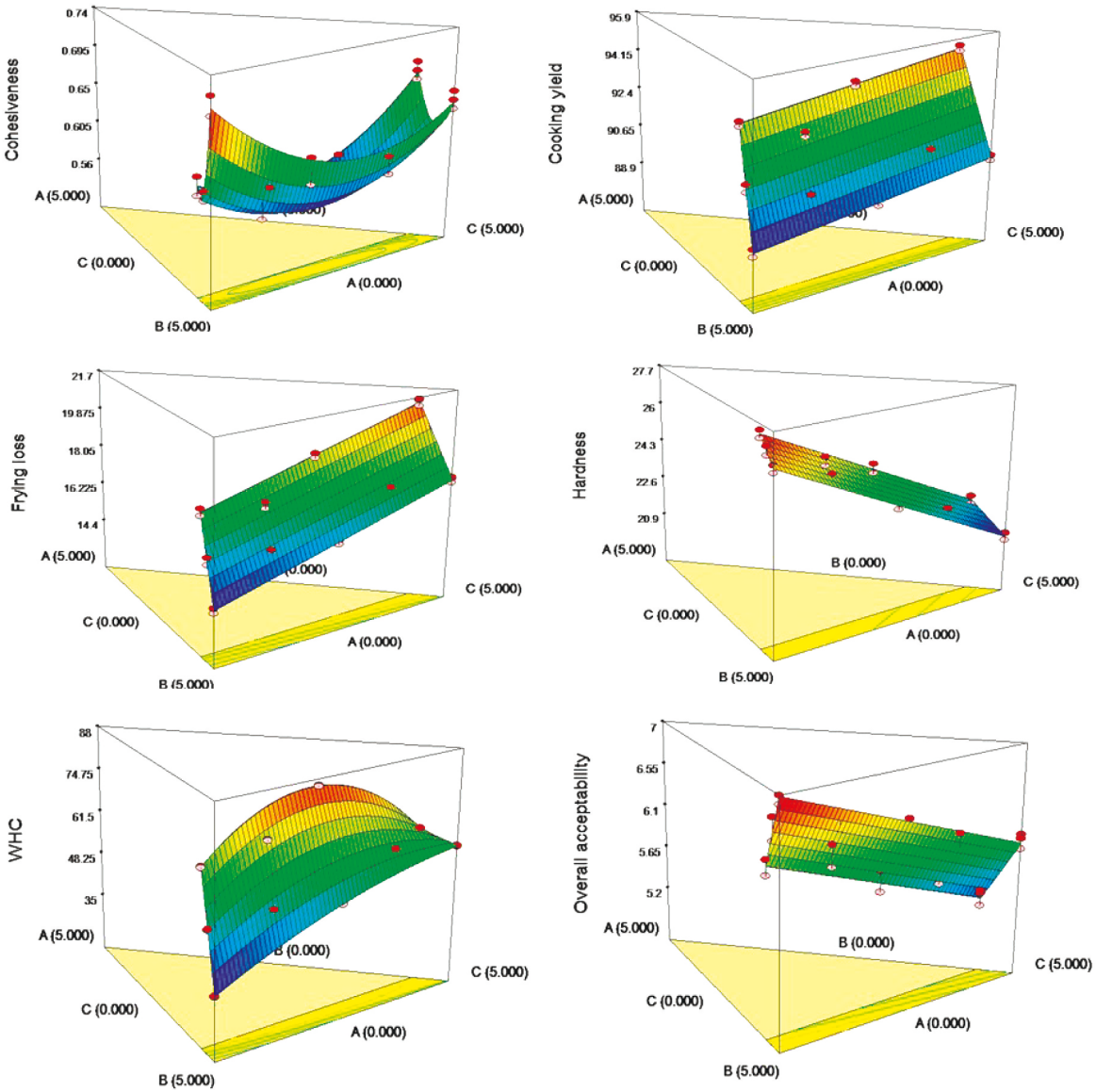

Fig. 1. Contour plots for effectы of konjac (A), inulin (B)б and starch (C) on water holding capacity (WHC), cooking yield, frying loss, hardness, cohesivenessб and overall acceptability of prebiotic sausage.

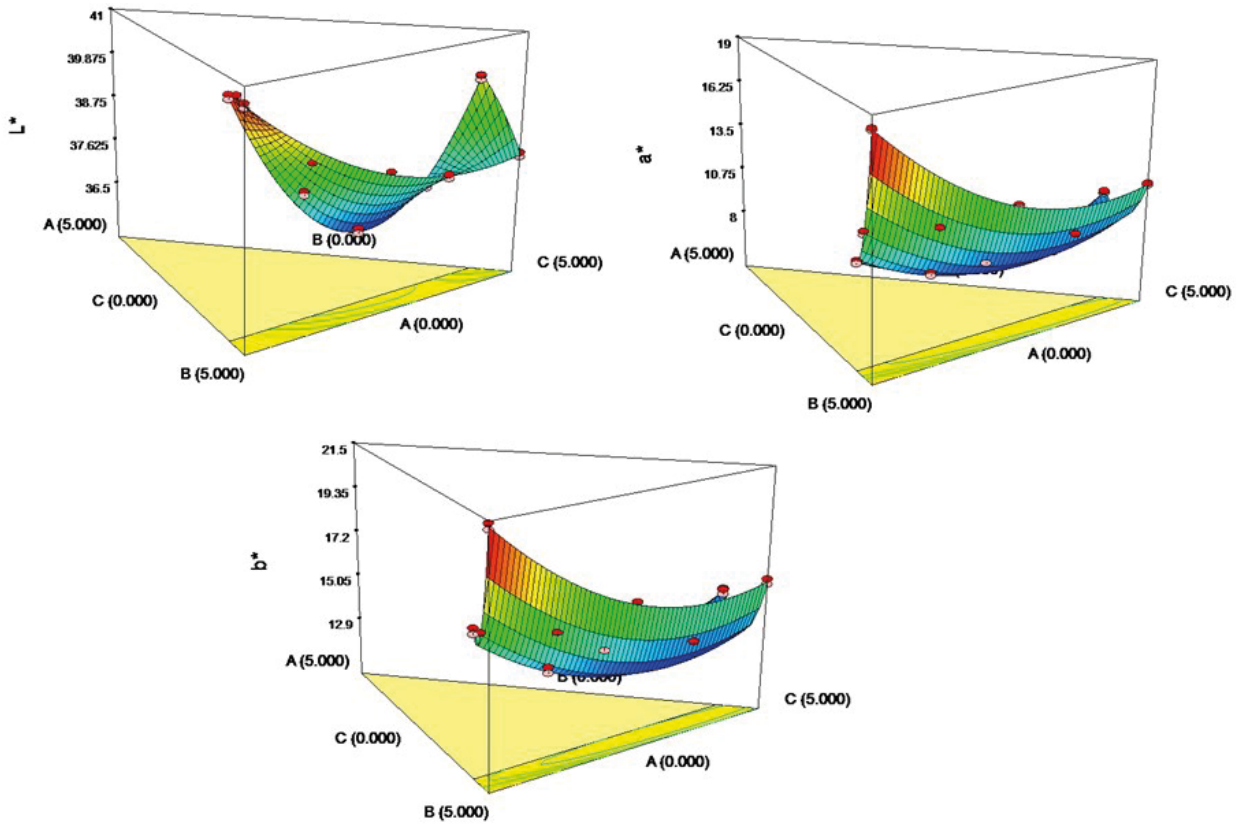

Fig. 2. Contour plots for effectы of konjac (A), inulin (B), and starch (C) on lightness (L*), redness (a*), and yellowness (b*) of prebiotic sausage.

ples are similar: one pair is samples no. 1 and 5, another one is the samples no. 3 and 7. These compositions were defined by Design Expert software to check the repeatability of findings. As it was expected similar compositions have displayed comparable results for cooking and sensory characteristics in Table 2, indicating the repeatability of findings.

Several studies suggested to use konjac in combination with other hydrocolloids. Emir *et al*. discussed that weak junction zones in konjac made it susceptible to heat, and its interaction with other hydrocolloids caused tights junction which made it resistant to cooking or frying [11].

According to regression coefficients (Table 3), inulin caused decrease in cooking and frying loss. The sample no. 8 (with the maximum level of inulin) displayed the least cooking yield and frying loss. This data is similar to that of Afshari *et al*. who indicated although inulin is able to increase WHC and decrease frying loss, but, at higher amounts, it reduced the moisture retention and cooking yield probably due to its porous structures and inability to form a tight gel [1].

Texture profile analysis (TPA). As the hardness analysis showed, konjac had a strong effect on the hardness of sausage, inulin also increased it, while starch, on the contrary, reduced the hardness of the product.

Several studies indicated that the addition of konjac into the food matrix increased the hardness of products but it depends to many factors that should be taken into consideration. These factors are the molecular weight and particularly the type of konjac (flour or as hydrolyzed), pH of a food system, presence of salts, and an amount of incorporated konjac and other food ingredients, specially gelling agents. All these studies are in agree that increase in amount of konjac increase the hardness that may not be accepted by consumers. Hu *et al*. reported that konjac glucomannan (KGM) affected functional properties of egg white protein and increased hardness, chewiness, and springiness of the gel samples at a certain concentration [13]. The investigation conducted by Emir *et al*. indicated that the bigger molecular weight of KGM caused the highest hardness and closely the lowest springiness, which had negative effect on the choice of panelists [11]. Akesowan reported that increasing of NaCl resulted increase in links between konjac/κ-carrageenan and konjac/gellan, leading to the increment in the hardness of the produced gel [2]. Purwandari *et al.* used konjac a noodle formulation. They found that the hardness and adhesiveness of noodle significantly increased ($p < 0.05$) and became three times harder than standard Chinese or Japanese wheat noodle [21]. The researchers also indicated that an increase in proportion of water in pregelatinised flour led to increased harness in konjac noodle.

Several studies also determined that the use of powdered inulin resulted in higher moisture loss during cooking. This can affect the texture of a product and increased hardness of burgers, frankfurter sausages and dry-fermented chicken sausages. [1, 18 and 19]. These results are in a good agreement with the results of this study.

Another texture parameter related to meat products is cohesiveness. Adhesiveness and cohesiveness are parameters that play an important role in handling of sau-

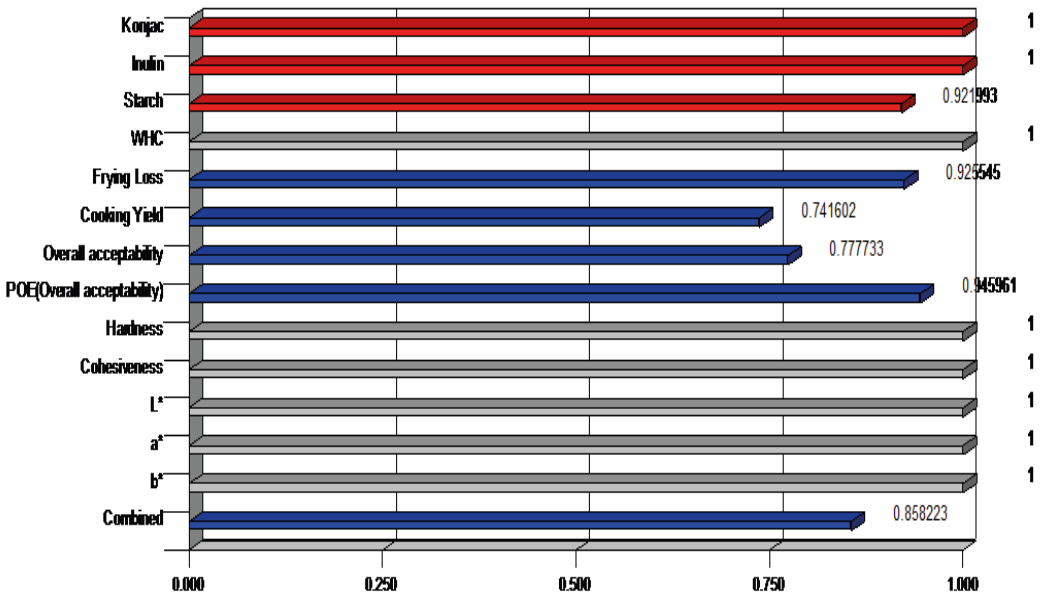

Fig. 3. Desirable plot for optimum formulation.

sages, particularly for the slicing of these products. If products are too adhesive or cohesive, they become undesirably sticky, and it cannot be easily to cut [20]. In the current investigation konjac showed a significant positive effect on cohesiveness of probiotic sausage. On the contrary, combination of konjac with inulin or starch reduced the undesirable effects of using konjac alone and maintained the appropriate adhesiveness and cohesiveness of sausages with improved textural properties. The study [9] documented that when konjac was used as a multi ingredient in the formulation of meat products, unwanted hardness and cohesiveness decreased significantly. The researchers suggested to incorporate konjac in combination with other hydrocolloids Purwandari *et al.* also confirmed that konjac had a substantial effect on the increase of adhesiveness and cohesiveness of noodle: konjac noodle was about ten times more cohesive and sticky than wheat flour noodle [21].

Colour. As shown in Table 3 and Fig. 2, all three component separately increased the lightness of the product, while their combination in special cubic model caused decrease in L* value in the sausages. The results indicated that inulin was meaningfully ($p < 0.05$) more effective in enhancing the lightness compared to starch and konjac. The samples no. 6 and 8, which containing the highest amount of inulin, illustrated the most lightness.

According to Table 3, konjac demonstrated a significant ($p < 0.05$) positive effect on a* and b* values causing more reddishbrown product, while its combination with starch or inulin decreased a* and b*. Trespalacios and Pla reported that if when myoglobin and fat content was maintained constant, the color of formulated products was mostly influenced by many factors, including additive ingredients [24]. In the present study, as the protein and fat content was invariable, the color was influenced mainly by mixing ingredients. Amini *et al.* mentioned konjac led konjac to a more reddishebrown of a product by its susceptibility to Maillard browning [4]. Jiménez-Colmenero *et al.* also indicated that the addition of konjac in frankfurter sausage caused decrease ($p < 0.05$) in lightness (L*) and an increase ($p < 0.05$) of yellowness (b*), compared to other samples [14]. The results of another investigation, conducted by Ruiz-Capillas *et al.*, are in agreement with the present study that konjac gel affected color parameter of sausages through decrease in L* and increase in yellowness (a*) [22]. Delgado-Pando *et al*. observed less red, paler ($p < 0.05$), and yellower pâtés as a result of adding konjac [10].

Sensory analysis. The experimental results obtained from the regression coefficient values of overall acceptability (Table 3 and Fig. 1) displayed that increase in the proportion of konjac had a significant ($p < 0.05$) negative effect on the overall acceptability of the product. Inulin showed a positive effect on the acceptability of the product, while starch was not significantly effective. Formulation 12 (contained 0.25; 4.75; and 0% konjac, inulin and starch, respectively) and formulation 9 (0.125; 3.68; and 1.18%) demonstrated the highest overall acceptability score.

Results obtained by sensory analysis highlighted that adding konjac in the amount of up to 0.2% could improve the appearance of sausage. On the contrary, increase in the amount of konjac (more than 0.2%) decreased the overall acceptability significantly ($p < 0.05$). The results emphasized that hardness and cohesiveness are the factors that significantly influence overall acceptability. Increase in proportion of konjac (more than 0.2%) can make the sausage harder and more cohesive than standard sausage which may not be acceptable by consumers. In the other words, consumers would not accept a product with extreme hardness or cohesiveness. Another explanation is that high amount of konjac probably enhances its typical fishy taste/odours. These findings are in a good agreement with the results of Purwandari *et al*. who reported that the addition of konjac glucomannan could improve sensory perception of wheat noodle, while a high level of this ingredient reduced preference, since noodle became too sticky [21]. Lin *et al*. also observed that 1% konjac in reduced-fat frankfurter sausages led to higher scores of sensory overall acceptability [15].

Liu *et al*. also assumed that functional properties of food products could be controlled by adding small amounts of KGM without causing undesirable sensory changes [16].

On the contrary, inulin showed positive coefficient on overall acceptability of sausages and an increase in portion of inulin improved the product flavour. Menegas *et al.* represented incorporation of inulin (maximum level of 7.5%) in reduced-fat sausages made the product more favorable and acceptable by consumers [19].

Mixture proportion optimization and desirability function

The optimisation was done in order to access the optimal amount of each component that had an excessive effect on quality properties of the sausages. The predicted values of the responses are shown in Fig. 3. Our aims were to maximize overall acceptability and cooking yield of the sausages, minimise frying loss, and, at the same time, to maintain WHC, hardness, and cohesiveness within normal range. Having all these criteria taken into consideration, we found that optimal amounts of inulin, starch, and konjak were 2.09; 2.76; and 0.146%, respectively (Fig. 3). The selected mixture achieved 0.858 desirability score. As the desirability value between 0.8 and 1.0 is recognized as acceptable and excellent product, the formulation with 0.858 desirability value was selected as optimal formulation that could provide valuable nutritional and technological properties.

CONCLUSION

In conclusion, the development of functional foods opens up new possibilities for the food industry and consumers. The development of healthier sausage with prebiotics inulin and konjac is a promising direction of research. The physicochemical and sensory characteristics of the prebiotic sausages are conditioned by the formulation. The study demonstrated that the sausage contained 0.146; 2.09; and 2.76% konjac, inulin, and starch, respectively, has high quality and sensorial properties.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

ACKNOWLEDGEMENTS

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Evaluation of rheological parameters of dough with ferrous lactate and ferrous gluconate

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Abstract: The aim of this study was to analyse the effect ferrous gluconate and ferrous lactate on the rheological behaviour of dough from a high extraction rate. For fortification of wheat flour, we used iron ions in a divalent form in amounts of 3, 4, and 5 mg/100 g. To record the rheological characteriscics of the fortified wheat flour dough, Farinograph, Amilograph, Falling Number, Rheofermentometer, and Thermo Haake Mars dynamic rheometer were applied. The Farinograph did not show significant changes in the water absortion values in the samples with ferrous salts. As for dough development time and dough stability, small amounts of ferrous additives increased and large amounts decreased those parameters. The effect was more significant in the samples with ions from gluconate form than from lactate salt. The Amylograph recorded an increased peak viscosity with an increasing ferrous salt quantity. That was the case for both ferrous salt forms. The increased was in a similar way for both types of ferrous salt forms used. The total CO_2 volume production and the retention coefficient obtained with the help of the Rheofermentometer device increased in the dough samples with 3 and 4 mg of iron/100 g. However, the addition of 5 mg of iron decreased those indicarors. The decrease was more significant for iron ions from ferrous ferrous gluconate than from ferrous lactate. The fundamental rheological properties of the dough were analysed by using a frequency sweep and oscillatory temperature sweep test. Ferrous lactate and ferrous gluconate influenced both the fundamental and empirical rheological properrties og the dough in similar way.

Keywords: Wheat flour, ferrous lactate, ferrous gluconate, rheological properties

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INTRODUCTION

Iron is a vital element for the humans, hence iron deficiency can seriously affect human's health [1, 2]. 60% of the world's population was estimated to be deficient in iron, while 33%, 30% and 15% are deficient in zinc, iodine, and selenium, respectively. Such a status is known as 'hidden hunger', due to diet that is poor in essential micronutrients [3, 4]. Iron deficiency has reached epidemic levels in numerous developing countries and affects people of all ages worldwide [5, 6]. The functional iron pool consists of such structural components in heme proteins as hemoglobin, myoglobin, and cytochromes [6, 7]. In addition, iron plays a part in nearly all redox reactions, and it is a vital component in several enzymes [3].

Iron aids the distribution of oxygen to the body, keeps the immune system strong, and helps the body to produce energy. Iron deficiency is caused by an insufficient iron intake, a poor absorption of iron or both. Iron deficiency exerts an adverse effect on mental and motor function, work productivity, immunity, cognitive development, and the quality of life in general [3, 5, 8, 9].

In 2012 WHO (World Health Organization) implemented a plan on maternal, infant, and young child nutrition to achieve a 50% reduction of anaemia by 2025 [10]. The food industry has initiated the use of iron into consumer products such as bread, breakfast cereals, biscuits, and energy bars. The food vehicles recommended to be fortified with iron, apart from staple foods, seasonings (i.e. table salt, soy sauce, fish sauce, broth, and curry powder) have been assayed owing to their extensive use in the various target populations [10]. The fortifying ingredients should however be used in the recommended amounts to prevent the risk of excessive consumption. Blanco-Rojo, Vaquero, and Hurrell [10, 11] reported that iron was the most difficult micronutrient to produce fortified foods. Many of the compounds used as iron forti-

ficants caused unacceptable colour and flavour changes in foods.

Cereal is the main source of food for humans, especially in developing countries where it takes half of the calorie intake. The most appropriate iron compounds recommended by the WHO to fortify cereals are ferrous sulfate, ferrous fumarate, ferric pyrophosphate, and electrolytic iron [10, 12]. Most nutrients are present in the outer layers of wheat and are lost during the milling process. Wheat flour is the raw material in the manufacturing of many foods: bread and bakery products, confectionery products, snacks, and biscuits. In Romania, wheat flour has been the only food item widely used for iron fortification at the national level [13]. An alternative source of this element for the treatment of iron deficiency can be iron fortified bread [3].

Besides flour, bread and other fortified products also contain a number of various ingredients and food additives. Iron can interact with these, which can cause taste, odour and colour changes, enchance the toxicity of added food additives, or decrease the vitamin and mineral content in the products. The rheological properties of fortified dough, among other parameters, change [14, 15] during the technological process.

For successful fortification programme, it is important that the combination of the fortificant and the food item to be easily accepted by the comsumer [16, 17]. This requirement includes not only sensory properties of the fortified food but also economic viability and efficacy (bioavailability) [17, 18]. The interactions between the iron fortificant, the food vehicle and the consumer acceptance can be the subject of a further investigation and a multidisciplinary approach [10].

Iron in other products of plant origin is non-heme, and its disadvantage is to interact with substances in foods that inhibit its absorption such as tannins, phytates, and polyphenols. Therefore, iron has a low bioavailability [19]. The most significant enhancer of iron bioavailability is ascorbic acid, which both reduces and chelates iron, rendering it soluble and availability for absorption in the gut [6, 20].

Fortification of wheat flour with iron is technically more difficult than that with other nutrients because iron is a pro-oxidant and therefore promotes lipid oxidation. Hence, the ideal iron compound for fortification of food should be one that ensures high iron bioavailability and does not affect the nutritional value or sensory properties of food [21–23]. Therefore, that waa the reason why we chose ferrous lactate and ferrous gluconate as an iron source. Theese froous salts ensure a high bioalvalabilty [24, 25], so they are widely reccommened as iron source for food products. In this paper we analysed an effect of fortification of wheat flour from a high extraction rate with iron ions in a divalent form from ferrous lactate and ferrous gluconate in amounts of 3, 4 and 5 mg/100 g on the rheological behaviour of the flour To our knowledge no such complex study on empirical (mixing, pasting, and fermentation) and fundamental rheological behaviour of gough was made using this type of iron ions.

STUDY OBJECTS AND METHODS

The wheat flour used in this study was provided by S.C. Dizing S.R.L. (Brusturi. Neam, Romania). The following characteristics of wheat flour were analysed according to Romanian or international standard methods: moisture (ICC 110/1)*, ash content (ICC 104/1)**, protein content (ICC 105/2)***, gluten deformation index (SR 90:2007), wet gluten (ICC 106/1), and Falling Number (ICC 107/1)****. The analytical characteristics for the wheat flour analysed were the following: 1.25 g/100 g for ash content, 12.8% for moisture, 14.3% for protein, 35% for wet gluten, 3 mm for gluten deformation index, and 262 s for Falling Number. Ferrous gluconate ($Fe(C_6H_{11}O_7)_2 \cdot 2H_2O$) and ferrous lactate ($Fe(CH_3CH(OH)COO)_2 \cdot 2H_2O$) were provided by Jost Chemical (Belgium). The ferrous salts were added in suce a way to achieve the iron ion concentration in wheat flour of 3 mg/100 g, 4 mg/100 g, and 5 mg/100 g.

The empirical rheological tests during mixing, pasting, and fermentation processes of wheat flour dough with and without iron ions addition were carried out using Farinograph, Amilograph, Falling Number, and Rheofermentometer devices.

Empirical rheological properties of the dough during mixing were evaluated by using a Farinograph device (Brabender, Duigsburg, Germany, 300 g capacity) according to ICC method 115/1*****. We analysed water absorption (WA, %), dough stability (ST, min), dough development time (DT, min), and degree of softening (DS, min) at 10 min.

Viscometric rheological properties of the dough were analysed with the help of a Falling Number device (Perten Instruments AB, Sweden) and an Amylograph device (Brabender OGH, Duisburg, Germany). ICC method 107/1 was used to evaluate the α-amylase activity of the wheat flour through the Falling number values (FN, s). Such parameters as gelatinization temperature (T_g, °C), peak viscosity (PV_{max}, BU), and temperature at peak viscosity (T_{max}, °C) were determined according to ICC method 126/1******.

Dough rheological properties during fermentation were measured with a Chopin Rheofermentometer (Chopin Rheo, type F3, Villeneuve- La- Garenne Cedex, France). The parameteres were: maximum height of gaseous production (H'm, mm), total CO_2 volume production (VT), volume of the gas retained in the dough at the end of the test (VR), and retention coefficient (CR, %).

Fundamental dough rheological properties were analysed using a HAAKE MARS 40 rheometer. The dough samples had the optimum dough consistency according to the water absortion values previosly

* Standard Method 110/1. Determination of the Moisture Content of Cereals and Cereal Products (Practical method).

** Standard Method 104/1. Determination of Ash in Cereals and Cereal Products.

*** Standard Method 105/2. Determination of Crude Protein in Cereals and Cereal Products for Food and Feed.

**** Standard Method 107/1. Determination of the 'Falling Number' according to Hagberg – Perten as a Measure of the Degree of Alpha-Amylase Activity in Grain and Flour.

***** Standard Method 115/1. Method for using the Brabender Farinograph.

****** Standard Method 126/1: Method for using the Brabender Amylograph.

established by the Farinograph device. Each sample was placed between the rheometer plates. The excess margins of the samples was removed and vaseline oil was used to prevent drying of the dough samples. The gap was setted to 2 mm, and a plate system with a diameter of 40 mm was used. Before analysis, the dough samples were left between plates for 10 min in order to allow its relaxation and to eliminate the stress rsulting from the mixing process. Frequency sweep tests from 0.00 to 20 Hz were performed at 25°C for all the dough samples. For the temperature sweep test, the samples were heated from 20 to 100°C at a heating rate of 4°C per min at a fixed frequency of 1 Hz and a strain of 0.001. During the frequency sweep tests and during heating storage modulus (*G'*) and loss modulus (*G''*) were analysed.

Statistical analysis of the triplicate results obtained was done using the XLSTAT statistical package (free trial version 2016, Addinsoft, Inc., Brooklyn, NY, USA), at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 demonstrates the empirical rheological properties of dough samples with or without iron ions during mixing which were analysed by the Farinograph device.

As one can see in Table 1, water absortion values did not signifcantly change in the samples with the iron ions. A slightly decrease of these values were noticed in the samples with large amounts of iron ions. This might be due to the fact that salt ions are able to modify hydrogen and hydrophobic interactions with the wheat flour components and lead to protein-water interactions instead of protein ones [26].

Increased amounts of iron ions addition decreased the dough development time significantly ($p < 0.001$) for both types of salts. An explanation of that was probably gluten proteins interactions modified by iron salts. They would possibly present more positive electric charge which might favor a less interaction in a shorter mixing time. Also, dough stability decreased more significqantly at high levels of iron ions addition in the case of gluconate salt than in the case of lactate one. This behaviour may be atribuited to the anion salt type.

Table 1. Effects of iron ions from the gluconate and lactate salts on Farinograph rheological properties

Iron ions, mg per 100 g/salt type	WA, %	DT, min	ST, min	DS, BU
0 (control)	65.0 ± 0.02	5.7 ± 0.01	7.3 ± 0.02	31 ± 0.02
3/FG	65.4 ± 0.01	6.2 ± 0.02	8.2 ± 0.02	28 ± 0.01
4/FG	64.9 ± 0.01	2.2 ± 0.02	7.9 ± 0.02	25 ± 0.01
5/FG	64.7 ± 0.02	2.2 ± 0.01	7.7 ± 0.03	24 ± 0.03
3/FL	65.1 ± 0.01	5.2 ± 0.01	7.8 ± 0.03	27 ± 0.02
4/FL	65.0 ± 0.02	2.0 ± 0.02	7.4 ± 0.02	24 ± 0.03
5/FL	64.6 ± 0.03	2.0 ± 0.03	7.0 ± 0.03	22 ± 0.03

Note: 0 is the sample without iron ions; FG is ferrous gluconate; FL is ferrous lactate; WA is water absorption; DT is dough development time; ST is stability; DS is degree of softening at 10 min

According to Codină *et al*. [27], the same level of iron ions addition contains lactate anion in a less amount that the gluconate anion. This will lead to a more compacted dough in the case of gluconate salt than in the case of lactate one. It is well known fact that the cation salt has a less effect on wheat flour components of dough system than the anion salt. As Miller and Hoseney reported [28], anion from a salt added in wheat flour might decrease electrostatic repulsion between gluten proteins, allowing them to connect and thus forming more stable dough. An increase in the dough stability with the increase in the level of iron salts has also been reported by Akhtar *et al.* and Rebellato *et al*. [29, 30].

The degree of softening values at 10 min decreased to a larger extent in the case of ferrous lactate than in the case of ferrous gluconate, which indicated a more weakening effect when lactate salt was incorporated in the wheat flour dough.

The dough viscometric rheological properties on Falling Number and Amylograph values are shown in Fig. 1. The value decreased with the increase in level of iron

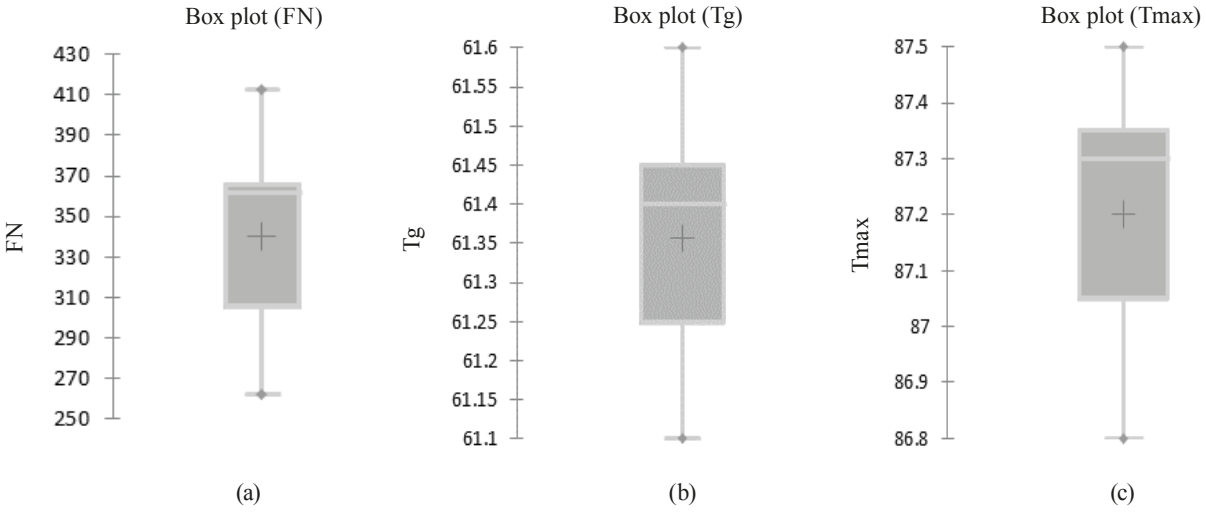


Fig. 1. Dough viscometric rheological parameters with different types and amounts of iron ions addition: (a) FN = falling number, s; (b) T_g = gelatinization temperature; (c) T_{max} = temperature at peak viscosity.

salts, with no significant differences between the dough samples with different types of iron ions incorporated (Fig. 1a). These decreased values indicated an increase in the wheat flour slurry viscosity, which could be correlated to decreased α-amylase activity in the wheat flours samples [31]. Falling number values increased up to 413 s and the mean values of the samples were slightly higher than 330 s. This indicated that the flour with iron ions additions showed a low α-amylase activity, which agreed well with the results obtained in [32].

All the parameters in the experimental samples analysed by Amylograph presented higher or similar values compared to the control sample (Fig. 1b and c). However, no significant difference were noticed between the samples with different type of iron salt addition. These results were somewhat predictable due to the fact that the Amylograph device was also a viscometric method which could be used to predict the α-amylase activity of wheat flour [27, 33] which is highly connected with these parameters. A lower α-amylase activity in wheat flour led to a lower starch hydrolysis and therefore to a lower amount of simple sugars and dextrins [34], which in turn caused an increase in all Amylograph parameters values [35].

Dough rheological properties during fermentation was analysed by a Chopin Rheofermentometer (Table 2). The maximum height of gaseous production were recorded by a Rheofermentometer pressure sensor, and the total CO_2 volume production were determined by means of a pneumatic circuit which measured an increase in the pressure of the fermentation gases. The iron salts addition increased the total CO_2 volume production from the dough system, which was probably due to the fact that iron ions stimulated the growth of yeast cells and therefore the total amount of the CO_2 volume production.

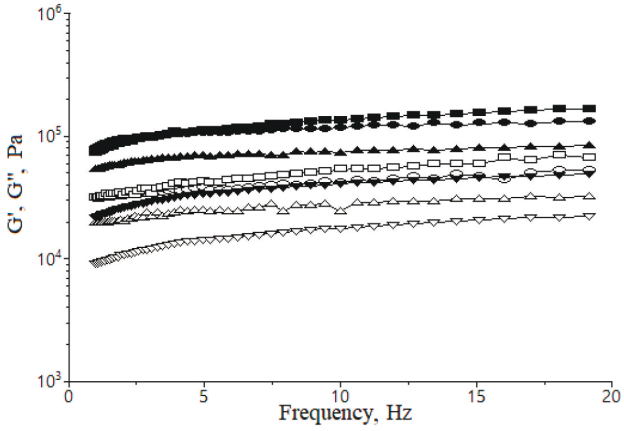
However, the volume of the gas retained in the dough at the end of the test (VR) and the retention coefficient (CR) decreased with the increased level of iron ions addition. This increase was greater in for the samples with ferrous lactate salt than for those with ferrous gluconate. The cause of that might be weakening effect that iron salts exerted on the wheat flour dough which was not capable to retain the gas formed. Similar results were alo obtained by Codină *et al.* [26]. The maximum height of gaseous production (H'm) varied with the increase in level of iron ions addition. This was probably due to the fact that iron ions additions in increased amounts initiated an increased gas production in the dough, but the wheat flour dough was not capabale to retain it.

Table 2. Effects of iron ions from the gluconate and lactate salt on Rheofermentometer rheological properties

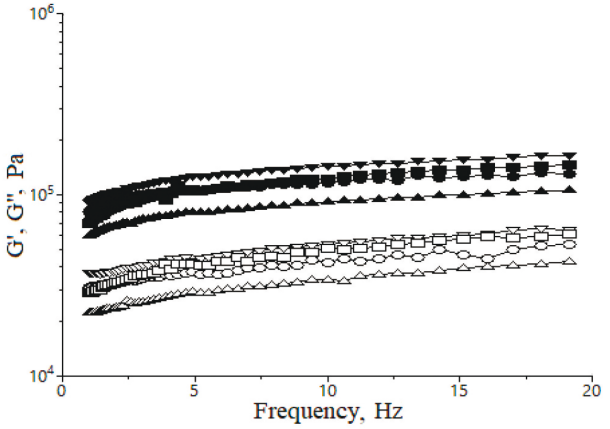
Iron ions addition, mg per 100 g/salt type	H'm, mm	VT, ml	VR, ml	CR, %
0 (control)	30.8 ± 0.02	1,400 ± 0.2	1,074 ± 0.2	76.7 ± 0.2
3/FG	30.1 ± 0.01	1,415 ± 0.3	1,051 ± 0.3	74.3 ± 0.3
4/FG	32.0 ± 0.01	1,504 ± 0.4	1,023 ± 0.4	68.1 ± 0.4
5/FG	30.6 ± 0.02	1,354 ± 0.2	860 ± 0.2	63.5 ± 0.2
3/FL	31.1 ± 0.02	1,424 ± 0.3	975 ± 0.2	68.4 ± 0.3
4/FL	30.6 ± 0.01	1,450 ± 0.2	989 ± 0.1	68.3 ± 0.2
5/FL	31.0 ± 0.02	1,198 ± 0.4	736 ± 0.2	61.4 ± 0.3

Note: 0 is the sample without iron ions; FG is ferrous gluconate; FL is ferrous lactate; H'm is maximum height of gaseous production; VT is total CO_2 volume production; VR is volume of the gas retained in the dough at the end of the test; CR is retention coefficient (%)

Fig. 2 shows effects of the iron ions additions from the two types of salts on the storage/elastic module *G'* and the loss/viscous module values *G"*. All the experimental dough samples, as expected, presented *G' > G"* at all frequency ranges, which indicated a solid elastic-like behavior of wheat flour dough according to [36]. The *G'* and *G"* values increased slightly with the increase in frequency from 1 to 20 Hz. The dough samples with 3 mg of iron ions addition showed a decrease in the *G'* and *G"*, which implied that the samples demonstrated visco-elasticity characteristics to less extent than the control sample. However, high levels of iron ions increased the *G'* and *G"* values compared to the control sample. An explanation of this might be dehydration effect that iron salts could exert on gluten network that might lead

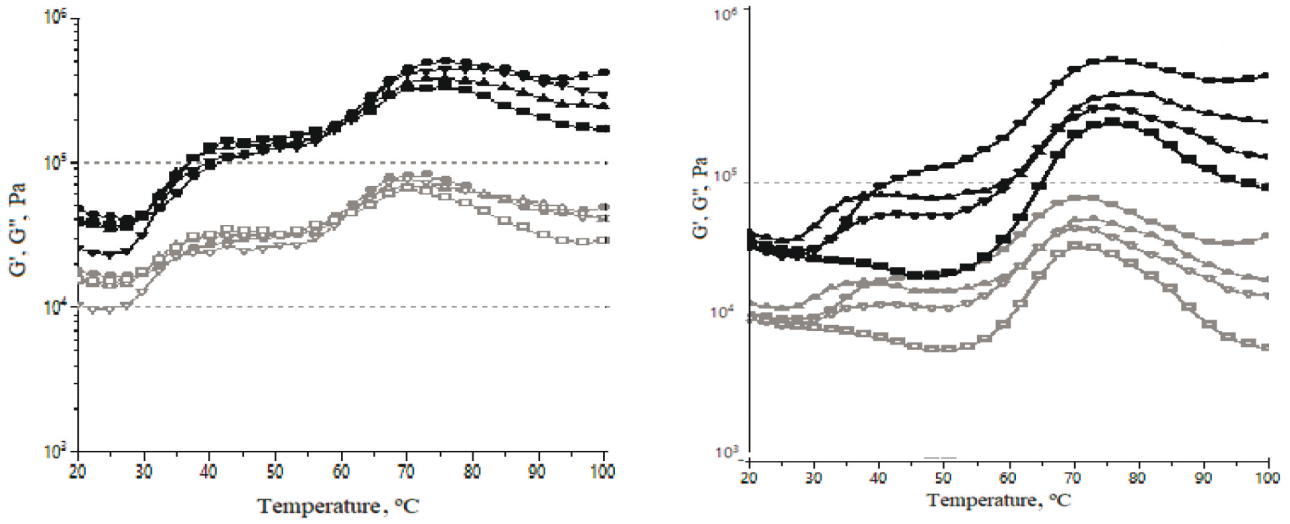


(a)



(b)

Fig. 2. Evaluation with frequency at 20°C of storage modulus G' values (represented by solid symbols) and loss modulus G" (open symbols) for samples with different amounts of iron ions addition: 0 mg/100 g (●), 3 mg/100 g (▼), 4 mg/100 g (▲) and 5 mg/100 g (■) from lactate salt (FL) (a) and gluconate salt (FG) (b)



(c) (d)

Fig. 3. Evaluation with temperature of storage modulus G' values (represented by solid symbols) and loss modulus G" (open symbols for dough samples during heating with different amounts of iron ions addition: 0 mg/100 g (●), 3 mg/100 g (▼), 4 mg/100 g (▲) and 5 mg/100 g (■) from lactate salt (FL) (c) and gluconate salt (FG) (d)

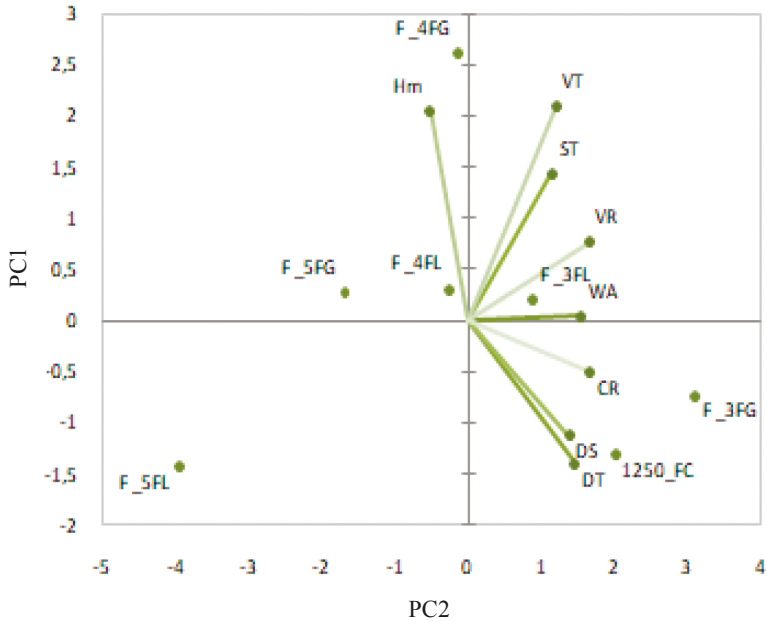


Fig. 4. Principal component analysis of dough sample characteristics (amounts of iron ions from gluconate (FG) and lactate salt (FL) were 3, 4, and 5 mg/100 g) analyzed from the Farinograph and Rheofermentometer devices. WA = water absorption; DT = dough development time; ST = stability; DS = degree of softening at 10 min; H'm = maximum height of gaseous production; VT = total CO_2 volume production; VR = volume of the gas retained in the dough at the end of the test; CR = retention coefficient, %.

to a more compacted dough with higher visco-elasticity properties.

The influence of the iron ions addition on dynamic moduli during heating is shown in Fig. 3. *G'* and *G"* values were lower in the samples with the maximum amount of iron ions (5 mg/100 g). It seemed that both ferrous lactate and ferrous glucanate displayed a significant effect during dough heating. At the begining of heating the moduli decreased for all the samples due to protein denaturation which seemed to increase with the increase in the amount of iron ion addition. Thus, proteins lost their capacity to retain water, starch granules began to absorb the water and to gelatinise as temperature increased. This fact is obvious, since an increase in dough elasticity and viscosity is manifasted in the increase of the *G'* and *G"* after the temperature exceeds 50°C.

The principal components analysis *(PCA*) of the wheat flour dough rheological characteristics determined by the Farinograph and Rheofermentometer is shown in Fig. 4. The two plots represent 99.72% of the total variance. The plot of PC1 vs. PC2 loadings shows a close association between the dough sample with 3 mg of iron ions from the lactate salt addition and the volume of the gas retained in the dough at the end of the test (VR). The dough samples with 3 mg/100 g addition from gluconate salt is closed positioned to the retention coefficient (CR). This facts shows that the samples with iron ions addition in the aount of

3 mg/100 g presents a prositive effect on the dough rheological properties during the fermentation process.

The second PC axis show a close association between the samples with 4 mg of the iron ions per 100 g of the wheat flour, which indicate that both types of salts at this amount have a similar effect on dough rheological properties. However, both ferrous lactate and ferrous gluconate in an increased amounts show a different effect, from a statistical point of view, on the dough rheological properties, since they are differently positioned in the PCA plot.

According to the dought rheological properties results obtained with the help of Farinograph and Rheofermentometer devices, good correlation may be observed between CR and DS, CR and DT, ST and VR, as well as between VT and VR Rheofermentometer values.

CONCLUSION

The effect of iron ions from lactate and gluconate salts in amounts of 3, 4, and 5 mg/100 g on wheat flour dough empirical and fundamental rheological properties was analyzed. It seems that the 3 mg/100 g iron ions addition did not affect adversely the dough rheological properties since dough stability, dough development time, and total CO_2 volume production increased. In addition, such dough rheological properties as the degree of softening at 10 min, Amylopgrah paramter values, volume of the gas retained in the dough at the end of the test, retention coefficient, and dynamic rheological properties did not decrease significantly. The 4 mg/100 g the iron ions addition weakened the dough rheological properties, namely decreased dough stability, the degree of softening at 10 min, and the retention coefficient value. Despite the increase of the total CO_2 volume production, the wheat flour dough was not capable of retaining a high amount of CO_2 released.

However, the 5 mg/100 g iron ions addition impaired the dough rheological properties in the case of lactate salt more significantly than in the case of gluconate salt. According to the data obtained, ferrous gluconate in the amount of up to 4 mg/100g was optimal to use in bread making wheat flour to ensure good rheological properties of dough.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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Environmental regulations in Russian food security

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Abstract: The present research features the legal effect of environmental regulation on food security in the Russian Federation. The author analyzed the system of environmental regulation together with its legal instruments, and gave a legal assessment of its efficiency in providing safe environment and food products. The efficiency of the mechanism of environmental regulation affects the safety of products. However, the system of environmental regulation is largely represented by sanitary and hygienic standards and does not fully meet modern challenges. Meant as a basis for environmental and food security, the current environmental regulation takes into account neither local conditions nor the level of aggregate anthropogenic load on environment, thus failing to ensure the production of safe, high-quality food products. The study proves that there is an inextricable link between environmental regulation and environmental safety, which has to be taken into account in state policy planning. By providing environmental safety, environmental regulation serves as the main means of food security. The author proposes to develop legislation on various types of environmental standards that would ensure food quality and security. The measures of food quality and security should be based on the principles of environmentalisation and sustainable agriculture.

Keywords: Ecology, law, environmental regulations, environmental protection, food security, food quality, environment, food, nutrition

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INTRODUCTION

Economic activity produces a negative effect on the natural environment. As a result of prolonged anthropogenic impact, soil, water and other natural resources accumulate harmful substances, which subsequently poison humans and other living organisms. Harmful substances enter human body directly, via air and water, and indirectly, when one consumes products produced in adverse environmental conditions. In the latter case, pollution can take hidden forms and accumulate. As a result, its negative effect on human health can manifest itself in the future.

Economic activity is bound to affect the environment. Thus, there are three main issues in this aspect: 1) how to measure the extent of this effect; 2) how to introduce a scientifically based criterion that would assess the effect on the natural environment and human health; 3) how to minimize the impact of agriculture, industry, energy, etc. on the natural environment and its components.

Environmental regulation seems to provide a legal instrument to handle the matters. According to Russia's 2025 Environmental Security Strategy, environmental regulation is one of its main mechanisms*. Ecological security means protection of the natural environment and vital human interests from the possible negative impact of economic and other activities, as well as emergency situations of natural and man-made character and their consequences [1]. Hence, ecological security ensures a certain level of life quality and allows people to live a full and safe life in the modern world. Ecological security exists along with other types of national security, such as economic, food, etc. No individual can be healthy and successful in an ecologically unfriendly environment. The quality of food one consumes largely depends on the natural factors connected with raw materials, e.g. how they were grown, processed, transported, etc. Even in a man-made environment, it is ecology that determines the effectiveness of all other types of safety.

* Strategiya ehkologicheskoy bezopasnosti Rossiyskoy Federatsii na period do 2025 goda 'O Strategii ehkologicheskoy bezopasnosti Rossiyskoy Federatsii na period do 2025 goda' [Strategy of Environmental Security of the Russian Federation for the period up to 2025. 'On the Strategy of the Environmental Security of the Russian Federation for the Period up to 2025']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2017, no. 17. (In Russ.).

The present study tries to solve the following conceptual problems: 1) Can environmental regulation, as the main legal instrument of environmental safety, ensure food security in modern conditions? 2) If so, what are its legal means and how effective are they?

STUDY OBJECTS AND METHODS

The study features a legal assessment of the efficiency of current environmental regulations that ensure food security in the Russian Federation.

It is based on the legislative idea of environmental regulation as a legal means of a) preserving a favourable environment, b) maintaining its safe level, c) reducing the negative impact of human activity on the environment. The methodological idea behind the research is the interaction of the natural and social environments in human habitat.

The author employed general methods, general scientific cognition methods, and such special legal methods as formal legal, comparative legal, interpretation of law, legal modelling, and legal forecasting methods.

RESULTS AND DISCUSSION

According to Art. 19 of the Federal Law 'On Environmental Protection', environmental regulation prevents and (or) reduces the negative impact of economic and (or) other activities on the environment**.

The legal institute of environmental regulation has a long history. However, disputes regarding its legal nature, legal consequences, and classification do not subside [2–4, 24].

Environmental regulation reached its peak in 1938-1991, when standards for water, air, soil, noise, and vibration were established. Their goal was to protect human health, as well as the genetic fund of flora and fauna. By the beginning of the 1990s, thousands of harmful substances had been standardized.

However, environmental regulation was limited to independent but obligatory sanitary-hygienic rules. They were so tough that business entities simply could not comply with them. As environmental problems became more acute in the late 1980s, the existing approaches to the legal regulation had to be changed.

As a result, 'a compromise was reached between economics and ecology' [5], which was reflected in the Law of the Russian Soviet Federative Socialistic Republic adopted on December 19, 1991, no. 2060-1 'On the Environmental Protection'***. Chapter IV 'Quality regulation of the environment' included the following standards:

– maximum permissible concentrations of harmful substances;
– maximum permissible emissions and discharges of harmful substances;
– maximum permissible levels of noise pollution, vibration, magnetic fields, and other harmful physical factors;
– maximum permissible level of radiation exposure;
– maximum permissible norms for the use of agrochemicals in agriculture;
– maximum permissible residues of chemicals in food;
– maximum permissible norms of environmental load;
– environmental requirements for products, i.e. standards for new equipment, technology, materials, substances, etc.;
– standards for sanitary and protected zones.

However, the standards for the use of natural resources provided by Art. 19 were not directly included in the system of environmental regulation. They were part of the system of limits set up for environmental management.

Most of the standards listed in the law still had a sanitary-hygienic character and defined environmental quality indicators in terms of its safety for human health. Others concerned various activities of economic entities and set environmental restrictions and other requirements for public health hazards. Their goal was to protect the environment and human health [4].

According to Petrov [6], the system of environmental regulation included three groups of standards. The first group included sanitary and hygienic standards. The second group presupposed production and economic standards, which included technology, construction, and urban planning. The third group involved complex standards, e.g. maximum permissible environmental standards, standards for sanitary and protective zones, etc.

Crassov [7] allocated the following types of standards:

1) Environmental standards for maximum permissible concentrations of harmful substances. They included maximum permissible levels of physical impacts on the environment; standards for maximum permissible emissions and discharges of harmful substances; maximum permissible norms of environmental load; standards for sanitary protection zones.

2) Sanitary and hygienic standards. Those stipulated by the Law 'On Environmental Protection' included norms for the use of agrochemicals and standards for maximum permissible residual quantities of chemicals in food. Others were provided by the Law 'On Sanitary-Epidemiological Well-Being of the Population and Sanitary Rules and Norms'.

3) Construction and urban planning regulations. They were part of regulatory and technical documents in the field of construction and included various rules, practices, and standards.

Brinchuk [8, 9] studied the legislation of the period of the Law 'On Environmental Protection' and existing state standards. He defined the following groups of environmental standards:

1) Environmental quality standards, such as maximum permissible concentrations of harmful substances, maximum permissible levels of harmful physical effects on the environment;

2) Standards for maximum permissible harmful effects on the environment, i.e. standards for maximum permis-

** Federal'nyy zakon № 7-FZ 'Ob okhrane okruzhayushchey sredy' [Federal Law No. 7-FL 'On Environmental Protection'. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2002, no. 2, Art. 133. (In Russ.).

*** Zakon RSFSR № 2060-1 'Ob okhrane okruzhayushchey prirodnoy sredy' [Law of the RSFSR No. 2060-1 'On Environmental Protection']. *Vedomosti S"ezda narodnykh deputatov i Verkhovnogo Soveta Rossiyskoy Federatsii* [News of the Congress of People's Deputies and the Supreme Council of the Russian Federation], 1992, no. 10, Art. 457. (In Russ.).

sible emissions and discharges of harmful substances;
3) Standards for levels of noise, vibration, magnetic fields, and other harmful physical effects; standards radiation exposure;
4) Maximum permissible norms for the use of agrochemicals and waste disposal limits;
5) Standards for the use of natural resources, established by natural resource acts, which took into account the specifics of individual natural resources, as well as limits on environmental management;
6) Environmental standards, i.e. such environmental requirements as standards for products and services;
7) Regulations for sanitary and protective zones, including security of industrial facilities.

Currently, environmental regulations and state standards in the field of environmental protection are separated from each other by the Federal Law 'On Environmental Protection'. It seems logic enough, in spite of the fact that both have a common goal, i.e. to define certain mandatory rules of conduct or requirements aimed at providing environmental security. Environmental regulation and standards are designed to ensure the quality of the environment and the permissible level of exposure. In this sense, they serve as a criterion for this quality. State standards act indirectly, through mandatory requirements for products, services, technologies, etc. Standards are one of the ways to ensure the normal operation of regulations. They help to ensure the quality, minimize the negative impact on the environment, and contribute to the stabilization of its quality.

In the modern institute of environmental regulation, the system of environmental standards is presented from the standpoint of the Federal Law 'On Environmental Protection'. It includes:
1) Environmental quality standards, e.g. standards established for chemical, physical, and biological indicators of the state of the environment, etc.;
2) Standards for permissible environmental impact of economic and other activities. They include standards for permissible emissions and discharges; technological standards; technical standards; standards for waste generation and limits on their disposal; standards for permissible physical impacts; standards for permissible removal of environmental components; standards for permissible anthropogenic load on the environment; and
3) Other environmental standards.

Art. 28 of the Federal Law 'On Environmental Protection' allows for the establishment of other types of environmental standards. It does not specify which kinds of standards belong to this group. They can be established both by the regulatory legal acts of the Russian Federation and the normative legal acts issued by the constituent entities of the Russian Federation.

The science of environmental law reveals no consensus on which standards can be attributed to this group. These may include both standards that are not named directly and those that meet the goals of ensuring environmental security and, thus, food security.

For instance, water quality objectives play an important role in maintaining a favourable environment****. They are established by schemes of integrated use and protection of water pools and include chemical, physical, biological, radiation, and bacteriological indicators. They are based on a) the maximum allowable concentrations established according to the type of water use; b) estimated conditional background concentrations of pollutants, determined by the results of systematic monitoring; c) environmental standards, actually reflecting the state of the water body in the water sector.

Other types of environmental standards may also include:
1) Maximum permissible norms for the use of agrochemicals, e.g. mineral fertilizers, plant protection products, growth stimulants, etc.;
2) Standards for chemical residues in food. Food must meet the requirements for the permissible content of chemical, biological substances and their compounds, microorganisms, and other biological organisms that pose a threat to the health of current and future generations.

The establishment of a non-exhaustive list of legal environmental standards has a number of advantages. First of all, it is impossible to predict what specific standards should be introduced with every new source of public hazard. Second, a flexible mechanism can make it possible to take into account not only the state of the environment in a particular area, but also such local factors as the state of human health, the standard of living, and the need for food products of some particular quality.

In populated areas, the level of anthropogenic pressure on environmental components and initial resources of food production can be extremely high. It is impossible to reduce the amount of harmful substances in the air, water, and soil without taking into account the cumulative or caused harm and its specifics. As a result, food security depends on how effective the mechanism of environmental regulation is.

The harmlessness of products consumed by the population is an important quality of life factor and a quality element of food security in general. The state of food security in Russia is determined not only by the availability of sufficient food resources, but also by the economic and physical availability of food for all groups and categories of the population. As follows from the Doctrine of Food Security of the Russian Federation*****, the safety of food supplied to the market ensures food security in general [10].

The problem of food security is believed to belong to the sphere of economic science [11, 12]. However, food safety issues are currently moving into the legal sphere and need to develop a legal support system adapted to modern challenges.

Very often, food security problems are considered

**** Vodnyy kodeks Rossiyskoy Federatsii [Water Code of the Russian Federation adopted]. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2006, no. 23, Art. 2381. (In Russ.).

***** Doktrina prodovol′stvennoy bezopasnosti № 120 'Ob utverzhdenii Doktriny prodovol′stvennoy bezopasnosti Rossiyskoy Federatsii' [The Doctrine of Food Security no. 120 'On the Approval of the Doctrine of Food Security of the Russian Federation']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2010, no. 5, Art. 502. (In Russ.).

from the point of view of food availability [13, 14]. According to legal literature, food security should be understood as a state of protection of citizens and the state from external and internal food threats. Domestic needs should be satisfied by guaranteed agricultural production, which provides every citizen with physical, economic, and social access to high-quality and safe foods for active and healthy lifestyle [15, 16].

Threats to food security are related both to global food security trends and domestic issues. Thus, the World Health Organization defines the following problems of global food safety: a) microbiological hazards; b) chemical food contaminants; c) new food technologies, e.g. genetically modified foods; d) unstable food safety systems in many countries that fail to ensure a safe global food chain [17].

De-environmentalisation of Russian legislation seems to be the main current national threat to domestic food security. Unfortunately, environmentalisation is one of the most vulnerable links in a food security system. According to the Economic Security Strategy******, excessive environmental requirements are considered as a threat to the national economic security of the Russian Federation. Therefore, it is extremely difficult to find a balance between environmental, economic, and social interests in this closed cycle of legal relations. It is very hard to determine when the environmental requirements are justified and when they are overcharged.

For many decades, Russia exercised a sanitary-hygienic approach to environmental regulation. Formally and legally, it resulted in a split in the legal regulation of the establishment of environmental requirements in the field of food security. There were two directions: 1) requirements for the primary state of the potential raw material (soil, water, etc.) in natural conditions; 2) requirements for the so-called result, i.e. extracted natural resource, products, and other anthropogenic objects. At the same time, there remains a general trend of substituting environmental requirements with sanitary and hygienic standards. It can be seen from the 2030 Strategy for Improving the Quality of Food Products in the Russian Federation*******.

Neither Russian science nor legislation has a clear view of the relationship between the concepts of 'product quality' and 'product security'. The Federal Law 'On the Quality and Security of Food Products' does not see them as equivalent. According to Art. 1, food quality refers to a set of characteristics of food products that can meet human needs for nutrition under normal conditions of their use. Food safety refers to a state of reasonable confidence that food products, under normal conditions of use, are not harmful and pose no health risks to current and future generations********.

At the same time, the abovementioned Strategy for Improving the Quality of Food Products does not see the concepts of quality and security of food products as identifiable. However, the document states a different relationship between them: the quality of food products, as a set of relevant characteristics, includes its security in order to preserve human health.

In any case, a question arises: what criteria are used to determine food quality, as well as its security? Approaches to the legal regulation of food quality requirements, including security, have changed.

According to the Federal Law 'On Technical Regulation'********, it is mandatory to observe technical regulations on food security for all producers, while it is optional whether food quality should comply with national standards or not. This general rule is valid unless there are established exceptions. There are currently many obligatory sanitary and hygienic requirements for quality and security of food products. Their violation may trigger administrative and other measures.

Undoubtedly, voluntary execution is the main disadvantage of the current legal regulation of quality and security of food products. The situation is getting even more aggravated since the mechanisms of state supervision and control in this area are highly inefficient. It seems advisable to tighten the existing requirements since the lifespan of every individual depends on the quality of food products.

The quality and security of food products is also affected by the state of the natural environment in which raw materials are produced. The lower environmental risk factors, the healthier the raw materials and food production conditions. Ecological regulation serves the purpose of preventing and minimising the negative impact on the environment and its components. It helps to minimize the accumulation of harmful substances in food raw materials.

The individual provisions of the Federal Law 'On Environmental Protection' contain environmental requirements at various stages of economic and other activities, as well as in various fields. According to Art. 47 of the Federal Law and Art. 14, 43 of the Federal Law 'On the Sanitary-Epidemiological Well-Being of the Population'********, production and handling of potentially hazardous chemicals, including radioactive, and microorganisms are regulated by law. The Articles also

****** Strategiya ehkonomicheskoy bezopasnosti Rossiyskoy Federatsii na period do 2030 goda № 208 [Strategy for Economic Security in the Russian Federation for the period up to 2030 No. 208]. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2017, no. 20, Art. 2902. (In Russ.).

******* Strategiya povysheniya kachestva pishchevoy produktsii v Rossiyskoy Federatsii do 2030 goda № 1364-r [Strategy for Improving the Quality of Food Products in the Russian Federation for the period up to 2030 No. 1364-p]. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2016, no. 28, Art. 4758. (In Russ.).

******** Federal'nyy zakon № 29-FZ 'O kachestve i bezopasnosti pishchevykh produktov' [Federal Law No. 29-FL 'On the Quality and Safety of Food Products']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2000, no. 2, Art. 150. (In Russ.).

******** Federal'nyy zakon № 184-FZ 'O tekhnicheskom regulirovanii' [Federal Law No. 184-FL 'On Technical Regulation']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2002, no. 52 (part 1), Art. 5140. (In Russ.).

******** Federal'nyy zakon № 52-FZ 'O sanitarno-ehpidemiologicheskom blagopoluchii naseleniya' [Federal Law No. 52-FL 'On Sanitary and Epidemiological Well-Being of the Population']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 1999, no. 14, Art. 1650. (In Russ.).

provide the necessary toxicological and hygienic studies of these substances, establish the procedure for handling them, set up environmental regulations, and state registration rules for potentially dangerous chemical and biological substances and certain types of products.

Ecological regulation is an important legal instrument that regulates the production and handling of potentially hazardous chemicals. However, as far as the content in food is concerned, it is mostly reduced to sanitary and hygienic standards. According to The Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor), the content of harmful substances in products in 2017 did not exceed the figures for 2016. On the contrary, there was a slight decrease in the total share of domestic and imported products that did not meet the regulations for chemical and microbiological pollutants. However, 2017 saw an increase in certain types of products that did not meet the standards for microbiological indicators, namely meat and meat products, flour and cereals, bakery products, canned food, and vegetables [18].

State registration is an effective legal instrument that prevents production, transportation, purchase, storage, sale, and use of potentially dangerous chemical and biological substances, as well as certain types of products. The Federal Register of Potentially Hazardous Chemicals and Biological Substances reflects potentially dangerous chemical, biological substances, and preparations, introduced into production or previously unused. It also reflects potentially dangerous products as well as certain types of products, including foods, imported into the territory of the Russian Federation for the first time.

The current plurality and fragmentation of legal norms is a significant drawback of the legal mechanism for regulating the production and handling of potentially dangerous chemical and biological substances. The ongoing debate on legal regulation does not make it easier. It seems expedient to expand the scope of legal regulation of environmental legislation. It should cover the protection of the natural environment, the favourable state of which contributes to the life quality, as well as to the protection of human life and health from adverse environmental risks. There is still a need to develop a special legislative act that would regulate the production and handling of potentially hazardous chemical and biological substances in the Russian Federation.

Art. 49 of the Federal Law 'On Environmental Protection' provides special requirements and environmental measures for the use of chemicals in agriculture and forestry. They are further developed in the Federal Law 'On the Safe Handling of Pesticides and Agrochemicals'********, as well as in registration tests, examinations, licensing, standardization, certification, state supervision, and control of pesticides and agrochemicals.

Agricultural lands are one of the most valuable categories of land and a strategic resource for ensuring food security. Legal measures for their protection are the main means of achieving the goals of food quality and security. Population obtains the overwhelming majority of food products (up to 99%) from agricultural soils. However, the basis of agricultural production is destroyed in the process of their exploitation, together with the environment. Hence, the National State Programme for the development of agriculture and the regulation of markets for agricultural products, raw materials, and food******** is ecologically oriented. It is aimed at preventing the effect of natural and anthropogenic factors on land, water, and other natural resources, including those involved in agricultural circulation. The mechanisms of state support which the Programme provides for agriculture are aimed at ensuring the quality of agricultural products. The Programme increases environmental safety, preserves the resource potential of soil, prevents water pollution, improves drainage and cultivation systems, and prevents uncontrolled breeding of plants and animals.

Soils are the most vulnerable type of land. The Federal Law 'On State Regulation of Ensuring Fertility of Agricultural Lands'******** has failed to improve the state of Russian soil. According to the Ministry of Agriculture of the Russian Federation, 35% of arable lands have high acidity, 31% demonstrate low humus content, 22% have phosphorus deficiency, and 9% reveal potassium deficiency. Large areas of land are subject to water and wind erosion, salinization, acidification, overgrowing by shrubs and underbrush, desertification, and other negative processes [19].

The agrochemical condition of the soil was aggravated by natural and man-made factors, including the low level of environmental management in the field of agriculture in 1990–2007. For instance, organic fertilizers were introduced into the soil twice as rarely as before. The area of pesticide-laden agricultural land is increasing. The Federal Law 'On the Safe Handling of Pesticides and Agrochemicals' seems to be of no help. So are the measures of legal liability, including criminal liability, for deterioration of land and for not conducting mandatory improvement activities.

Apparently, the reasons lie in the absence of real action on the implementation of agrarian policy and a lack of state support for environmental-oriented economic entities and agricultural producers. According to Ignatieva [20], the fragmented nature of economic regulation does not give positive practical results. The norms of environmental legislation are nothing but declarations that express the future intentions of the state.

******** Federal′nyy zakon № 109-FZ 'O bezopasnom obrashchenii s pestitsidami i agrokhimikatami' [Federal Law No. 109-FL 'On the Safe Handling of Pesticides and Agrochemicals']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 1997, no. 29, Art. 3510. (In Russ.).

******** Postanovlenie Pravitel′stva Rossiyskoy Federatsii № 717 'O Gosudarstvennoy programme razvitiya sel′skogo khozyaystva i regulirovaniya rynkov sel′skokhozyaystvennoy produktsii, syr′ya i prodovol′stviya' [Resolution of the Government of the Russian Federation No. 717 'On the State Programme for the Development of Agriculture and Regulation of Agricultural Products, Raw Materials, and Food Markets']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2012, no. 32, Art. 4549. (In Russ.).

******** Federal′nyy zakon № 101-FZ 'O gosudarstvennom regulirovanii obespecheniya plodorodiya zemel′ sel′skokhozyaystvennogo naznacheniya' [Federal Law No. 101-FL 'On State Regulation of Ensuring Fertility of Agricultural Lands']. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 1998, no. 29, Art. 3399. (In Russ.).

The Ministry of Natural Resources and Environment of the Russian Federation reported on the state of the environment and proposed some directions for improving the environmental security in the field of agriculture:
– to consider natural and climatic features while improving the legislation in the sphere of the agro-industrial complex;
– to recognize natural and environmental factors as critical for effective agriculture and rational distribution of agricultural production;
– to give priority to environmental management over traditional, short-term benefit-oriented agricultural economy;
– to set up balance between agricultural activities and the ecological capacity of a specific area, i.e. sustainable agriculture;
– to increase the amount of perennial grasses and legumes;
– to ensure stability of agricultural production by improving soil fertility with the help of natural perennial protective cover;
– to develop the system of agrolandscape-ecological zoning;
– to optimise the type and structure of cultivated areas by analysing local climatic conditions, landscape features, and soil properties;
– to subsidise some agricultural producers to let them undertake measures for land care and soil fertility recovery;
– to issue state orders for the development of new-generation agricultural technologies in the system of adaptive landscape farming;
– to restore the management system of agricultural land resources, introduce new functions and improve the existing ones, i.e. land management, agro-ecological monitoring of land, inventory, agro-ecological regulation, development and updating of targeted schemes for the use and protection of land in various regions of the Russian Federation and municipalities;
– to prioritise protection of valuable agricultural lands;
– to adapt agriculture to global climate change;
– to create an assessment system of agro-climatic resources for their rational use; and
– to develop and improve scientific research in the system of agrochemical services, including environmental risk assessment, regulations, chemical safety, etc.

Environmentalisation and sustainable agriculture are the main principles of the system of measures aimed at improving the efficiency of organization and farming. Without them, food quality and security cannot be improved.

Other documents on the socio-economic development of the Russian Federation and its regions also feature environmentalisation of socio-economic development. Environmentalisation is meant to create a unified system of economic mechanisms for environmental management and environmental protection. It should result in innovative projects, energy efficient and resource-saving technologies, and environmentally safe technological processes. In addition, environmentalisation is aimed at developing scientifically based environmental norms and standards. Environmental legal mechanisms have to be introduced into the system of state policy measures in agriculture. The natural environment is the basis of life and human activities. It provides opportunities for the socio-economic development of every individual. Thus, natural environment and resources deserve respect and careful attitude. Clean environment provides safety and life quality, which includes food.

Legal regulation in environmental sphere is extremely complex – for two reasons. First, it should make the established patterns of environmental relations favourable both for the environment and the population. Second, it has to resolve socio-economic problems identified by strategy-planning.

The principle of sustainable agriculture reflects the global trend towards balanced and harmonious social, economic, and environmental development. 'Sustainable development' means continuously supported, self-sustaining, admissible, and balanced development. The term was introduced by the United Nations International Panel on Environment and Development in 1987. This kind of development meets the needs of the present without compromising the ability of future generations to meet their needs [1].

At the International Conference in Rio de Janeiro (1992), sustainable development was recognized as a process of environmental changes in which economy, exploitation of natural resources, investment, science and technology, and personality development work together to strengthen the present and future potential of humanity to meet its needs********. This is how the term was explained in the final document of the Conference, after which it became global [21]. However, the term can be interpreted differently and remains dynamic, open, and changing. There are more than 80 uses of the term 'sustainable development' in scientific literature.

The principles of sustainable development have been put into practice in different countries – with variable success. Nevertheless, as it was noted at the RIO+20 Conference *The Future We Want*, people must continue to fulfil the tasks set in 1992 to harmonize the relations between the society and the nature, economic and social development in the interests of present and future generations.

Russia adopted the traditional approach to sustainable development as a balanced, environmentally sound social and economic development. As evidenced in practice, the implementation of legal norms on sustainable development is extremely inconsistent, and their mere formal legal fixation is not enough.

There are independent legal regulations concerning environmental requirements for genetically modified organisms in the Federal Laws 'On Environmental Pro-

******** Deklaratsiya Rio-de-Zhaneyro po okruzhayushchey srede i razvitiyu. 3–14 iyunya 1992 g. Povestka dnya na XXI vek (Povestka 21) [Rio de Janeiro Declaration on Environment and Development. June 3-14, 1992, Agenda 21]. Available at: http://www.un.org/ru/documents/decl_conv/declarations/riodecl.shtml. (27 March 2019).

tection' (Art. 50) and 'On State Regulation of Genetic Engineering Activities'********. The rapid development of genetic research in biology and medicine makes it possible to use their results in agriculture, food industry, and pharmacy. Hence, the direction has acquired particular relevance. Since the very onset of genetic engineering, scientists all over the world have been discussing the possible risks for the natural environment and humans. Abroad, the legal regulation of GMOs is developing in different directions. On the one hand, the emphasis is on the legal regulation of their production. On the other, the focus is on the legal regulation of the product and its properties.

In spite of the fact that the GMO issue remains unresolved, the Russian legislators have developed a whole range of legal instruments for handling GMOs. These include licensing certain types of GM activities, certification, registration of GMOs, and legal liability (including criminal) for violating the rules.

In Russia, GMOs cannot be used for some categories of population and products. For instance, GMOs cannot be included in dairy products and juices designed for children. Also, there are the technical regulations complied by the Customs Union on the safety of certain types of specialized food products********. They ban GMO from foods meant for pregnant and lactating women, as well as from dietary therapeutic and prophylactic foods.

Thus, if a direct ban on the use of GMOs is not installed, their use is permissible. However, it is the matter of consumer rights protection. Producers must inform the consumer on the presence of GMOs in the raw materials or products. Consumers then choose by themselves whether to purchase such products or not. As a result, consumers are made responsible for their health, regardless of whether they are aware of the possible benefits or harms of GMOs or not.

This is a matter of a deeper legal problem. According to the constitutional provision of Art. 42, everyone has the right for a favourable environment, reliable information about its condition, and for compensation for health or property damage caused an environmental offence******** [22]. In addition, the use of GMOs is also related to environmental legislation. GMOs might damage the natural properties of ecological systems and their elements or upset the natural balance. This may lead to previously unknown direct or indirect risks to the natural environment and human health. Considering various aspects of the nature – man sphere [23], it seems incorrect that the legislator appeals to civil law and shifts the burden of responsibility for their health to the consumers themselves.

In the framework of the current legislation, Rospotrebnadzor regularly checks economic entities for the use of GMOs in their food products. According to Rospotrebnadzor, 26,019 food samples were examined for the presence of GMOs in 2017. Of these, 17 were found to contain GMOs, which is a little more than in 2016. In 2017, the share of samples with identified GMOs was 0.07%, in 2016 – 0.05%. At the same time, the share of samples in imported products in 2017 increased significantly from 0.06% to 0.77%, compared to 2016. In the vast majority of cases, producers inform about the content of GMOs: only one product out of the abovementioned 17 samples lacked information on the presence of GMOs. In 2012, there was no GMO-related information in 13 cases out of 22 [18].

According to the idea of sustainable development, food security should be environmentally oriented. It means that not only the so-called environmentally friendly raw materials should be used, but also that waste generation should be avoided.

Art. 24 of the Federal Law 'On Environmental Protection' establishes standards for production and consumption waste and its disposal. The negative impact of production and consumption waste cannot be underestimated. Its causes soil, air, and water pollution and affects human health via pollution of natural resources and biological contamination. Huge areas of forested, agricultural, and populated lands are occupied by landfills. The legislation regarding the disposal of production and consumption waste is currently undergoing some changes. It is important to restructure the existing landfills to improve waste storage and disposal. However, it is of even greater importance to eliminate accumulated harm and prevent it in the future.

The efficiency of production and consumption waste management requires well-coordinated and progressive work in relevant areas and depends on the following factors.

1) Environmental factor takes into account: a) the natural characteristics of regions and municipalities, b) the state of the local environment and its components, c) the anthropogenic load on the environment and its components according to the population size, etc.

******** Federal′nyy zakon № 86-FZ 'O gosudarstvennom regulirovanii v oblasti genno-inzhenernoy deyatel′nosti' [Federal Law No. 86-FL 'On State Regulation of Genetic Engineering'. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 1996, no. 28, Art. 3348. (In Russ.).

******** *Reshenie Soveta Evraziyskoy ehkonomicheskoy komissii № 34 'O prinyatii tekhnicheskogo reglamenta Tamozhennogo soyuza 'O bezopasnosti otdel'nykh vidov spetsializirovannoy pishchevoy produktsii, v tom chisle dieticheskogo lechebnogo i dieticheskogo profilakticheskogo pitaniya' (vmeste s 'TR TS 027/2012. Tekhnicheskiy reglament Tamozhennogo soyuza. O bezopasnosti otdel'nykh vidov spetsializirovannoy pishchevoy produktsii, v tom chisle dieticheskogo lechebnogo i dieticheskogo profilakticheskogo pitaniya')* [Decree of the Council of the Eurasian Economic Commission adopted No. 34 'On the adoption of the technical regulations of the Customs Union 'On the safety of certain types of specialized food products, including dietary medical and dietary preventive foods' (together with TRCU 027/2012 'Technical Regulations of the Customs Union 'On the safety of certain types of specialized food products, including dietary medical and dietary preventive foods')]. Available at: http://www.tsouz.ru. (27 March 2019).

******** Konstitutsiya Rossiyskoy Federatsii, prinyata vsenarodnym golosovaniem 12.12.1993 [Constitution of the Russian Federation adopted by popular vote on December 12, 1993]. *Sobranie zakonodatel'stva Rossiyskoy Federatsii* [Collected Legislation of the Russian Federation], 2014, no. 31, Art. 4398. (In Russ.).

2) Financial and economic factor includes: a) real state support of environmentally oriented businesses; b) targeted expenditure of environmental fees; c) co-financing of waste management measures.
3) Legal factor requires adoption of a significant number of legal acts on a) national standards aimed at turning the sphere of waste disposal into an innovation industry; b) reclaiming disturbed lands; c) a stricter legal liability for violations in this area; d) garbage sorting, e) role of municipalities in solving the problems of waste disposal and landscaping.
4) Social factor establishes the partial responsibility of local citizens for cleaning adjacent territories from wastes and maintaining them in proper condition.

Methodological invariance remains a significant drawback of environmental regulation development. Unfortunately, current environmental legislation takes components of the natural environment and the sources of environmental hazard separately. The severe anthropogenic load on the environment results in the fact that there is no differentiation between environmental hazards from various sources. Taken together, the consequences cause a greater damage to environment and human health than if the standards were observed separately. These specifics are not taken into account when establishing environmental and sanitary standards. The combined effects of chemicals and other substances may cause more harm to nature and human health, both directly and indirectly, including via food consumption.

CONCLUSION

The research resulted in the following conclusions:
1) Ecological regulation is a legal way of determining the quality of the environment and regulating the permissible impact of economic and other activities on the environment. It maintains the ability of nature to restore itself. Ultimately, ecological regulation provides favourable conditions for human life, prevents harm to human life and health, thus contributing to food security.
2) Environmental regulation and food security are closely and inextricably linked. Environmental regulation ensures the state of environmental security and, as a result, is the main means of ensuring food security.
3) To be efficient, food safety measures should be ecological. When establishing standards that ensure environmental and food security, it is necessary to take into account local natural and geographical conditions, the amount of accumulated harm and the total anthropogenic load on the territory. Such precociousness will make it possible to calculate the prospective environmental risks and their consequences for the nature and human health in a certain area.
4) Legislative and law enforcement practices show that most of the existing standards in food security are of sanitary and hygienic character. They form the quality of the human environment to protect human health. However, the current state of affairs requires unification of legal regulation in the field of environmental and sanitary-hygienic regulation. A unified system will extend environmental regulation into all types of environmental standards, including those that ensure the food quality and security.
5) Agriculture is a strategic resource for ensuring food security. Thus, the idea of sustainable development in agriculture fits perfectly into the general world trend of modern socio-economic development, which is based on an ecological approach. This provision should be taken into account when shaping the state policy in the sphere of food security.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this article.

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Developing freeze-dried bioproducts for the Russian military in the Arctic

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Abstract: The Arctic is currently a strategic region of Russia and it requires military protection. Good nutrition is a prerequisite for successful performance of the servicemen in such extreme conditions. The Russian armed forces do not have a special ration for the Arctic region. The existing rations lack products enriched with biologically active substances, probiotics, prebiotics, or vitamin and mineral complexes. Subcaloric nutrition, even with short-term physical exertion, in combination with low temperatures deprives the human body of vitamins and minerals. Therefore, the development of freeze-dried bioproducts with functional properties is highly relevant. This article presents the results of a study aimed to develop new freeze-dried milk-based bioproducts for the Russian military in the Arctic. For this, we created a microbial consortium of lactic acid bacteria with a wide spectrum of antimicrobial activity. We also used a range of functional ingredients, namely pectin, pine nut meal, a 'Lactobel' prebiotic protein-and-carbohydrate product, rowan puree, and rosehip syrup in the amounts established during the study. The new freeze-dried milk-based bioproducts named 'Pobeda', enriched with combiotics and metabiotics, will provide the Russian military with better nutrition in the extreme conditions of the Arctic.

Keywords: Military nutrition in the Arctic, biotechnology, freeze-dried milk-based bioproducts, probiotic bacteria consortium, synbiotics, combiotics, metabiotics

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INTRODUCTION

Gaining control over the Arctic region is currently becoming not only a matter of geopolitics, but also a matter of survival in the future multipolar world. Its geographical and transport conditions, as well as ample energy resources, make the Arctic zone of Russia an area of increased interest not only for the Arctic states, such as the USA, Norway, Canada, and Denmark, but also for some non-Arctic countries, such as Japan, China, South Korea, and others. Therefore, this strategic region requires Russian military presence to protect the northern state border and ensure national security. Good nutrition of the Russian military is a prerequisite for the successful implementation of their duty in the extreme Arctic conditions [1–4].

In the Arctic, the human body is affected by a complex of adverse factors even if living conditions are quite satisfactory. These factors disturb almost all types of metabolism and greatly reduce the content of vitamins and glucose in the blood. According to scientists, even short-term physical exertion in combination with low temperatures and a subcaloric diet can lead to a deficiency of vitamins (C and group B), as well as macro- and microelements (potassium, calcium, magnesium, fluorine, selenium, and iodine). Moreover, low temperatures disturb the metabolism of vitamin C and the B vitamins [5–8, 34].

Currently, there are no special rations for the Russian military serving in the Arctic zone. Their diet is regulated by the Decree of the RF Government of December 29, 2007 'On food supplies for servicemen and other categories of staff, as well as feed (food) supplies for animals in military units and organisations in peacetime'. According to this decree, food rations for the military are as follows: ration no. 1 (common ration) with extra products for units in the Extreme North (canned

fish 40 g, butter 15 g, biscuits 40 g, and condensed milk 25 g); ration no. 2 (summer ration); and ration no. 5 (medical ration) [9]. During the cold season (from October 1 to March 31), 100 g of rye and wheat bread is replaced with 20 g of salo (cured fatback) [10]. Thus, the limited range of products and a complete replacement of fresh fruits and vegetables with canned ones, cannot provide the Arctic servicemen with adequate nutrition.

The current system of army rations is based on the theory of sensible nutrition. This explains why they lack foods enriched with biologically active substances, eubiotics, prebiotics, vitamin-and-mineral complexes, and phytoprotectors. Therefore, their inclusion in the rations is perceived as an innovation.

The theory of functional nutrition is better suited for the Arctic conditions. Functional nutrition contains a variety of essential elements such as:
– fat-soluble and water-soluble vitamins (A, D, E, B_1, B_2, B_6, B_{12}, B_9, C, and PP);
– vitamin-like substances (choline, L-carnitine, ubiquinone, lipoic and orotic acids);
– essential amino acids;
– polyunsaturated fatty acids (omega-3, omega-6);
– phospholipids;
– antioxidants (vitamins C, E, carotenoids);
– macro- and microelements (calcium, magnesium, potassium, iodine, selenium, iron, zinc, etc.);
– probiotics (probiotic microorganisms);
– prebiotics (dietary fibres, carbon-containing compounds of microbial and non-microbial origin, plant and microbial polysaccharides, etc.);
– synbiotics (probiotics + prebiotics);
– combiotics (synbiotics + functional ingredients, such as vitamin-and-mineral premixes, phenol-based and other plant compounds); and
– metabiotics (containing active metabolites, metabolic products of probiotic cultures) [3, 8, 11–13].

The Military Medical Academy, the RF Ministry of Defence is currently developing nutrition standards for military personnel serving in the Extreme North. They may include foods from local raw materials, such as plants (cranberries, lingonberries, blueberries, etc.), algae, mushrooms, and lichens [14].

The priority here is to develop various freeze-dried bioproducts, since they have a low weight, a small size, and a long shelf life. They also ensure the vitality of vitamins and probiotic microorganisms at low temperatures. Freeze-dried bioproducts are especially important as a survival ration for military personnel serving in remote areas, away from logistics bases, as well as for raid groups and special forces whose work involves increased physical activity and long-distance transitions. This makes the development of milk-based freeze-dried functional bioproducts a promising direction.

Thus, we aimed to develop a biotechnology for producing milk-based bioproducts with functional ingredients to improve the diet of servicemen in the Arctic.

STUDY OBJECTS AND METHODS

The study objects were Siberian starter cultures of lactic acid bacteria with a high content of viable cells, not less than 10^9 CFU/cm^3. The cultures were adapted for Russians and had proven health-benefitting effects. They were:
– *Lactobacillus acidophilus (L-1)*, *Lactobacillus casei (L-2)*, and *Lactobacillus delbrukii subsp. bulgaricus (L-4)* produced by ZAO Vektor-BiAlgam, Novosibirsk;
– *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. diacetilactis*, and *Streptococcus salivarius thermophilus (BK-Altai-Snzh)* produced by the Barnaul Biofactory, Barnaul;
– their microbial consortium obtained during the study; and
– liquid and dry synbiotic bioproducts developed during the study.

Titratable acidity was determined according to State Standard 3624-92* by titration with a 0.1N sodium hydroxide with phenolphthalein and expressed in Turner degrees (°T)

Active acidity was determined by a potentiometric method according to State Standard 32892-2014** on a SG8-ELK SevenGo pro pH-meter / ion meter (Mettler Toledo, Switzerland).

The quantity of lactic acid bacteria cells was measured by the method of limiting dilutions on a dense agar medium MRS according to State Standard 10444.11-89*** and State Standard 10444.11-2013****.

The morphology of the bacteria was studied by making preparations stained with methylene blue, followed by microscopy in an immersion system with a 100x objective lens using a MIKMED-5 binocular microscope and photographing with a LOMO TC-500 camera.

The concentration of exopolysaccharides (EPSs) was determined by the anthrone method [15]. The action of concentrated sulfuric acid on carbohydrates forms furfural, 5-methylfurfural, or 5-hydroxymethylfurfural. Reacting with anthrone, they produce intensely coloured green or blue-green products. During the experiment, 4 volumes of distilled water and 10 volumes of freshly prepared anthrone reagent were added to 1 volume of the culture liquid of the bioproduct. The mixture was then incubated for 10 min at 1,000°C in a water bath. The concentration of EPSs was determined by spectrometry on a Unico-2800 single-beam scanning spectrometer at a wavelength of 620 nm. Various concentrations of glucose solutions were used as a standard.

The selection of starter cultures in the microbial consortium was based on the microorganisms' probiotic properties and their ability to synthesise EPSs.

The antagonistic activity of starter cultures of lactic acid bacteria and the microbial consortium against

* State Standard 3624-92. Milk and milk products. Titrimetric methods of acidity determination. Moscow: Standards Publ., 2004. 8 p.
** State Standard 32892-2014. Milk and dairy products. Method of pH determination. Moscow: Standartinform Publ., 2015. 13 p.
*** State Standard 10444.11-89. Food products. Methods for determination of the lactic acid bacteria. Moscow: Standartinform Publ., 2010. 14 p.
**** State Standard 10444.11-2013. Microbiology of food and animal feeding stuffs. Methods for detection and enumeration of mesophilic lactic acid bacteria. Moscow: Standartinform Publ., 2014. 23 p.

pathogenic and potentially pathogenic microorganisms was determined by the method of serial dilutions. The mixed populations were compared with the growth of test cultures in a liquid nutrient medium, followed by inoculation in solid media [16]. *E.coli*, *S.aureus*, *Pr.vulgaris*, *Ps.mirabilis*, *Kl.pheumoniae*, *Sh.flexneri*, *Sh.sonnei*, *and S.cottbus* were used as test microorganisms.

Antibiotic resistance of starter cultures of lactic acid bacteria and the microbial consortium was measured by the method of serial dilutions in a liquid nutrient medium.

Physical and chemical indicators of bioproducts were studied by standard methods.

The solubility index of freeze-dried bioproducts was determined according to State Standard 30305.4-95*****.

The moisture content of freeze-dried bioproducts was determined on an OHAUS MB-35 moisture analyser.

Sensory characteristics were evaluated according to State Standard 28283-89****** and State Standard R ISO 22935-2-2011*******.

Safety indicators of the bioproducts were assessed against the medical and biological requirements established by the regulatory documents [17].

The statistical processing of experimental data derived from 3–5 replications was performed with Statistica 12. The differences were considered significant with $p < 0.05$.

RESULTS AND DISCUSSION

Our earlier studies found that fermented milk products based on microbial consortia of lactic acid bacteria that synthesised exopolysaccharides had plenty of benefits. In addition to improved rheological properties, they had a wider spectrum of antimicrobial activity, compared to starter cultures. This promoted adhesion of beneficial probiotic bacteria to the intestinal walls and ensured maximum effectiveness for the human body.

Numerous studies by Khamagaeva *et al.* proved that multi-strain starters had a higher biotechnological potential and were more resistant to adverse environmental factors. Therefore, for this study, we created a consortium of lactic acid bacteria with the greatest ability to synthesise EPSs (Fig. 1) and a wide spectrum of antimicrobial activity.

The synthesis of EPSs usually depends on the composition of the microbial consortium and the conditions of its cultivation. For example, an increase or decrease in the optimal growth temperature causes an active synthesis of EPSs. This was confirmed by our studies with a cultivation temperature of 38 ± 1°C for bioproducts with the consortium. This cultivation temperature was not optimal for all the lactic acid bacteria making up the microbial consortium.

In addition, a greater synthesis of EPSs leads to closer intercellular contacts between rod-shaped and coccoid microorganisms in the microbial consortium. Fig. 2 shows the morphology of microflora in bioproducts with starter cultures and their microbial consortium.

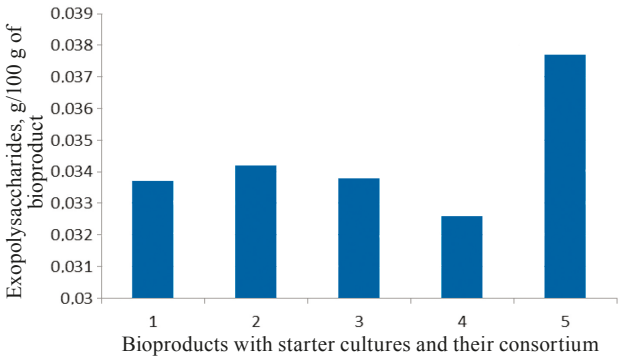

Fig. 1. Microbial exopolysaccharides content in bioproducts. 1 – bioproduct with *Lactobacillus acidophilus (L-1)*, 2 – bioproduct with *Lactobacillus delbrukii subsp. bulgaricus (L-2)*, 3 – bioproduct with *Lactobacillus casei (L-4)*, 4 – bioproduct with *Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetilactis, Streptococcus salivarius thermophilus (BK*-Altai-Snzh*)*, 5 – bioproduct with the microbial consortium (1:2:3:4 in the ratio of 1:1:0.8:1.2).

Increased formation of EPSs makes the bioproduct more viscous and enhances cohesion (aggregation). As shown in studies [18–21], cohesion is an accumulation of cells, a kind of a supra-organism system similar to a multicellular organism. It is a cooperation of individual cells whose concerted activity is aimed at achieving the same result. The exchange of information and signals in this system is carried out by extracellular metabolites that regulate the activity of bacteria. Such interactions ensure physiological and adaptive resistance of cells to negative environmental effects.

As can be seen in Fig. 2, the aggregation of microorganism colonies in the microbial consortium demonstrates their ability for multicellular organisation, which is characteristic of bacteria in the gastrointestinal tract. In addition, a bio-

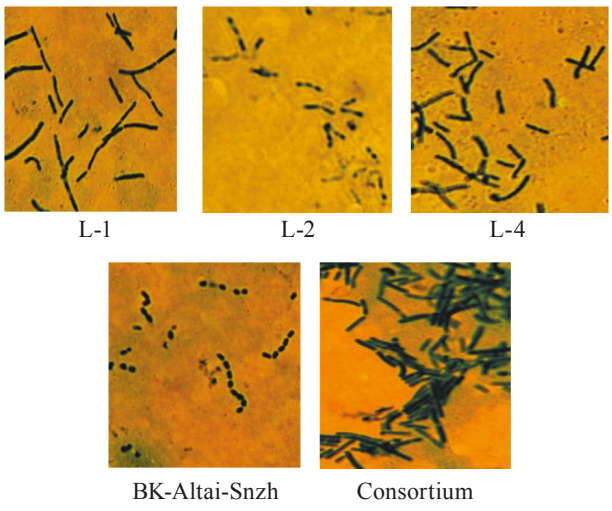

Fig. 2. Morphology of microflora in bioproducts with starter cultures and their microbial consortium (magn. 1000×).

***** State Standard 30305.4-95. Dry dairy products. The procedure of measurement of solubility index. Moscow: Standards Publ., 2001. 4 p.
****** State Standard 28283-89. Milk. Sensory analysis. Determination of odour and taste. Moscow: Standartinform Publ., 2009. 6 p.
******* State Standard R ISO 22935-2-2011. Milk and milk products. Sensory analysis. Part 2. Recommended methods for sensory evaluation. Moscow: Standartinform Publ., 2012. 20 p.

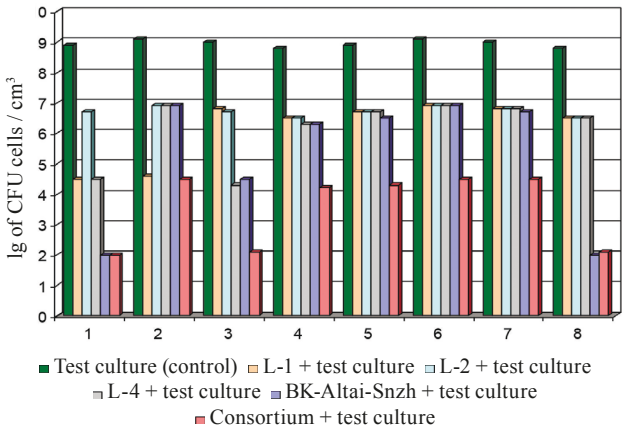

Fig. 3. Antagonistic activity of microflora in bioproducts with starter cultures and their microbial consortium against pathogenic and potentially pathogenic microorganisms: 1 – e.coli, 2 – s.aureus, 3 – pr.vulgaris, 4 – ps.mirabilis, 5 – kl.pheumonia, 6 – sh.flexneri, 7 – sh.sonnei, 8 – s.cottbus.

product with a microbial consortium has a wider spectrum of antagonistic activity against pathogenic and potentially pathogenic microorganisms (Fig. 3), compared to the starter cultures present in it. Such products are also more resistant to various antibiotics (Table 1) and can therefore be used in antibiotic therapy.

As can be seen in Fig. 3, the microflora in the bioproduct with a microbial consortium displayed the highest antagonistic activity against *E.coli*, *Pr.vulgaris* and *S.cottbus*, with the number of viable cells being 10^2 CFU per 1 cm^3. This was probably due to the ability of lactic acid bacteria to produce antimicrobial substances, such as nisin (*Lactococcus lactis subsp. lactis*), diplocin (*Lactococcus lactis subsp. cremoris*), bulgaricin (*Lactobacillus delbrukii subsp. bulgaricus*), and lactocins B, F, G, and M (*Lactobacillus acidophilus*).

Table 1 shows a greater resistance of microflora in bioproducts with a microbial consortium to all the antibiotics, compared to the starter cultures. This is probably due to the acquisition of new genetic information through the transfer of plasmids during conjugation and transduction by not only closely related bacterial species, but also by bacterial genera. Changes in the cultivation temperature appear to cause rearrangements in the lactococcal genome. Lactococcus plasmids are able to encode such properties as the production of antibiotic substances and the preservation of antibiotic resistance.

At the next stage, we studied the influence of various functional prebiotic substances on the growth of microflora in the microbial consortium of dairy bioproducts. Namely, we used pectin, 'Lactobel' (a milk protein-and-carbohydrate product), pine nut meal, 'Sibirskaya' rowan puree (developed by Kemerovo State University), and rosehip syrup. In the course of experimental studies, we established the most optimal process parameters and amounts of the above prebiotic substances for producing new bioproducts. The combined process flow chart is shown in Figure 4.

The biotechnology for producing freeze-dried products involves standard operations used in the production of liquid fermented dairy products [22]. After fermentation, the finished bioproduct is cooled and freeze-dried.

People can consume both liquid bioproducts and freeze-dried bioproducts in the form of tablets, highly water-soluble dry powders, or bio-sweets.

Combining dairy raw materials with plant components, we can create functional bioproducts with a balanced composition that have a health-benefitting effect and are better absorbed by the human body. Pectin was chosen as a polysaccharide with prebiotic properties that is present in all algae and land plants. It is a common stabilising and gelling agent used in the food industry to give products good rheological properties. Pectin is one of few natural organic compounds that does not undergo chemical degradation when passing through the acidic and alkaline enzymatic barriers of the human body. It maintains its chemical structure and properties in the gastrointestinal tract, helping to restore its mucous membranes. In addition, pectin normalises colonic motility, regulates intestinal microflora, inhibits putrefactive microflora, and participates in most of essential physiological processes. Therefore, most countries recognize

Table 1. Antibiotic resistance of microflora in bioproducts based on starter cultures and their microbial consortium

Name of antibiotic	Antibiotic activity unit	Antibiotic dose	Concentration of the antibiotic to which the microflora of starter cultures and their microbial consortium is resistant				
			L-1	L-2	L-4	Snzh	Consortium
Bicillin	units/cm^3	100; 10; 1; 0.1; 0.01	1	1	1	0.1	10
Gentamycinum	μg/cm^3	80; 40; 8; 4; 0.8	80	40	40	8	80
Clarbact	μg/cm^3	1,000; 100; 10; 1; 0.1	100	100	10	100	1,000
Levomycetin	μg/cm^3	2,500; 250; 25; 2.5; 1.25	250	250	25	25	2,500
Oletetrin	μg/cm^3	250; 25; 2.5; 0.25; 0.025	2.5	2.5	25	250	250
Sparfloxacinum	μg/cm^3	1,000; 100; 10; 1; 0.1	1,000	1,000	1,000	1,000	1,000
Sulfadimethoxinum	μg/cm^3	1,000; 100; 10; 1; 0.1	1,000	1,000	1,000	1,000	1,000
Tetracyclinum	μg/cm^3	1,000; 100; 10; 1;0.1	1,000	100	1,000	100	1,000
Furadonin	μg/cm^3	1,000; 100; 10; 1; 0.1	1,000	100	1,000	1,000	1,000
5-NOK	μg/cm^3	50; 25; 2.5; 0.25; 0.025	25	2.5	25	0.25	50

Note: L-1 – bioproduct with *Lactobacillus acidophilus*; L-2 – bioproduct with *Lactobacillus delbrukii subsp. bulgaricus*; L-4 – bioproduct with *Lactobacillus casei*; Snzh – bioproduct with *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. diacetilactis*, *Streptococcus salivarius thermophilus*; Consortium – bioproduct with the microbial consortium (1:2:3:4 in the ratio of 1:1:0.8:1.2)

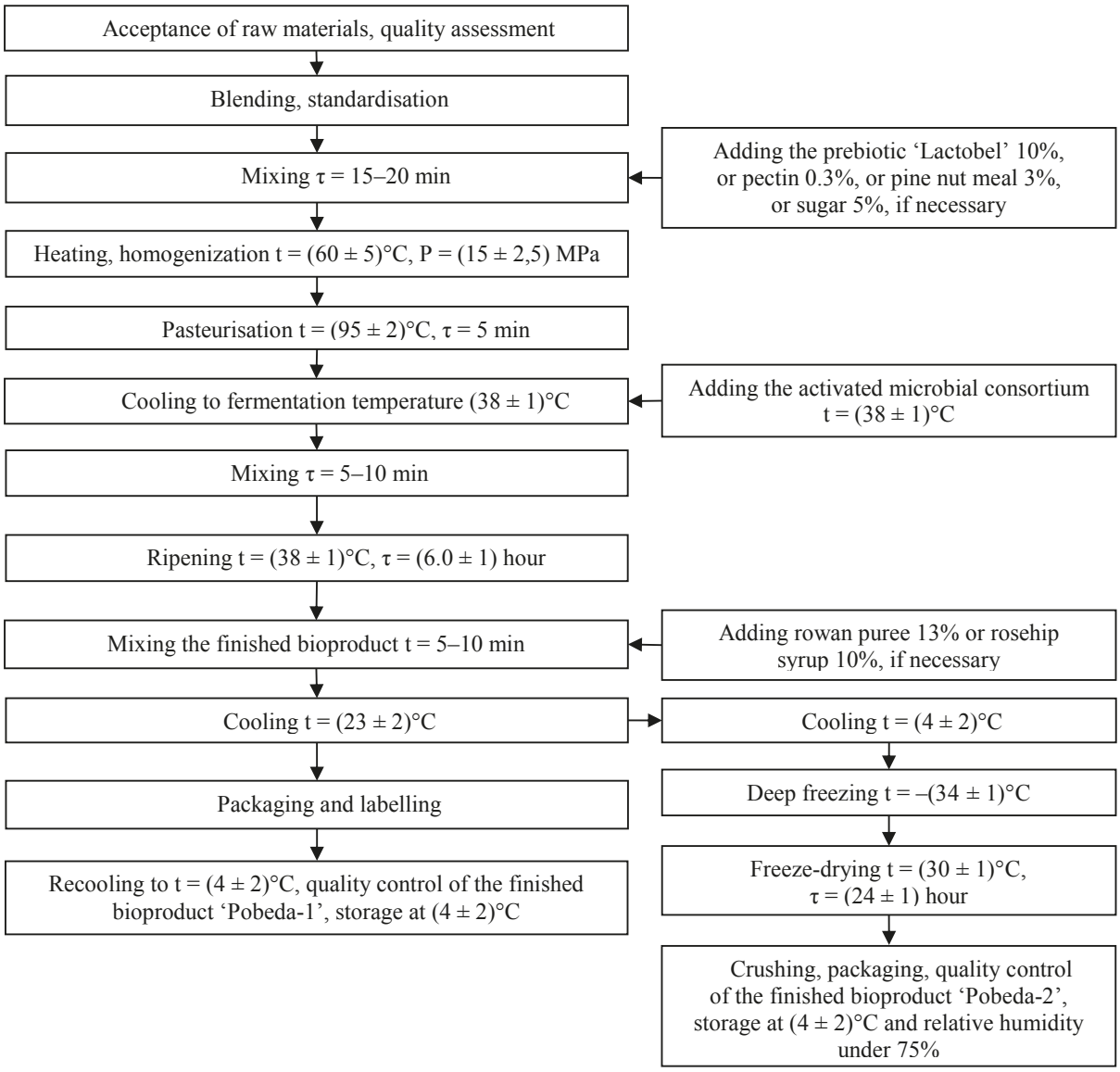

Fig. 4. Combined process flow chart for producing bioproducts

pectin as a physiologically valuable functional ingredient that improves human health when present in conventional diets [23–29].

The choice of 'Lactobel', a Russian milk protein-and-carbohydrate product with prebiotic action, was determined by its properties. This slightly yellow powder with a sweetish taste of powdered milk is highly soluble in warm water and skim milk. Pasteurisation does not change its technological properties, i.e. it does not cause proteins to precipitate, coagulate, or denature. In addition, 'Lactobel' contains albumin, caseinates, lactose, whey proteins, and a prebiotic (lactulose). Its prebiotic and bifidogenic properties revitalise microflora in the microbial consortium and beneficial microflora in the human gastrointestinal tract. As a result, its use ensures a synbiotic bioproduct with highly nutritional and biological values.

The use of Russian pine nut meal creates a synbiotic dairy bioproduct with functional properties. According to some studies [30–32], pine nut meal is a granular powder with the taste of a pine nut that activates beneficial microflora in the gastrointestinal tract and has prebiotic and bifidogenic properties. Its protein composition includes 19 amino acids: tryptophan, leucine, isoleucine, valine, lysine, methionine, glutamic acid, histidine, proline, serine, glycine, threonine, alanine, aspartic acid, phenylalanine, cystine, cysteine, arginine, and tyrosine. Of these, 70% are essential and conditionally essential amino acids, which indicates a high biological value of the protein. Pine nut meal proteins contain more methionine (up to 5.6 g/100 g of protein), cysteine and tryptophan (3.4 g/100 g of protein) than the ideal protein. Their digestibility (95%) is similar to that of chicken egg proteins.

The carbohydrate composition of pine nut meal includes polysaccharides (starch, fibre, pentosans, and dextrins) and water-soluble sugars (glucose, fructose, sucrose, and raffinose). A high content of dietary fibre gives pine nut meal prebiotic properties. Its vitamin composition includes mainly fat-soluble vitamins (A, E, D, K, and F), group B vitamins, and folic acid. Its content of macro- and microelements makes pine nut meal a unique natural source of essential minerals that play

Table 2. Key quality indicators of 'Pobeda' freeze-dried bioproducts

Indicators	Characteristics of 'Pobeda' freeze-dried bioproducts			
	with pectin and rowan puree	with pine nut meal	with 'Lactobel'	with rowan puree and rosehip syrup
Fat mass fraction, %	25.0 ± 0.5	25.0 ± 0.5	25.0 ± 0.5	25.0 ± 0.5
Protein mass fraction, %	25.0 ± 0.4	35.0 ± 0.4	35.5 ± 0.4	24.0 ± 0.4
Carbohydrate mass fraction, %	40.4 ± 0.5	45.5 ± 0.5	36.5 ± 0.5	50.4 ± 0.5
Moisture content, %	3.7 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.8 ± 0.1
Solubility index, cm^3	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
	Sensory indicators			
– taste and aroma	pure fermented milk taste with rowan flavour	pure fermented milk taste with nutty flavour loose powder	pure fermented milk taste	pure fermented milk taste with rowan and rosehip flavour
– texture	loose powder	creamy, uniform	loose powder	loose powder
– colour	light pink, uniform throughout the product	throughout the product	creamy, uniform throughout the product	light brown, uniform throughout the product
Cell count at the end of shelf life, not less than, CFU/g: lactic acid bacteria	1×10^9	1×10^9	1×10^9	1×10^9
Shelf life, days	180	180	180	180
Safety indicators	Meet Technical Regulations of the Customs Union 033/2013			

an important role in many biochemical processes in the human body. It contains up to 5% of mineral substances such as copper, manganese, vanadium, potassium, phosphorus, calcium, molybdenum, nickel, iodine, boron, zinc, iron, phosphorus, and magnesium. Only 100 g of pine nut meal meets the daily human need for magnesium, manganese, copper, zinc, cobalt, and iodine. In addition, Siberian pine nut meal contains barium, titanium, silver, aluminium, iodides, and sodium. Its lipids are represented by phospholipids, cerebrosides, sulfolipids, and unsaturated fatty acids (oleic, linolenic, and gamma-linolenic). They give the bioproduct an attractive appearance, natural taste, smell, and aroma of the raw material, thus increasing its sensory characteristics.

Siberian rowan berries and rosehips were chosen due to their healing properties and ubiquity in Western and Eastern Siberia. Rowan berries contain a complex of important vitamins such as C, E, K, P (rutin), PP (niacin), B group (thiamine, riboflavin, and folic acid), vitamin A, and biologically active substances. They have a higher content of vitamin C (70 mg) than blackcurrant and lemon and are richer in carotene (vitamin A) than carrots. Rowan berries contain such minerals as magnesium (331 mg), potassium (323 mg), as well as calcium (58 mg), phosphorus (36 mg), and sodium (9 mg). Their trace elements include copper, manganese, iron, and zinc. They also contain organic acids (citric, tartaric, and ursolic), easily digestible sugars (sucrose, sorbose, fructose, and glucose), hemicellulose, fibre, and pectic substances. We developed a special technology to make rowan puree.

Russian rosehip syrup is a pleasant-tasting, sweet, viscous dark brown liquid rich in vitamins C and P, pectins, and flavonoids. It has a tonic effect, boosts immunity, prevents hypovitaminosis and malignant neoplasms, and restores energy in fatigued and physically exhausted people. Also, it enhances vision, improves the functioning of blood-making organs, and normalises blood pressure. Finally, rosehip syrup has powerful antioxidant properties, participates in the synthesis of collagen, prevents the destruction of bones, has a positive effect on the regeneration of tissues and bones in cases of injuries or fractures, and accelerates recovery from colds [33].

Table 2 shows the key quality indicators of new 'Pobeda' freeze-dried bioproducts developed during the study.

According to the data obtained during the study, the 'Pobeda' freeze-dried bioproducts have good sensory characteristics and contain a sufficient number of viable lactic acid bacteria cells. These new freeze-dried bioproducts can be produced in the existing production facilities, on fermented milk and freeze-dried biofoods production lines.

CONCLUSION

The 'Pobeda' freeze-dried bioproducts of protein and fat orientation with combiotics and metabiotics can enrich the diet of the Russian military performing special tasks in the Arctic. They can also be used in rations of servicemen performing long-transition raids with increased physical activity and in survival rations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Antipathogenic effects of emulsion and nanoemulsion of cinnamon essential oil against *Rhizopus* rot and grey mold on strawberry fruits

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Abstract: Application of essential oils in controlling plant pathogens is generally associated with difficulty due to low solubility in water, strong odor, physical and chemical instability. One of the ways to minimise these effects is to use a nanoemulsion system. It also increases the antimicrobial properties. In this research, after preparation of cinnamon (*Cinnamon zeylanicum* L.) essential oil (CEO), nanoemulsion of the essential oil was prepared and its physical and chemical properties were determined. The particle size of nanoemulsion was determined to be 115.33 ± 3.97 nm. Emulsification and nanoemulsion of the essential oil along with thiabendazole as an antifungal agent at various concentrations of active ingredient were studied for control of *Rhizopus stolonifera* and *Botrytis cinerea* fungi, strawberry fruit decay. Results in solid Potato Dextrose Agar (PDA) medium indicated that emulsion and nanoemulsion of CEO have a significant difference in antifungal activity against *B. cinerea* and *R. stolonifera*. The minimum inhibitory concentration was 500 and 1,000 µl fungi per liter of culture medium. According to the results of the research, essential oil nanoemulsion had a significant effect on the reduction of a fungal cartilage of strawberry fruit. In general, nano-emulsions of the essential oil showed more antifungal activity than essential oil. There was no significant difference in decay control between thiabendazole and CEO. The nano-emulsion of cinnamon oil at a concentration of 0.2% proved significant effect in reducing fruit decay and showed the lowest fruit infection (5.43%). Consequently, nano-emulsion of essential oil is recommended for the production of natural fungicides.

Keywords: Cinnamon essential oil, strawberry, control of fungal rot, nanoemulsion, *Botrytis cinerea*

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INTRODUCTION

Strawberries have a very short shelf life because of the high susceptibility to fungal agents. The use of artificial antifungal chemical compounds has been a concern for increasing the shelf life of this fruit. For this reason, it is necessary to use safe methods to control decomposition and maintain the quality of strawberry fruit during storage.

The strawberry belongs to the family Rosaceae and belongs to the genus Fragaria [1]. Due to the presence of ascorbic acid and anthocyanin, it has a high antioxidant effect and has many healing properties, such as enhancing the immune system and reducing the incidence of cancers [2], Its susceptibility to fungal rot, especially *Rhizopus stolonifer*, and grey mold produced by *Botrytis cinerea*, as well as high respiration rate, high water content (about 91%), and high metabolic activity have made strawberry one of the most corrosive fruit with short lifespan [3]. Therefore, the use of chemical pesticides to reduce the damage caused by pathogens after harvest is a matter of course [4–6].

The use of fungicides is the best way to control post-harvest diseases. Spraying fruits with Benomyl before and with Thiabendazole immediately after harvesting is the most commonly used control method for post-harvest disease. Another effective method is washing fruits with sodium ortho-phenyl phenate or Imazalil as soluble or homogeneous treatments in packaging and packing lines of factories [7]. The use of these chemical pesticides results in acne or chronic toxicity to non-target organisms, including humans, which are associated with cumulative properties of living organisms or carcinogenicity [8].

Today, due to the special attention to human health and the environment, incentives to find alternatives to pesticides have become much more advanced. Therefore, it is

also essential to strengthen natural methods, using of antifungal herbal products, Gyawali and Ibrahim [9]. Essential oils also provide cropping of agricultural products and food due to their influence on the vapor phase and the possibility of controlling afterbirth [1, 10, 11].

An emulsion is a mixture of two immiscible and suspended liquid phases. Depending on the dispersed phase, the emulsion is divided into two groups: oil-in-water (O/W), where oil droplets are suspended in water, and water-in-oil (W/O), where the water is suspended in oil. The emulsion systems used for conveying compounds are divided into conventional macromolecular emulsions and nanoemulsions [12]. Some of their properties are similar, including composition, structure, and thermodynamic properties, but some of the characteristics are different because of differences in particle size. The size of nanoemulsion particles is 20–50 nm, which is much less than the wavelength of the light, so they do not diffuse the light intensity and thus are transparent or somewhat foggy [13].

Nanoemulsions have used in the manufacture of chemicals, pharmaceuticals, and cosmetics. In cosmetics, rice bran oil is used in the preparation of sunscreens, anti-aging creams, and for skin disease treatment. The size and diffusion of particles can affect the properties of nanoemulsion, such as stability, rheological properties, colour, and texture [14].

Unfortunately, some of the characteristics, including low solubility in water, volatility and strong odor of essential oils, have restricted the use of these natural compounds in foods, especially drinking materials [15, 16]. Moreover, essential oils in foods make unpleasant smells and tastes to consumers, so nowadays we try to reduce the undesirable effects of essential oils.

One of the ways to minimise these adverse effects is to use the nanoemulsion that increases the stability of volatile compounds, protects them against interactions with other compounds, and increases antimicrobial properties by increasing cellular absorption [17, 18].

In this research, we tried to investigate the effect of emulsion and nanoemulsion of cinnamon on controlling the cartilage and grey mold rot of strawberry. The aim of the research was to minimise the strawberry strain losses between the manufacturer and the consumer and the remaining residue of chemical pesticides. If desired, it can be used as an alternative to chemical fungicides to reduce post-harvest lesions of strawberry fruit.

STUDY OBJECTS AND METHODS

Plant material and essential oil extraction. The dried bark of the Cinnamon tree (*Cinnamomum zeylanicum* Blume) was provided from the laboratory sample maintenance unit of RUDN University, Moscow, Russia and then grounded. The essential oil was extracted via distillation by water during three hours using a Clevenger apparatus. Fifty grams of herbal sample was used for essential oil extraction for each treatment. Experiments were carried out in three replications. The obtained essential oil was dewatered by dry sodium sulfate and kept in dark glasses at 4°C until antifungal test [6].

Formulation of nanoemulsion essential oil. Formulation of 10% nanoemulsion essential oil of the Cinnamon was provided from chemistry engineering group of plants and pharmaceutical raw materials from Shahid Beheshti University in Tehran, Iran [18]. In order to obtain nanoparticles of required size, the ultrasonic transducers of a Swiss MTI model (400 W, 220 V, 20.5 kHz, 30% amplitude, and 19 cm probe diameter) was used. Span 80 (Sorbitan monooleate), Tween 80 (Polysorbate 80), and Lecithin were used to make nanoemulsion. Finally, the containers with the nanoemulsion were covered and kept in the fridge.

The particle size of nanoemulsions. The particle size of nanoemulsion was determined with the help of a DLS device. The device obtained the range of particle distribution, as well [19, 20].

Providing the propagule of pathogen. Two most common fungi as the decay factor of strawberry fruits namely *B. cinerea* SBU205 and *R. stolonifera* SBU205 were taken from a collection of microbes of RUDN University. In order to supply the intended isolated propagule, it was cultivated for a week on PDA medium at 25°C. A 12-hour light/darkness period was used. The spore suspension was prepared in sterile distilled water containing 0.05% of Tween 80 and then reached to concentration of 10^4 spores per millimeter of sterile distilled water by a hemocytometer [11].

Antifungal effect of emulsion and nanoemulsion of the Cinnamon essential oil. The antifungal effect of emulsion and nanoemulsion of CEO on two fungi of strawberry fruits, namely grey mold and soft rot was investigated by the method of mixing the essential oil and PDA cultivation medium. For this purpose, a 10% emulsion was prepared from the intended essential oil in a solution of Tween 80 (0.5%). Moreover, the Tween 80 solution (0.05%) was considered as the control treatment. Flasks containing the PDA cultivation medium were kept at room temperature after being put in the autoclave until 42–45°C. Concentrations of 0.125, 0.25, 0.5 and 1.0% of CEO emulsion and nanoemulsion, which equaled 125, 250, 500 and 1,000 µl of essential oil respectively, were transferred separately into flasks with the PDA medium and were mixed to be unified.

The samples obtained were immediately put into Petri dishes with the diameter of 9 m^{-2}. After they got solid, the cork borer provided fungal disks with the diameter of 5 mm from young cultivations of the stated fungus. One fungi disk was then put in the middle of the Petri dishes with cultivation medium which were put in an incubator at 25°C. After 24 hours, the vegetative growth of fungi was measured until the surface of the cultivation medium of control Petri dishes were totally occupied by fungi. In this test, three replications were carried out for each treatment.

Antifungal effects on strawberry fruits. Strawberry fruits of Selva variety (*Fragaria* × *ananassa* cv. Selva) without any physical or chemical problems and equal in size and ripeness index were provided by a greenhouse situated in Moscow, Russia. For superficial disinfection, the fruits were immersed in 0.1% hypochlo-

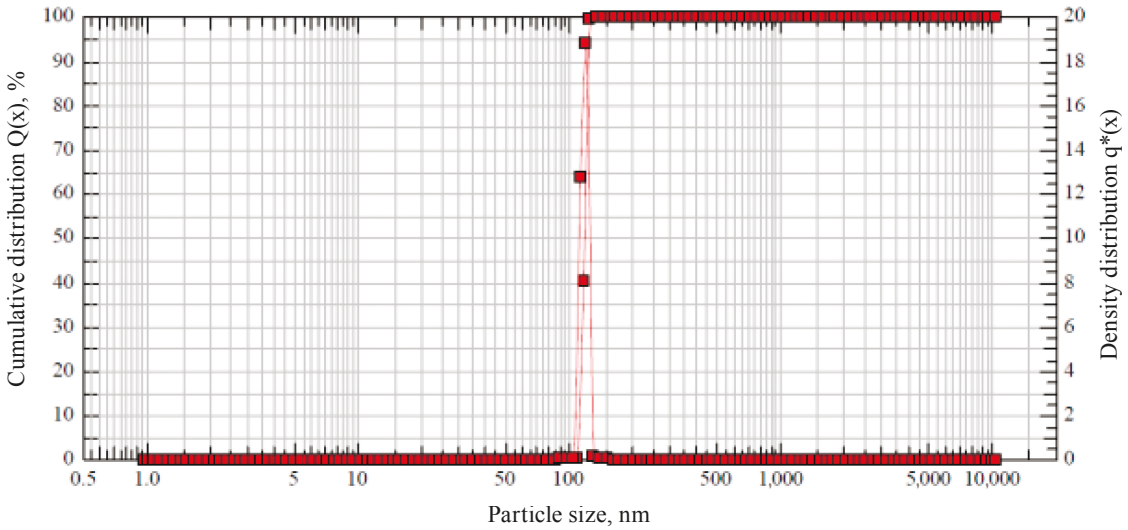

Fig. 1. Particle size and distribution of nanoemulsion obtained by DLS analysis.

rite sodium for 30 s and then they were washed twice by sterile distilled water. After getting dry, in sterile conditions, they were immersed in spore suspensions of *R. stolonifera* and *B. cinerea* for one minute via two separate tests. The concentration was 1×10^4 of spores per a millimeter of sterile distilled water. Further, CEO emulsion and nanoemulsion at concentrations of 0.5, 1 and 2% were added into sterile distilled water and were used for treatment of the inoculated fruits via spraying.

Thiabendazole fungicide (tacto 60) in sterile distilled water (at the concentration of 0.05, 0.1, and 0.2% active ingredient) was used as a control. Other control treatments included infected samples (fruits inoculated by pathogen spore and sprayed by sterile distilled water containing 0.5% of Tween 80) and sound/safe samples (healthy fruits sprayed by sterile distilled water containing 0.5% of Tween 80) [21]. Every five fruits were put as a repetition in one plastic transparent dish with dimensions of 14×11×14 cm [22].

Percentages of *Rhizopus* rottenness and grey decay in strawberry fruits were measured after five and 10 days of storage at 25°C, respectively. The infection of each fruit was based on its mashing, rottenness of fruit's surface; as such that each fruit was divided into eight parts, the symptoms of infection in each part estimated as 12.5% [23, 24]. In other words, the intensity of disease among the infected strawberry fruits ranged between 0 and 8. Here, zero indicates healthy fruit and numbers 1, 2, 3, 4, 5, 6, 7, and 8 shows rottenness to be 12.5%, 12.5–25%, 25–37.5%, 37.5–50%, 50–62.5%, 62.5–75%, 75–87.5%, and 87.5%, respectively. In all treatments, the infection rate of fruit was recorded and rate of control in different treatments was computed by using the proportion of infection in treatment to infection rate in infected samples minus figure of one [23, 24].

Statistical Analysis. A completely randomized design was conducted for testing among treatments with three replications. The normality of data set was determined by Kolmogorov-Smirnov test. The GLM method was used for analysis of variance. After the analysis of variances, the mean comparison of the data was done at significance levels of 1 and 5% with the help of Duncan's multiple range test. All statistical analyses were performed using SPSS software.

RESULTS AND DISCUSSION

Size dispersion and distribution of nanoemulsion. According to Fig. 1, the mean size of the Cinnamon nanoemulsion was equal to 115.33 ± 3.97 nm. The distribution range of particle size of the Cinnamon nanoemulsion was 15.56 ± 3.90 nm.

Antifungal effect of essential oil emulsion and nanoemulsion in laboratory. The results obtained from investigating the antifungal effect of CEO emulsion and nanoemulsion on the growth of fungus showed that generally, an increase in the concentration of essential oil has led to increasing in antifungal activity. The intensity of increase in the deterrence property was observable on *B. cinerea* fungi (Table 1). Regarding the results, emulsion and nanoemulsion of CEO with minimum complete deterrence concentration equaled 500 µl per litre of cultivation medium demonstrated the antifungal activity against *B. cinerea* fungi. It also showed complete deter-

Table 1. Effect of different concentrations of emulsion and nanoemulsion of Cinnamon essential oil on *R. stolonifera* and *B. cinerea* fungus in PDA cultivation medium

Treatment	Active ingredient, µl/l	Deterrence percentage*	
		Rhizopus stolonifer	Botrytis cinerea
Essential oil emulsion	125	3.16 ± 0.33	41.59 ± 0.25
	250	29.42 ± 0.92	65.88 ± 0.85
	500	63.8 ± 0.97	100^{nd}
	1,000	100^{nd}	100^{nd}
Essential oil nanoemulsion	125	11.25 ± 0.19	49.15 ± 0.44
	250	45.68 ± 0.75	68.14 ± 0.82
	500	72.35 ± 1.12	100^{nd}
	1,000	100^{nd}	100^{nd}

Note: nd = not determined (lack of measurable fungal colony)

* The presented number is the mean value of three repetitions

Table 2. Effect of different concentrations of emulsion and nanoemulsion of Cinnamon essential oil on strawberry grey mold after 10 day of storage in darkness at 25°C

Treatment	Fruit infection, %					
	0.05%		0.1%		0.2%	
Essential oil emulsion	41.7 ± 1.79	b	11.84 ± 1.79	e	7.58 ± 1.79	fg
Essential oil nanoemulsion	12.5 ± 1.52	d	7.58 ± 1.52	fg	5.43 ± 0.85	g
Tween 80 (0.05%)	98.43 ± 0.85	a	96.65 ± 0.85	a	97.48 ± 0.85	a
Thiabendazole	23.52 ± 2.65	c	14.13 ± 0.85	e	11.54 ± 1.59	e

Note: Comparing the means has been done in all levels of treatments.
Dissimilar letters indicate significant difference ($p \leq 5\%$)

rence against *R. stolonifera* fungi in the concentration of 1,000 µl per litre of cultivation medium (Table 1). Results of sub-culturing of fungal disks in the treatments where no fungal growth was observed showed that CEO emulsion and nanoemulsion at the concentration of 1,000 µl of active ingredient per one liter of cultivation medium had strong fungicide effect against both fungi.

Reduction of grey mold on strawberry fruits. Amount of strawberry grey mold in infected control samples was computed as 98.33%. There is a significant difference among treatments in terms of grey rottenness amount (Table 2). Generally, an increase at the concentration of the essential oil emulsion and nanoemulsion led to increasing in antifungal activity. Essential oil emulsion at the concentration of 0.2% and essential oil nanoemulsion at concentrations of 0.1 and 0.2% showed the highest amount of deterrence from the grey mold of fruit. There was no significant difference between them, but they had the significant difference with thiabendazole. CEO nanoemulsion at the concentration of 0.2% showed no significant difference with the sound control samples where the infection was zero. Thiabendazole fungicide at the highest concentration (0.2%) with 11.54% infection showed less impact than nanoemulsion.

Reduction of soft rot on strawberry fruits. Emulsion and nanoemulsion of the essential oil had a significant effect on the rate of infection due to the reduced activity of the *R. stolonifera* fungus (Table 3). The results obtained from examining the essential oil showed a decline of rottenness due to *R. stolonifera* on the fifth day of storage. Generally, an increase in the concentration of the essential oil led to increasing in antifungal activity and the essential oil at the concentration of 0.2% demonstrated the highest deterrence from fungi growth. Other concentrations of CEO were not so effective. The antifungal activity of nanoemulsion enhanced with increased concentration, thus Cinnamon nanoemulsion at the concentration of 0.2% displayed the highest rate of control by 13.50% of infection.

The use of medicinal herbs in the pharmaceutical and food industries are expanding due to the active biological compounds in herbs. In addition, extensive research has showed that secondary metabolites of certain medicinal plants are effective in preventing the growth of pathogenic fungi. This makes them an appropriate alternative to chemical pesticides [25].

A number of studies report about the effectiveness of essential oils of some medicinal herbs, such as Sour, Thyme, Cinnamon, Marjoram, Basil, etc., in the control of post-harvest disease in storehouses [10, 26–28]. In Iran, the use of essential oils such as basil, fennel, green substitute, sweet pepper, and peppermint to control of *B. cinerea* and *R. stolonifer* growth is proved to be effective [29–32]. Similarly, the use of herbal essential oils such as marjoram and cinnamon in vegetable washings has had great success in controlling molds and vegetable pathogens [33, 34]. In the study by Saranya *et al.* [7] about the antibacterial properties of a nanoemulsion of eucalyptus oil against *Proteus mirabilis*, the size of particles produced was on average 20 nm. Differences in the size of nanoparticles were due to differences in the types of production methods, while physicochemical properties of the dispersed phase were constant [7, 35].

In our research, CEO also showed significant antifungal activity in controlling grey caries and strawberry fruiting. The preventive effect of essential oils on the growth of the fungus was due to the presence of its active substances. Results of the analysis of essential oils have shown that the main component of cinnamon is cinnamaldehyde which has had antifungal and antimicrobial activity [28, 36].

Unfortunately, essential oils are difficult to use due to low solubility in water, high vapor pressure, and physical and chemical instability. Also, essential oils also

Table 3. Effect of different concentrations of emulsion and nanoemulsion of cinnamon essential oil on strawberry soft rot after five days of storage in darkness at 25°C

Treatments	Fruit infection, %					
	0.05%		0.1%		0.2%	
Essential oil emulsion	92.35 ± 0.85	a	63.33 ± 2.27	d	31.66 ± 1.69	e
Essential oil nanoemulsion	92.35 ± 2.27	a	65.00 ± 1.72	d	13.50 ± 1.72	g
Tween 80 (0.05%)	92.35 ± 2.27	a	92.35 ± 2.27	a	92.35 ± 2.27	a
Thiabendazole	87.50 ± 1.72	b	69.16 ± 0.85	c	20.18 ± 3.17	f

Note: Dissimilar letters indicate significant difference ($p \leq 5\%$)

have an adverse effect on sensory characteristics of the product [37]. One of the ways to minimise this effect is to use nanoemulsion, which allow increasing the stability and half-life of the active substance as well as the ease of reaching the targeted spot on the surface, provided size of the particles is small. In addition, it improves the antimicrobial properties by increasing cellular uptake [12, 17].

On the other hand, the particle size of a nanoemulsion indicates the degree of stability: the larger the particle size, the greater particle size distribution. Thus, the nanoemulsion is more unstable because interconnection of larger particles cause aggregate formation and lead to the instability of the nanoemulsion [38, 39].

In this study, the particle size distribution of the essential oil was about 115 nm, indicating its stability. In fact, the physical and chemical properties of nanoemulsions can be quite different from those of emulsions, as we demonstrate herein. The use of solvents provides the nanoemulsion of essential oils with appropriate viscosity and stability [15]. In addition, the small size of the particles is also very important for increasing the stability and half-life of the active substance and the ease of reaching the workplace [35, 40]. In this research, nanoemulsion of CEO compared to essential oil showed higher antifungal activity against grey caries and soft carcass strawberries.

Similar results were obtained by [18, 20, 41] about the effectiveness of CEO nanoemulsion, savory, and thyme in controlling human pathogenic bacteria. Also, the application of nanoemulsions of *Thymus vulgaris L.* and *Satureja khuzistanica* L. has been effective in controlling strawberry's soft deciduous caries [16]. Nanoemulsions are used as lipophilic transfer systems in the pharmaceutical industry, as flavoring and antibacterial agents in the food industry, as solvents for solving insoluble pesticides in water in the agriculture, as well as carriers of skin care and personal care products in cosmetic products [19]. Particles of nanoemulsions contact the patient's cell wall and begin to destroy them. This non-specialised mechanism does not cause resistance to strains.

On the other hand, regarding the application of essential oils as plant fungicides, the production of a stable, homogeneous, organic aquatic system facilitates its commercial use. The ease of use is possible due to the rapid dilution of emulsions in water by farmers and other consumers [16]. The results of our research allow us to suggest cinnamon nanoemulsion for controlling strawberry storage fungus under post-harvest conditions.

CONCLUSION

Larger particle size is known to lead to unstable nanoemulsion because particles form aggregates. In the current research, essential oil of cinnamon showed considerable antifungal activity against the grey *Rhizopus* rots of strawberry. The preventive effect of essential oil against fungi growth is due to their active ingredients. The nanoemulsion technology may be a potential method to satisfy the demands of commercialization formulations.

In most cases, CEO nanoemulsion in comparison to essential oil indicated greater antifungal activity in controlling the soft rot of strawberry. Generally, increasing the concentration of essential oil raised the antifungal activity and essential oil at the concentration of 0.2% showed the highest rate of deterrence from fungi growth that had a significant difference with other concentrations. In nano essential oil, increasing the concentration led to increasing in antifungal activity, as such that cinnamon nanoemulsion in the concentration of 0.2% with 13.5% infection represented the highest rate of control of antifungal activity against the grey *Rhizopus* rots of strawberry.

According to the results of this research, the cinnamon nanoemulsion is suggested to control the growth of fungus on strawberry in a post-harvest period. In conclusion, improved control activity and maintenance of strawberry quality by cinnamon nanoemulsion indicate that such a formulation is very promising. In addition, further experiments to explain the relationships between antimicrobial activity and chemical composition are underway. After completing the studies about its effects on qualitative characteristics of fruit and obtaining desirable results, it is proposed to produce and use it commercially as an alternative for chemical fungicides in order to reduce the postharvest wastes for strawberry.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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(5) put together the sentences and phrases you have selected into one paragraph in the following sequence: introduction, objects and research methods, research results, conclusions;

(6) make sure the paragraph does not include: information not mentioned in the article; abbreviations or names of organizations; references to sources in the list of references or citations; unimportant details of research methods;

(7) after you have removed all unnecessary information and put together the sentences, make sure that the information is correct (the following order is preferred: relevance, purpose of work, research structure, research methods, main results, conclusion and research prospects);

(8) make sure that the information in the abstract corresponds to the information in your article;

(9) ask a colleague to have a look at your abstract;

(10) check if your final version of the abstract meets the requirements of the Journal (number of words, recommended subtitles, etc.).

Recommendations for the representation of physical quantities

Dimensions are separated from the figure by a space (100 kPa, 77 K, 10.34(2) A), except degrees, percents and per mille signs: 90°, 20°C, 50%, 10‰. Fractional dimensions: 58 J / mol, 50 m/s.

For complex dimensions, use either negative degrees (J mol-1 K-1) or brackets {J / (mol K) or J (mol K) -1}, to facilitate reading. Please follow the principle of uniformity.

In enumeration, as well as in numerical intervals, the dimension is given only for the last number (18–20 J/mol), with the exception of angular degrees.

Degrees Celsius: 5°C, not 5°. Angular degrees: 5–10°, not 5–10°.

Dimensions of variables are separated by a comma (E, kJ/mol), those with sub-logarithmic values are given in square brackets, without comma: ln t [min].

Spaces between words

References to figures and tables should be typed with spaces (Fig. 1, Table 2)

Quotes and brackets are not separated by spaces from the words enclosed in them: (at 300 K).

There is a space after № and §: (№ 1; § 5.65).

Numbers with letters in the notation are typed without spaces: (IVd; 1.3.14a; Fig. 1d).

Recommendations for references

(1) If a Russian article has no title in English, and there is no English name of the journal on its website:

– authors (transliteration); in case of two authors, put *and* between their surnames without a comma; in case of 2–5 authors, put *and* preceded by a comma before the last name; if there are more than five authors, indicate the first three authors and put *et al*. preceded by a comma;

– the title of the article (transliteration);

– [translation of the title into English in square brackets];

– the name of the Russian-language source (transliteration, ***italics***);

– publishing data in English (vol., No., Pp.);

– link to DOI (if available);

– indicate the original language (In Russ.).

Example: Babenyshev S.P. and Evdokimov I.A. Pereraspredelenie chastits dispersnoy fazy zhidkikh polidispersnykh sistem pri ul'trafil'tra-tsionnom razdelenii [Redistribution of the dispersed phase liquid of polydisperse systems in ultrafiltration separation]. *Khranenie i pererabotka sel'khozsyr'ya* [Storage and processing of farm products], 2007, no. 7, pp. 77–79. (In Russ.).

(2) If a Russian-language article has an English title, and the journal has an English-language name):

– authors (transliteration); in case of two authors, put *and* between their surnames without a comma; in case of 2–5 authors, put *and* preceded by a comma before the last name; if there are more than five authors, indicate the first three authors and put *et al*. preceded by a comma;

– title in English;

– source name in English, ***italics***;

– publishing data in English (vol., No., Pp.);

– link to DOI (if available);

– the original language (In Russ.).

Example: Korotkaya E.V. and Korotkiy I.A. Effect of freezing on the biochemical and enzymatic activity of lactobacillus bulgaricus. *Foods and Raw Materials*, 2013, vol. 1, no. 2, pp. 9–14.
DOI: https://doi.org/10.1007/s13762-017-1381-4. (In Russ.).

(3) Articles from an English-language journal:

– authors (transliteration); in case of two authors, put *and* between their surnames without a comma; in case of 2–5 authors, put *and* preceded by a comma before the last name; if there are more than five authors, indicate the first three authors and put *et al*. preceded by a comma;

– the title of the article;

– source name, ***italics***;

– publishing data in English (vol., No., Pp.);

– Link to DOI (if available).

Example: Ajibola C.F., Oyerinde V.O., and Adeniyan O.S. Wholesome Wheat Biscuits Incorporated with Moringa oleifera Leaves and Cocoa Powder. *Journal of Scientific Research and Reports*, 2015, vol. 7, no. 3, pp. 195–206. DOI: https://doi.org/10.9734/JSRR/2015/18070.

(4) Monographs, books, collections of articles:
– authors (transliteration); in case of two authors, put *and* between their surnames without a comma; in case of 2–5 authors, put *and* preceded by a comma before the last name; if there are more than five authors, indicate the first three authors and put *et al*. preceded by a comma;
– title (transliteration, ***italics***);
– [English translation of the title in square brackets];
– publishing data: place of publication in English (Moscow, St. Petersburg); Publisher in English, if it is an organization (Tomsk Univ. Publ.), or transliteration, if the publisher has its own name, with an indication that it is a publisher (Eskulap Publ., Standartinform Publ.);
– the number of pages in the publication (365 p.).

Example: Berezov T.V. and Korovin B.F. *Bioorganicheskaya khimiya* [Bioorganic Chemistry]. Moscow: Meditsina Publ., 1990. 221 p. (In Russ.).

A book chapter:

Diósay L.L. and Venkatesh-Mannar M.G. Vitamin A Fortification of Cooking Oils. *In: Preedy V.R., Srirajaskanthan R., and Patel V. (eds) Handbook of Food Fortification and Health: From Concepts to Public Health Applications, Volume 1*. New York: Springer, 2013, pp. 275–290.

A translated book:

Timoshenko S.P., Young D.H., and Weaver W. *Vibration problems in engineering.* 4th ed. New York: Wiley, 1974. 521 p. (Russ. ed.: Timoshenko S.P., Iang D.Kh., and Uiver U. *Kolebaniia v inzhenernom dele*. Moscow: Mashinostroenie Publ., 1985. 472 p.).

An article from an ongoing publication (collection of papers):

Astakhov M.V. and Tagantsev T.V. Eksperimental'noe issledovanie prochnosti soedinenii "stal'-kompozit" [Experimental study of the strength of joints "steel-composite"]. *Trudy MGTU* "*Matematicheskoe modelirovanie slozhnykh tekhnicheskikh sistem*" [Proc. of the Bauman MSTU "Mathematical Modeling of Complex Technical Systems"], 2006, no. 593, pp. 125–130. (In Russ.).

Conference proceedings:

Chernenkova A.A. Issledovaniye soderzhaniya vitaminov i mikroelementov v pyl'tse i v gotovom produkte [Investigation of vitamins and trace elements in pollen and in the finished product]. *Materialy mezhdunarodnoy nauchno-prakticheskoy konferentsii « Perspektivy innovatsionnogo razvitiya APK*" [Proc. of the Intern. Sci. and Prac. Conf. "Prospects of innovative development of agriculture"]. Ufa, 2014, pp. 28–31. (In Russ.).

Dissertation, author's abstract:

Semenov V.I. *Matematicheskoe modelirovanie plazmy v sisteme kompaktnyi tor* [Mathematical modeling of the plasma in the compact torus]. Dr. phys. and math. sci. diss., Moscow, 2003. 272 p. (In Russ.).

Abramov S.Yu. *Vliyaniye vlazhnosti i temperatury zerna krupyanykh kul'tur na effektivnost' ego pererabotki* [Influence of temperature, of humidity and groat crops on the efficiency of their recycling]. Cand. eng. sci. thesis, Moscow, 1984. 20 p. (In Russ.).

An article from an online journal:

Swaminathan V., Lepkoswka-White E., and Rao B.P. Browsers or buyers in cyberspace? An investigation of electronic factors influencing electronic exchange. *Journal of Computer - Mediated Communication,* 1999, vol. 5, no. 2. Available at: http://www. ascusc.org/ jcmc/ vol5/ issue2/. (accessed 28 April 2011).

An internet resource:

Pravila Tsitirovaniya Istochnikov [Rules for the Citing of Sources]. Available at: http://www.scribd.com/ doc/1034528/. (accessed 7 February 2011).

Government standards:

GOST 8.586.5–2005. Metodika vypolneniia izmerenii. Izmerenie raskhoda i kolichestva zhidkostei i gazov s pomoshch'iu standartnykh suzhaiushchikh ustroistv [State Standard 8.586.5–2005. Method of measurement. Measurement of flow rate and volume of liquids and gases by means of orifice devices]. Moscow: Standartinform Publ., 2007. 10 p. (In Russ.).

Patents:

Palkin M.V. and Kulakov A.V. *Sposob orientirovaniia po krenu letatel'nogo apparata s opticheskoi golovkoi samonavedeniia* [The way to orient on the roll of aircraft with optical homing head]. Patent RF, no. 2280590, 2006. (In Russ.).

Other documents:

Rasporiazhenie Pravitel'stva Rossiiskoi Federatsii ot 25.10.10 goda. № 1873 – r "*Osnovy gosudarst-vennoi politiki Rossiiskoi Federatsii v oblasti zdorovogo pitaniia naseleniia na period do 2020 goda*" [Instruction of the Government of the Russian Federation "Fundamentals of public policy of the Russian Federation in the sphere of healthy nutrition of the population up to 2020"]. (In Russ.).

Abbreviations:

– editor – (ed.), editors – (eds) (without a full stop)
– edition – edn, 2nd edn (without a full stop)
– number of pages – p.
– one page – p.
– page interval – pp.
– volume – vol., Volumes - vols (without a full stop)
– number – no., Numbers - nos (without a full stop)
– part – part
– issue – iss.
– article number – Article number.