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FOOD PRODUCTION TECHNOLOGY

ANALYSIS OF THE ENERGY EFFICIENCY OF THE FAST FREEZING OF BLACKCURRANT BERRIES

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Abstract: In this paper, some results of studying the energy efficiency of the fast freezing of different varieties of blackcurrant berries in a fluidized-bed fast freezer were reported. A method of calculating the energy expenditures on the fast freezing of different varieties of blackcurrant berries in an air fast freezer was proposed. The energy expenditures on the circulation of air at a rate required to create fluidization were determined depending on the air temperature. The energy consumption in the production of artificial cold for the provision of required heat-withdrawing air medium temperatures was calculated. The performed studies were used as a basis to determine the energy-efficient regimes of the low-temperature treatment of blackcurrant berries in an air fast freezer and also the types of a refrigerating machine and a refrigerant, which provided the least energy-consuming fast freezing of blackcurrant berries.

Keywords: blackcurrant, fast freezing, energy efficiency of freezing processes

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INTRODUCTION

The deficit of vitamins and minerals is currently the most widespread and, at the same time, most unwholesome deviation of nutrition from the rational physiologically substantiated standards [1].

Being a natural concentrate of bioactive substances, berries manifest physiologically active properties after entering a human organism and produce an essential effect on its metabolism and vital activity. Blackcurrant is one of the most valuable vitamin-containing plants of the Russian flora. It is rich in ascorbic acid, vitamins B and P. The outstanding value of blackcurrant berries is explained by that vitamins C and P contained in them in great amounts mutually potentiate their health-promoting effects [2].

Freezing is one of the simplest and most widespread methods for the preservation of moisture-containing products. Frozen berries can be stored for many months, as the moisture in them has been brought into a solid state. A decrease in temperature and dewatering during the transition of the moisture contained in berries into a solid state create unfavorable conditions for the development of biochemical reactions in a frozen object, and their rate is abruptly decelerated [3].

The formation of ice crystals alongside with the freezing of a moisture-containing object leads to the destruction of its structure. The destruction of a product's structure is induced by both mechanical and osmotic factors. Ice crystals formed outside cells deform and destruct their membranes, growing in size during the process of freezing. Moreover, the growth of ice crystals in the intercellular space leads to the diffusion of cellular moisture through membranes and the dewatering of cells.

The intensity and character of transformations in a product under freezing depends on the conditions and parameters of the process and the qualitative characteristics of a low-temperature treatment object. The intensification of heat withdrawal in the process of freezing is accompanied by an increase in the amount of crystallization seeds, and this in turn promotes the formation of a microcrystalline structure. The more intense is heat withdrawal, the smaller will be the crystals in a frozen product [4]. In this case, the crystalline structure will be uniform, and ice crystals will form in both the intercellular space and the cells themselves.

The withdrawal of heat in the freezing of berries can be intensified either by decreasing the temperature of a heat-withdrawing medium or by accelerating its circulation. In the first case, an increase in heat withdrawal will have an extensive character due to an increase in the temperature difference between a low-temperature treatment object and a heat-withdrawing medium. In the second case, an increase in heat withdrawal will have an intensive character due to an increase in the coefficient of heat transfer between a freezable object and a refrigerating medium [5].

The intensification of heat exchange in the freezing of berries is accompanied by the growth of energy consumption in both the first and second cases. To intensify the heat withdrawal in real berry freezing processes, the cumulative effect of the two above listed factors is used.

An important question in the development of a low-temperature treatment technology is the energetic component in the primecost of the finished fresh-frozen fruit and berry products. For this reason, the ratio of the thermal and convective factors is a determinative element in the optimization of energy consumption in

the low-temperature treatment of berries.

The objective of this work was to determine the low-temperature treatment regimes, which would allow the minimization of energy consumption in the production of fast-frozen blackcurrant berries.

OBJECTS AND METHODS OF STUDY

The fast freezing of berries is performed in fast freezers, which represent devices able to provide a high air circulation rate and required low-temperature treatment temperatures.

To determine the critical and optimal parameters of air motion in a freezer, it is necessary to have the data characterizing the mass and volumetric parameters of fruits and berries. Such characteristics of the studied varieties of berries are listed in Table 1.

Table 1. Mass and volumetric characteristics of the studied varieties of fruits and berries

Variety	Product unit mass, g	Product density, kg/m^3	Bulk density, kg/m^3	Bed porosity	Product unit diameter, mm
Pamyat' Lisavenko	1.4	1067	741	0.298	13–14
Seyanets Golubki	1.1	1082	751	0.293	12–13
Pamyat' Shukshina (Olimpiiskaya)	0.9	1070	743	0.290	11–12
Chernyi zhemchug	1.7	1075	746	0.306	14–15
Krasa Altaya	1.1	1059	735	0.295	12–13
Pushistaya	0.8	1063	738	0.291	11–12

The critical air velocities (w'_{cr}, w''_{cr}) are determined in compliance with the following method [6].

The critical air velocity w'_{cr} characterizes the beginning of fluidization.

$$w'_{cr} = \frac{\nu_{air}}{d_e} \times \frac{\mathrm{Ar}}{1400 + 5.22\sqrt{\mathrm{Ar}}}, \quad (1)$$

where ν_{air} is the air kinematic viscosity, m^2/s, and d_e is the spherical product part's diameter.

The Archimedes number is determined by the formula

$$\mathrm{Ar} = \frac{g \cdot d_e \cdot \rho_{pr}}{\nu_{air}^2 \cdot \rho_{air}}, \quad (2)$$

where g = 9.8 m/s^2 is the gravity acceleration, and ρ_{pr} and ρ_{air} are the product and air density, respectively.

The critical air velocity w''_{cr} is a velocity, at which the entrainment of a product is possible, and

$$w''_{cr} = \frac{\nu_{air}}{d_e} \times \frac{\mathrm{Ar}}{18 + 0.6\sqrt{\mathrm{Ar}}}. \quad (3)$$

To determine the freezing time for moisture-containing food products, the Planck formula is most widely used [7]:

$$\tau_{fr} = \frac{q_{fr} \cdot \rho_{pr}}{t_{cryo} - t_{air}} \times \frac{d_e}{6}\left(\frac{d_e}{4\lambda_{fr}} + \frac{1}{\alpha}\right), \quad (4)$$

where α is the product-to-air heat-transfer coefficient, W/(m^2·K), λ_{fr} is the heat conductivity of the product's frozen part, W/(m·K), ρ_{pr} is the product density, kg/m^3, q_{fr} is the specific heat withdrawn from a product in the process of freezing, J/kg, t_{cryo} is the cryoscopic temperature, °C, and t_{air} is the air temperature.

The heat-transfer coefficient is determined depending on the velocity and regime of the air flow, its thermodynamic parameters, and the shape and size of a product.

According to the theory of similarity, the heat-transfer coefficient can be calculated by the formula [8]

$$\alpha = \mathrm{Nu} \cdot \lambda_{air} / d_e, \quad (5)$$

where λ_{air} is the air heat conductivity, and Nu is the Nusselt number.

The Nusselt number for the heat transfer in a fluidized bed can be determined from the empirical equation [9]

$$\mathrm{Nu} = 0.03 \cdot \mathrm{Pr}^{1/3} \cdot \mathrm{Re}, \quad (6)$$

where Pr = $c_p \cdot \mu_{air}/\lambda_{air}$ is the Prandtl number, μ_{air} is the air dynamic viscosity, Pa·s, c_p is the air specific heat capacity, J/(kg·K), Re = $\omega \cdot d_e \cdot \rho_{air}/\mu_{air}$, ω is the air velocity, m/s, and ρ_{air} is the air density, kg/m^3.

The air amount m_{air} (kg) required to freeze a kilogram of berries is determined by the formula

$$m_{air} = \frac{\Delta h}{c_p \cdot \Delta t_{air}}, \quad (7)$$

where Δh is the difference between the enthalpies of berries before and after freezing (at 10 and –18°C, J/kg), and Δt_{air} is the air heating, K.

The air heating in the freezing of berries is found as

$$\Delta t_{air} = \alpha \cdot F_{pr} \cdot \Delta t_m, \quad (8)$$

where F_{pr} is the surface area of berries, m^2, and Δt_m is the logarithmic mean temperature difference between the air and freezable berries. In turn, Δt_m can be calculated as

$$\Delta t_m = \frac{t_{air2} - t_{air1}}{\ln \dfrac{t_{cryo} - t_{air1}}{t_{cryo} - t_{air2}}}, \quad (9)$$

where t_{cryo} is the cryoscopic temperature of berries, t_{air1} is the initial air temperature, t_{air2} is the final air temperature, and $\Delta t_{air} = t_{air2} - t_{air1}$.

Since the initial air temperature (t_{air1}) is specified, the final air temperature (t_{air2}) can be determined by Eq. (9).

The air volume required to freeze a kilogram of berries is calculated as

$$V_{air} = m_{air}/\rho_{air}. \quad (10)$$

The energy (L_{fan}), which should be spent on the circulation of this air volume at a required rate can be found as

$$L_{fan} = V_{air} \cdot \Delta P/\eta_{fan}, \quad (11)$$

where η_{fan} is the fan efficiency coefficient, which is nearly $\eta_{fan} = 0.76$ for centrifugal fans operating under the conditions of a fast freezer, ΔP is the aerodynamic resistance to the motion of air in the circuit of a fast freezer.

The aerodynamic losses during the motion of air in a fast freezer occur in the fluidized bed, the supporting grid, the finned air cooler sections, upon the turns of the air flow at the fan's inlet, and in the fan's diffuser. The greatest aerodynamic losses take place in the fluidized bed, the supporting grid, and the air cooler sections. The other listed regions of the air circuit structurally belong to the fan, and the aerodynamic losses in them are small in comparison with the other regions, so they may be included into the fan efficiency coefficient instead of being taken into consideration in particular calculations.

The aerodynamic resistance of the fluidized bed (ΔP_{fl}, Pa) can be determined by the following method [6]:

$$\Delta P_{fl} = 1.67 \left(\mathrm{Re} \frac{H_{fl}}{d_e} \right)^{0.2} \times \frac{G_{pr}}{F_{pr}}, \quad (12)$$

where H_{fl} is the fluidized product bed height, G_{pr} is the product mass, and F_{pr} is the surface area occupied by a product on the grid, m^2.

$$H_{fl} = H_0 \left(\frac{1-\varepsilon_0}{1-\varepsilon} \right), \quad (13)$$

where ε_0 is the bulk bed porosity (Table 1), $\varepsilon = \left(\frac{18\,\mathrm{Re} + 0.36\,\mathrm{Re}^2}{\mathrm{Ar}} \right)^{0.21}$ is the fluidized bed porosity, and H_0 is the bulk product bed height, m.

The aerodynamic resistance of the supporting grid with meshes of 3 × 3 mm in size and the free air flow area $E = 0.308$ (ΔP_g, Pa) is

$$\Delta P_g = 13.72 \cdot w^2 - 43.12 \cdot w + 119.36, \quad (14)$$

where w is the air flow velocity, m/s.

The aerodynamic resistance of the finned air cooler section (ΔP_{ac}, Pa) is found from the dependence

$$\Delta P_{ac} = 1.35 \cdot A \cdot \mathrm{Re}^{-0.24} \rho_{air} \cdot w^2, \quad (15)$$

where A is the coefficient taking into account the structural features of the air cooler.

Hence, the aerodynamic resistance to the air flow in the circulation circuit of the air cooler (ΔP, Pa) can be found from the equation

$$\Delta P = (\Delta P_{fl} + \Delta P_g + \Delta P_{ac})\alpha, \quad (16)$$

where $\alpha = 1.1$ is the coefficient taking into account the friction resistances to the air flow.

The working fluid in the cycles of refrigerating machines participates in different thermodynamic processes. The efficiency of refrigerating machines depends on the fashion, in which these processes are performed. The problem of thermodynamic analysis based on the first and second laws of thermodynamics is to ascertain the possible efficiency of refrigerating machine cycles [10].

The refrigeration efficiency of one-stage, two-stage, and cascade refrigerating machines operating on freons R-134a, R-22, R-404a, R-23, and ammonia was studied. The energy expenditures on the production of artificial cold were estimated by the method described in [11].

The ambient medium is of great importance in the thermodynamic theory of refrigerating machines. The ambient medium is first of all characterized by the independence of its parameters on the operation of a considered refrigerating machine. This means that any action of a refrigerating machine produces no changes in the ambient medium. The atmospheric air with a temperature from 15 to 35°C was considered as such a medium.

An important condition for the implementation of a refrigeration engineering solution is the organization of heat exchange between a working fluid and the ambient medium, and also a cold-carrying agent, which performs the transfer of heat from a freezable object to a working fluid without appreciable expenditures. To perform the thermal analysis of the efficiency of refrigeration cycles, the temperature difference between a working fluid and the ambient medium in the evaporator was taken equal to 10°C, and the temperature difference between a working fluid and the air leaving the air cooler was also set equal to 10°C.

The working cycle of a one-stage compression refrigerating machine is shown in Fig. 1a in the *p–i* (pressure–enthalpy) coordinates. In this cycle, *1–2* is compression in the compressor, *2–3′* is cooling of a cooling agent and condensation in the condenser, and *3′–3* and *a–1* are heat regeneration in the recuperative heat exchanger of freon refrigerating machines. Heat regeneration in ammonia refrigerating machines is unreasonable. Process *3–4* is expansion in an expanding device. Process *4–a* is boiling of a cooling agent in the evaporator.

The overheating of a working fluid before the compressor is estimated by the formula $\Delta T_{oh} = T_1 - T_a$. For ammonia refrigerating machines, the overheating of a working fluid at suction onto the compressor is taken to be ΔT_{oh} = 5–10°C. Let us set ΔT_{oh} = 10°C. For freon refrigerating machines, ΔT_{oh} = 15–35°C. Let us set ΔT_{oh} = 30°C.

The overcooling before expansion is calculated as follows. For regeneration cycle refrigerating machines, it is determined from the energy balance of a recuperative heat exchanger

$$h_L^{in} - h_L^{out} = h_V^{out} - h_V^{in}. \quad (17)$$

For non-regeneration cycle (ammonia) refrigerating machines, the temperature of the liquid cooling agent before the expanding valve is taken to be 1–3°C lower than the temperature of the liquid leaving the condenser (t_C), i.e., $t_L = t_C - (1–3°C)$.

The specific refrigeration capacity of a refrigerating machine q_0, the specific adiabatic compression work in the compressor l_s, and the real mass and volumetric capacities of the compressor G_{real} and V_{real} are then determined.

The specific refrigeration capacity of a refrigerating machine will be

$$q_0 = h_a - h_4 . \quad (18)$$

The specific adiabatic work of the compressor is determined from the formula

$$l_{ad} = h_2 - h_1 . \quad (19)$$

The power of a refrigerating machine N_e (kW), which should be provided to withdraw a certain amount of heat per unit time Q_0 (kW) from a freezable object is determined by the following method:

$$N_e = N_i + N_{fr} , \quad (20)$$

where N_i is the indicated power of the compression of a working fluid in the compressor, and N_{fr} is the power spent on friction and the driver of auxiliary devices,

$$N_i = G_{real}\, l_{ad}/\eta_i , \quad (21)$$

where G_i is the mass flow rate of a cooling agent circulating in a refrigerating machine (kg/s), and η_i is the indicated efficiency coefficient of the compressor.

The friction power is determined from the empirical formula

$$N_{fr} = p_{i,fr} V_t , \quad (22)$$

where $p_{i,fr} = (40–90)\cdot 10^3$ Pa is the friction pressure, and V_t is the theoretical volumetric capacity of the compressor (m^3/s).

The mass flow rate of a cooling agent is determined as

$$G_{real} = Q_0/q_0 . \quad (23)$$

The theoretical volumetric capacity is found by the formula

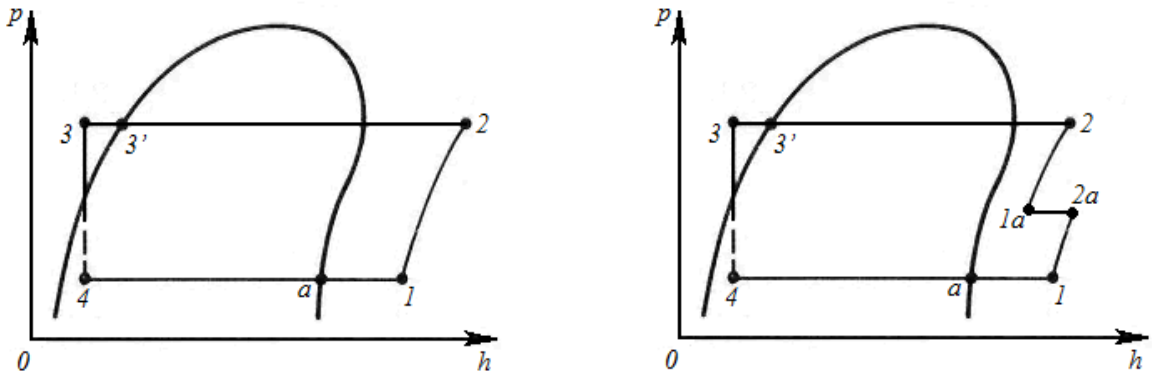
$$V_t = G_{real}\, v_1/\lambda , \quad (24)$$

where v_1 is the specific volume of a working fluid sucked into the compressor, λ the delivery coefficient of the compressor, $\lambda = f(p_{frcd}/p_{suct})$. Here p_{frcd} is the pressure, to which a working fluid is compressed immediately in the working members of the compressor, and p_{suct} is the pressure of the working fluid entering immediately into the working space of the compressor.

The method of the estimation of λ is described in [11].

A vapor-compression refrigerating machine can be implemented in different designs. We have considered a refrigerating machine in the simplest and, consequently, most acceptable implementation for the supply of a self-contained fast freezer with cold.

The working cycle of a two-stage compression single-expansion refrigerating machine is shown in Fig. 1b in the *p–i* coordinates. In this cycle, *1–2a* is compression in the compressor of stage *I*, *2a–1a* is cooling in the interstage cooler due to the transfer of compression heat to the ambient medium, *2–3′* is compression in the compressor of stage *II* and cooling of a cooling agent and condensation in the condenser, and *3′–3* and *a–1* are regeneration of heat in the recuperative heat exchanger of freon refrigerating machines. In ammonia refrigerating machines, $t_3 = t_3 - (1–3°C)$. Process *3–4* is expansion in the expanding device. Process *4–a* is boiling of the cooling agent in the evaporator.



(a) (b)

Fig. 1. Theoretical cycle of a refrigerating machine in the *p–h* (pressure–enthalpy) coordinates: (a) one-stage compression cycle, (b) two-stage compression cycle.

The method of calculating the cycle of a two-stage compression refrigerating machine principally corresponds to the method of calculating the cycle of a one-stage compression refrigerating machine, but has some peculiarities.

The interstage pressure is selected by the formula

$$p_{int} = \sqrt{p_1 p_2} , \quad (25)$$

or from the condition of the maximally admissible compression end temperature for a studied cooling agent.

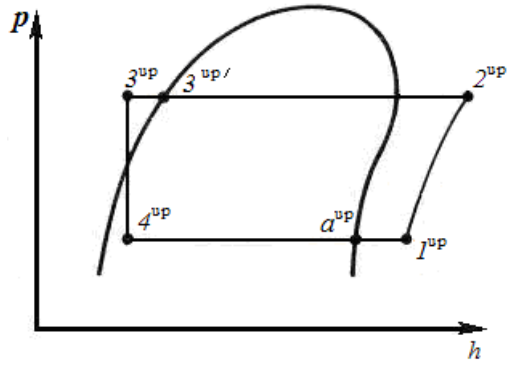
The temperature of point *1a* is determined from the ambient temperature and the condition of the

undercooling of a cooling agent in the interstage cooler due to heat underrecuperation.

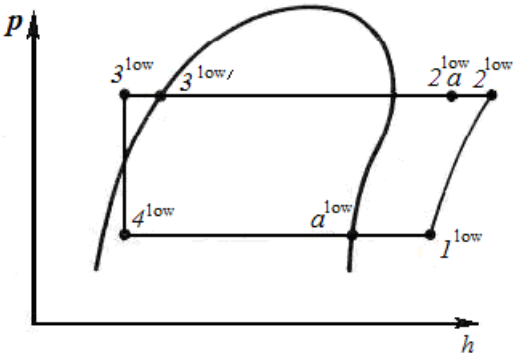
The two-stage compression work is determined by the formula

$$l_{comp} = l_I + l_{II} = (h_{2a} - h_1) + (h_2 - h_{1a}) . \quad (26)$$

In cascade refrigerating machines, at least two working fluids are used. A two-cascade refrigerating machine consists of two independent refrigeration cycles called branches. The interaction between the branches of a cascade refrigerating machine occurs in heat exchangers. In the lower low-temperature branch, a high-pressure working fluid is used (we used freon R-23 with a normal boiling temperature of –82.14°C). The cooling agent of the upper branch was freon R-22 with a normal boiling temperature of –40.81°C. The upper branch of a cascade refrigerating machine is designed for the withdrawal of condensation heat from the cooling agent of the lower branch and its transfer to the ambient medium. The working cycle of a cascade refrigerating machine is shown in Fig. 2.



(a)



(b)

Fig. 2. Theoretical cycle of a two-cascade refrigerating machine: (a) upper (high-temperature) cascade branch, (b) lower (low-temperature) cascade branch.

Here, 1^{low}–2^{low} is compression in the compressor of the lower cascade branch, 2^{low}–$2^{low}a$ is cooling of a cooling agent in the heat exchanger due to the withdrawal of heat into the ambient medium, $2^{low}b$–$3^{low\prime}$ is condensation of the cooling agent of the lower cascade branch in the evaporative condenser due to the withdrawal of condensation heat by the upper cascade branch, $3^{low\prime}$–3^{low} is overcooling of the liquid cooling agent of the lower branch in the regenerative heat exchanger due to heat exchange with the cooling agent sucked into the compressor (a^{low}–1^{low}), 3^{low}–4^{low} is expansion in the expanding device, 4^{low}–a^{low} is boiling in the evaporator of the lower branch. The upper cascade branch works by the cycle similar to the cycle of a one-stage compression refrigerating machine, but process 4^{up}–a^{up} is boiling of the cooling agent of the upper cascade branch in the evaporative condenser due to the delivery of heat from the condensing cooling agent of the lower cascade branch. The temperature difference between the cooling agents of the branches due to underrecuperation is 3–5°C.

The refrigerating capacity of a cascade refrigerating machine is controlled by the refrigerating capacity of the lower cascade branch, and the work expenditures correspond to the total work expenditures in both cascades.

When determining the work expenditures on the freezing of fruits and berries, it was necessary not only to take into account the work expenditures on the withdrawal of crystallization heat from a product and the heat spent on the cooling of fruits and berries before and after crystallization. It was also necessary to take into consideration the heat inflow through the heat-insulating enclosures of a freezer Q_1 and the heat inflow from the air entering a freezer through the charge and discharge ports Q_4.

The heat inflow Q_1 is determined by the formula

$$Q_1 = \Sigma(k_i F_i)\Delta t , \quad (27)$$

where k is the heat-transfer coefficient of heat-insulating enclosures and depends on the type and thickness of heat insulation, the averaged value k = 0.21 W/(m^2 k) is taken in our calculations; F is the surface area of heat-insulating enclosures and depends on the dimensions and shape of a freezer, and Δt is the temperature difference between the ambient air and the air in a fast freezer.

Since the surface area of heat-insulating enclosures can not be taken into account without the knowledge of the real dimensions of a fast freezer, the parameter $\Sigma(k_i F_i)$ was replaced by the specific heat flux through the enclosures per unit mass of a freezable product q_1. This parameter was taken to be q_1 = 90 W/(kg K) from averaged values.

The heat inflow from the air entering and leaving a freezer through the charge and discharge ports was taken from the recommendations $Q_4 = 0.4Q_1$.

RESULTS AND DISCUSSION

The experimental data given in Table 1 and Eqs. (1)–(3) were used to obtain the critical velocities of the studied fruit varieties. The results of calculations are plotted in Fig. 3.

From the performed calculations for the studied varieties of berries and fruits, it follows that the range of velocities, at which the phenomenon of fluidization takes place (from the appearance of a fluidized bed to the velocity, at which the entrainment of fruits and berries is possible), is 1.24–17.7 m/s at temperatures from –43 to –13°C for all the studied varieties. Since every variety of fruits and berries has its own range of air velocities, at which the process of fluidization is stable, we have used for studies the range of velocities, at which the process of fluidization without the entrainment of fruits and berries is ensured for all the studied varieties. These air velocities were from 2 to 11.5 m/s.

Using Eqs. (4)–(6), the freezing time was calculated for the studied varieties of blackcurrant berries at different air velocities and temperatures, and the results of calculations were plotted in Fig. 4.

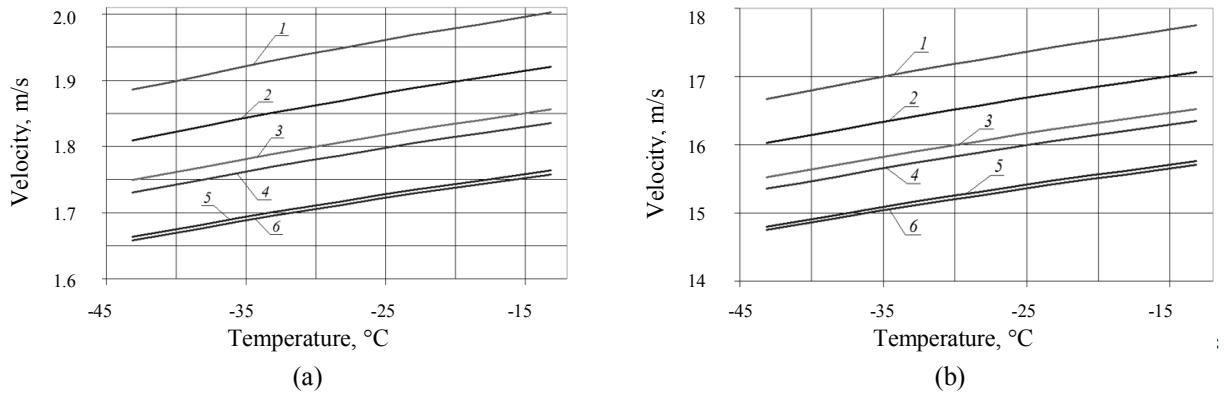


Fig. 3. Critical fluidization velocities of blackcurrant berries versus temperature: (*1*) Chernyi zhemchug, (*2*) Pamyat' Lisavenko, (*3*) Seyanets Golubki, (*4*) Krasa Altaya, (*5*) Pamyat' Shukshina, (*6*) Pushistaya, (a) fluidization beginning velocity, (b) berry entrainment velocity.

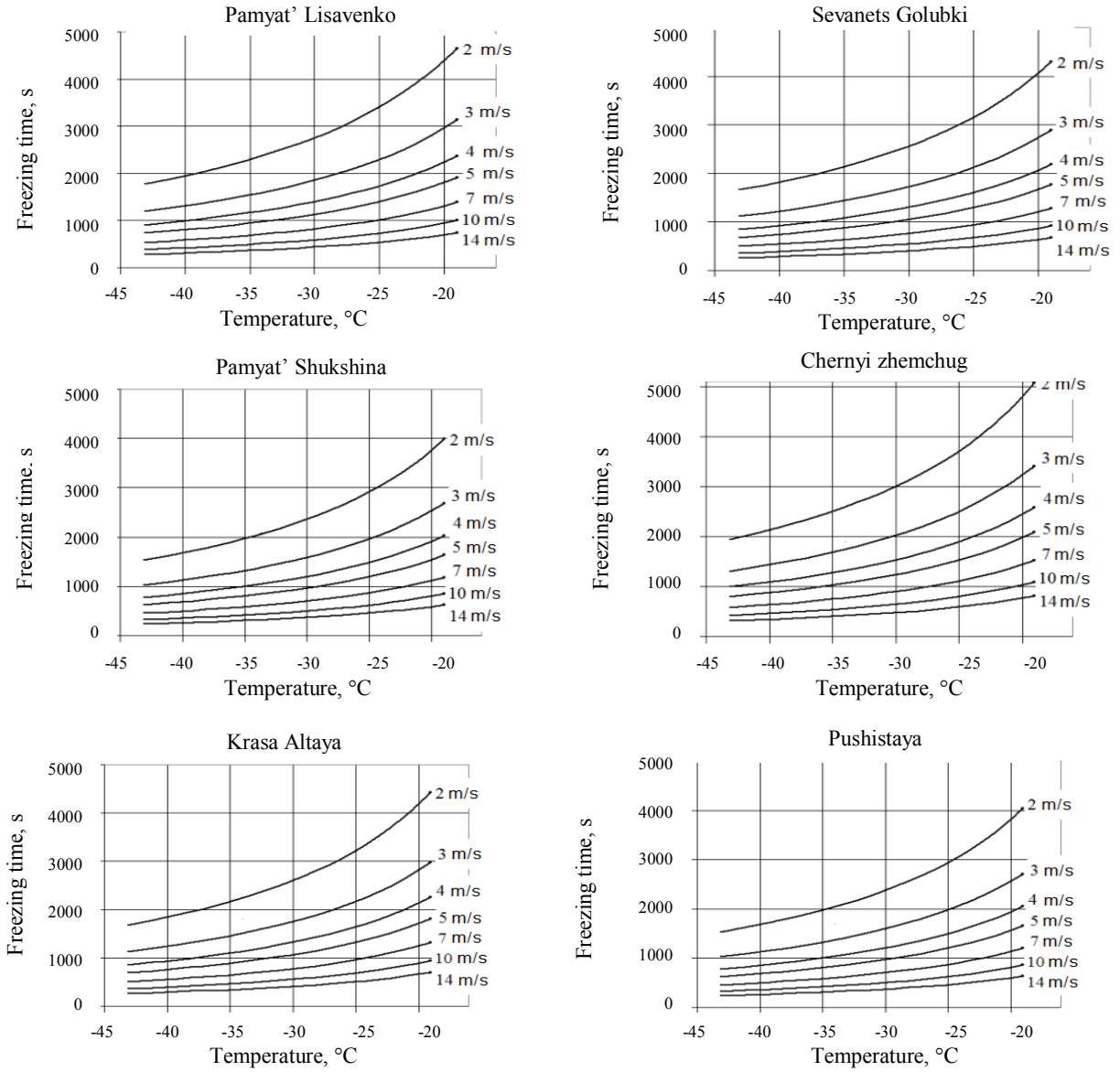


Fig. 4. Freezing time versus temperature for the freezing of the studied varieties of blackcurrant berries from the initial temperature of 10°C to the temperature of –18°C in a fluidized-bed fast freezer at different air velocities.

Using Eqs. (7)–(16), the energy expenditures on the circulation of air in a fast freezer were calculated depending on the air temperature and velocity. The energy expenditures were calculated for the freezing of blackcurrant berries from the initial temperature t_{init} = 10°C to the temperature t_{fin} = –18°C. The results of calculations are plotted in Fig. 5.

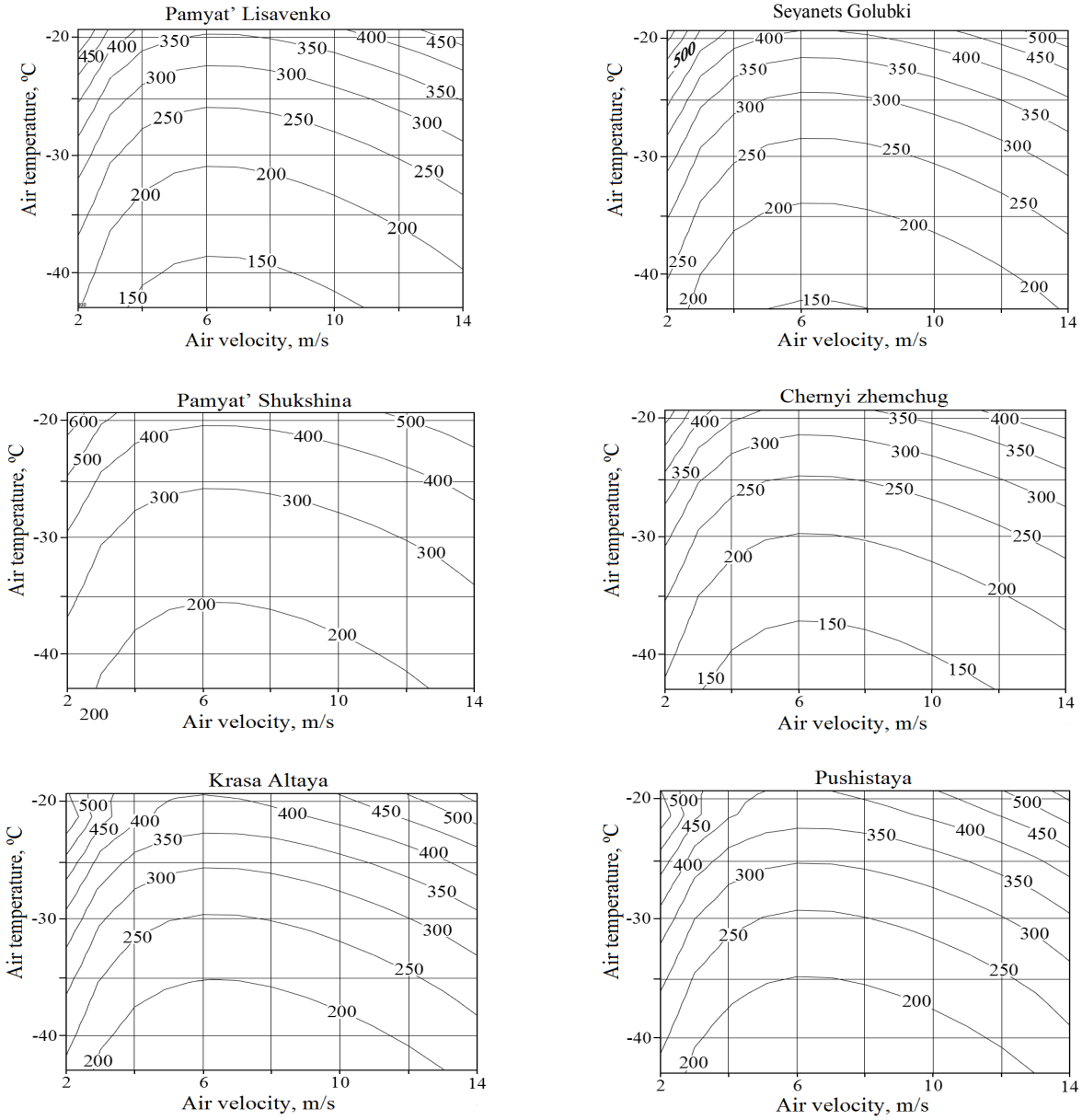


Fig. 5. Energy expenditures (kJ/kg) on the circulation of air in a fast freezer depending on the air velocity and temperature for the freezing of a kilogram of blackcurrant berries of studied varieties from the initial temperature of 10°C to the temperature of –18°C.

The results of calculations indicate the existence of a certain range of air velocities, at which the air circulation energy expenditures are minimal at a certain freezing air temperature for the studied varieties of blackcurrant berries. Thus, the optimal range of velocities for blackcurrant berries is 6–7 m/s.

An increase in the velocity of air passing through the bed of berries intensify the transfer of heat from freezable fruits and decreases the freezing time, so a smaller circulating air amount is required for the freezing of berries. However, the aerodynamic losses grow proportionally to the squared air velocity. Hence, an increase in the air velocity decreases the required air flow rate for the withdrawal of heat in the process of freezing and, consequently, the energy expenditures on the transport of a necessary air amount. At the same time, an increase in the air velocity leads to the growth of energy expenditures due to the need to overcome aerodynamic resistances. Hence, at the initial stage, a decrease in energy expenditures due to a reduction in the amount of circulating air compensates an increase in the energy spent to overcome aerodynamic resistances. When the air velocity attains the values exceeding the optimal level, the growth of the energy expenditures required to overcome aerodynamic resistances become quicker than a decrease in the

energy expenditure due to a reduction in the required amount of circulating air, so the total energy expenditures on the circulation of air grow.

The calculated required specific refrigeration capacity of a refrigerating machine for the freezing of a kilogram of blackcurrant berries as a function on the air temperature in a fast freezer and the ambient air temperature is plotted in Fig. 6.

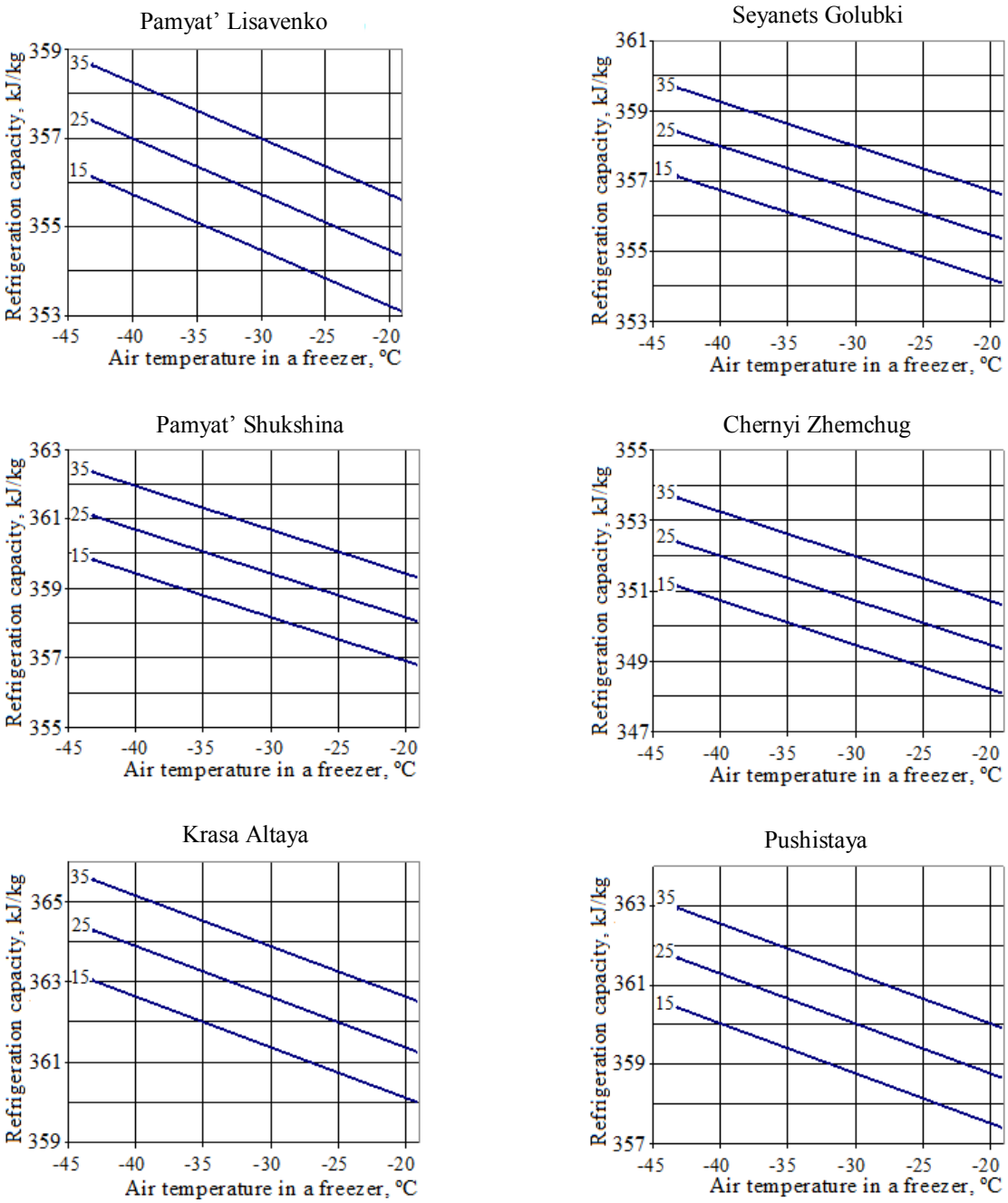


Fig. 6. Required refrigeration capacity (kJ/kg) of a refrigerating machine for the freezing of a kilogram of blackcurrant berries from the temperature of 10°C to the temperature of –18°C depending on the air temperature in a fast freezer and the ambient air temperature.

The calculation results plotted in Fig. 6 for the required refrigeration capacity of a refrigerating machine depending on the ambient medium temperature and the air temperature in a freezer indicate that the required refrigeration capacity of a refrigerating machine for the freezing of different varieties of the same species of berries and fruits differs slightly.

The required refrigeration capacity of a refrigerating machine for the freezing of a kilogram of blackcurrant berries differs by less than 1.9% depending on their variety.

The comparative analysis of the energy efficiency of different refrigeration schemes used for the freezing of fruits and berries was performed using Pamyat' Lisavenko blackcurrant berries.

The results of the comparative calculations of the energy expenditures on the freezing of berries in one-stage refrigerating machines are shown in Fig. 7.

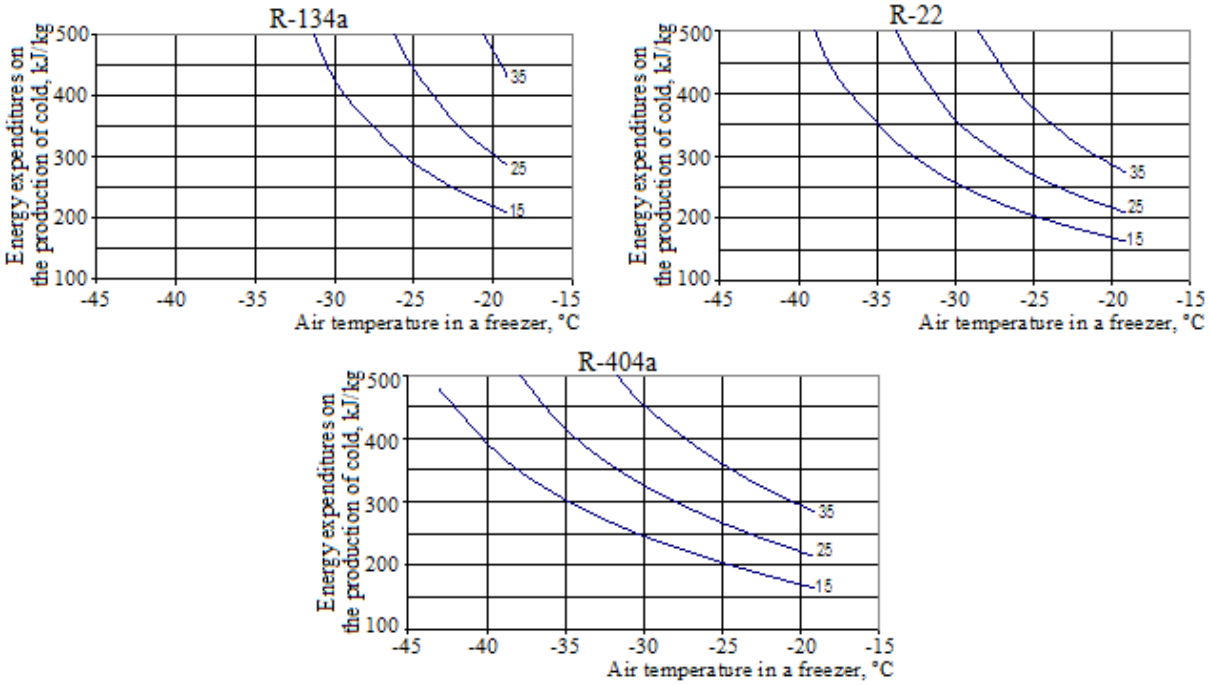


Fig. 7. Energy expenditures (kJ/kg) on the driver of a one-stage refrigerating machine for the freezing of a kilogram of Pamyat' Lisavenko blackcurrant berries from the temperature of 10ºC to the temperature of –18ºC depending on the air temperature in a fast freezer and the ambient air temperature.

The use of a one-stage refrigerating machine operating on freon R-134a is unreasonable, as the production of cold of a temperature level below –20°C in a refrigerating machine of this type is attended by unjustifiable high energy expenditures.

The energy expenditures on the production of artificial cold with a cooling source temperature above –25°C in a one-stage refrigerating machine operating on freons R-22 and R-404a are at the same level.

A decrease in the temperature of a cooling medium in a fast freezer below –25°C leads to the abrupt growth of energy expenditures in a refrigerating machine operating on freon R-22. A one-stage refrigerating machine operating on freon R-404a can be used to obtain a temperature level above –30ºC in a fast freezer.

The results of calculating the energy expenditures on the production of cold required to freeze a kilogram of berries in a two-stage refrigerating machine depending on the air temperature in a fast freezer and the ambient medium temperature are shown in Fig. 8.

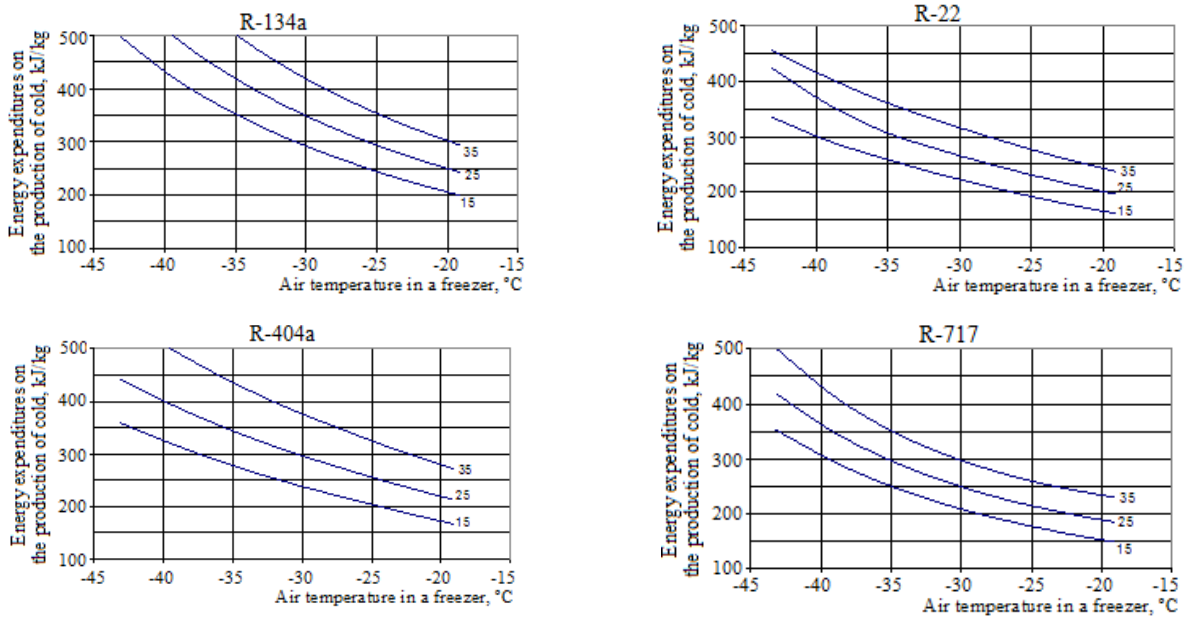


Fig. 8. Energy expenditure (kJ/kg) on the driver of a two-stage refrigerating machine for the freezing of a kilogram of Pamyat' Lisavenko blackcurrant berries from the temperature of 10°C to the temperature of –18°C depending on the air temperature in a fast freezer and the ambient air temperature.

From the preformed calculations it can be seen that the use of freon R-134a is unreasonable for the fast freezing of fruits and berries, and the use of freon R-134a in a two-stage compression refrigerating machine leads to the highest energy consumption in comparison with the other two-stage refrigerating machines.

The energetic efficiency of a two-stage refrigerating machine operating on freon R-404a is lower than for a refrigerating machine operating on freon R-22. The energy efficiencies of an ammonia refrigerating machine and a refrigerating machine operating on freon R-22 nearly correspond to each other. A two-stage refrigerating machine has insignificant advantages within a temperature range to –35°C, and a two-stage refrigerating machine operating on freon R-22 is more energe-tically profitable at temperatures below –35°C.

However, a freon refrigerating machine has better performance characteristics and a lower cost in comparison with an ammonia refrigerating machine, so the use of a two-stage refrigerating machine operating on freon R-22 is more efficient in comparison with an ammonia refrigerating machine.

The energy expenditures on the supply of a fast freezer with cold from a cascade refrigerating machine are plotted in Fig. 9.

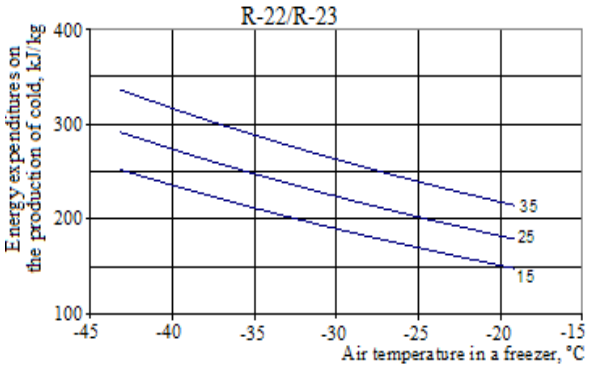


Fig. 9. Energy expenditures (kJ/kg) on the driver of a cascade refrigerating machine with freons R-23 and R-22 in the lower and upper cascade branches, respectively, for the freezing of a kilogram of Pamyat' Lisavenko blackcurrant berries from the temperature of 10°C to the temperature of –18°C depending on the air temperature in a fast freezer and the ambient air temperature.

Hence, a cascade refrigerating machine with freons R-22 and R-23 in the upper and lower cascade branches, respectively, has the best energetic characteristics among all the considered refrigeration schemes. How-ever, the practical implementation of such a scheme is most complicated, but has a number of advantages, the most important of which is lower energy expenditures on the production of artificial cold.

The energy expenditures on the attainment of temperatures below –30°C in the freezable volume of a cascade refrigerating machine and a two-stage refrigerating machine operating on freon R-22 differ slightly. As the refrigeration and operation scheme of a two-stage refrigerating machine is simpler and more reliable, it is possible to recommend the use of a two-stage refrigerating machine operating on freon R-22 for temperatures above –30°C. To attain a temperature level below –30°C, it is more preferable to use a cascade refrigerating machine.

The energy spent on the freezing of fruits and berries in a fast freezer is consumed by the driver of a refrigerating machine, i.e., for the organization of heat withdrawal from a heat-transfer agent into the ambient medium, and by the driver of fans, i.e., for the intensification of heat withdrawal from a freezable object to an intermediate cold-carrying agent—the air circulating in the freezer.

The performed studies show that the best energy efficiency can be attained at an optimal air medium velocity, which depends on the physical parameters and component composition of fruits and berries. A decrease in the air velocity, as well as an increase in this parameter with respect to an optimal value, leads to the growth of energy expenditures on the organization of heat withdrawal from a freezable object to the air.

A decrease in the air temperature in a fast freezer increases the efficiency of heat withdrawal. At the same time, a decrease in the air temperature in a fast freezer is accompanied by the growth of energy expenditures on the driver of a refrigerating machine. The cumulative effect of these two factors on the given energy expenditures on the freezing of a kilogram of berries is demonstrated in Fig. 10.

The regimes with minimum energy expenditures on the freezing of the studied varieties of fruits and berries at different ambient medium temperatures for cascade and two-stage refrigerating machines are characterized in Tables 2 and 3.

Table 2. Energetically optimal regimes of the freezing of the studied varieties of fruits and berries in a fast freezer with a cascade refrigerating machine

Variety	Air temperature in a freezer,°C			Air velocity, m/s			Energy expenditures on the freezing of a kilogram of berries, kJ			Freezing time, s		
	Ambient medium temperature, °C											
	15	25	30	15	25	35	15	25	35	15	25	35
Pamyat' Lisavenko	–39	–39	–39	6	6	6	379	417	459	695	695	695
Seyanets Golubki	–39	–39	–39	6	6	6	399	436	479	645	645	645
Pamyat' Shukshina	–43	–39	–39	6	6	6	410	448	491	527	593	593
Chernyi zhemchug	–39	–39	–35	6	6	6	369	406	447	761	761	866
Krasa Altaya	–43	–39	–39	6	6	6	410	448	491	578	651	651
Pushistaya	–43	–39	–39	6	6	6	406	444	487	529	596	596

Table 3. Energetically optimal regimes of the freezing of the studied varieties of fruits and berries in a fast freezer with a two-stage refrigerating machine

Variety	Air temperature in a freezer,°C			Air velocity, m/s			Energy expenditures on the freezing of a kilogram of berries, kJ			Freezing time, s		
	Ambient medium temperature, °C											
	15	25	30	15	25	35	15	25	35	15	25	35
Pamyat' Lisavenko	−31	−31	−31	6	6	6	429	473	524	913	913	913
Seyanets Golubki	−35	−31	−31	6	6	6	453	499	550	734	846	846
Pamyat' Shukshina	−35	−31	−31	6	6	6	466	515	567	675	778	778
Chernyi zhemchug	−31	−31	−31	6	6	6	416	460	510	998	998	998
Krasa Altaya	−35	−31	−31	6	6	6	466	515	567	742	857	857
Pushistaya	−35	−31	−31	6	6	6	462	510	562	679	784	784

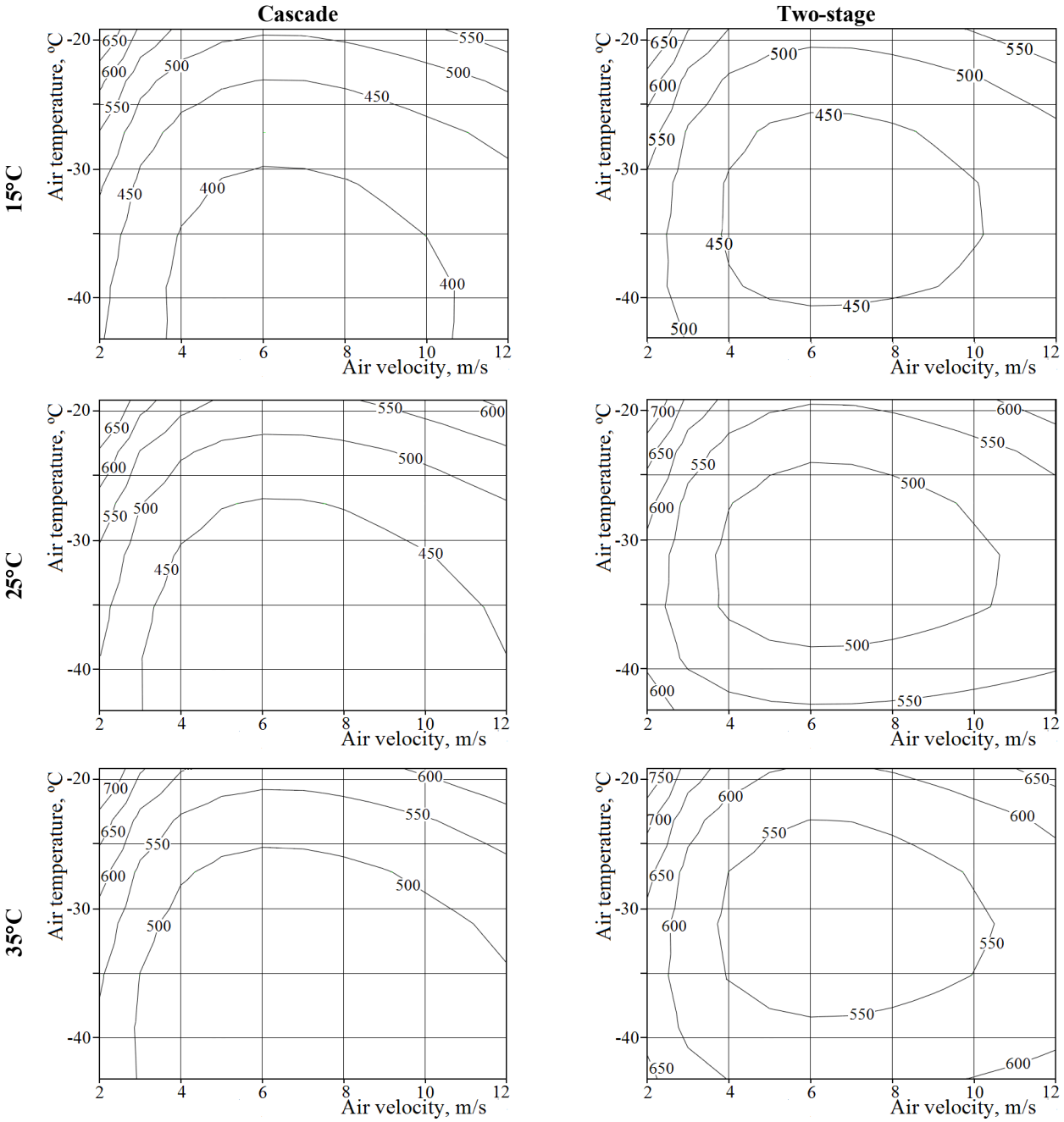


Fig. 10. Total energy expenditures (kJ/kg) on the freezing of Pamyat' Lisavenko blackcurrant berries in a fast freezer supplied with cold from cascade (R-23/R-22) and two-stage (R-22) refrigerating machines depending on the air temperature and velocity in a freezer and the ambient medium temperature.

From the presented results it can be seen that the air velocity of 6 m/s in a fluidized-bed fast freezer is least energy consuming for the freezing of all the varieties of blackcurrant berries. The air temperature in a freezer of –39ºC and –31ºC is the optimal temperature regime of fast freezing in the case of cold supply from a cascade refrigerating machine and a two-stage refrigerating machine with freon R-22 as a cooling agent, respectively.

The energy expenditures on the freezing of blackcurrant berries in a two-stage refrigerating machine are in average 14.2% higher than in a cascade refrigerating machine. The average time of the freezing of blackcurrant berries is 863 s at a temperature of –31°C and 657 s at a temperature of –39°C.

Hence, the performed calculations and the analysis of obtained results allow us to conclude that the use of cascade refrigerating machines is much less energy consuming in comparison with the other types of refrigerating machines for the fast freezing of fruits and berries. Moreover, the freezing of fruits and berries in a fast freezer supplied with cold from a cascade refrigerating machine is performed at lower temperatures, which considerably accelerate low-temperature treatment. This increases the productivity of a fast freezer and improves the qualitative characteristics of frozen fruits.

A cascade refrigerating machine is more expensive than a two-stage refrigerating machine. Its installation and maintenance are also more expensive and require a higher qualification of maintenance personnel. However, an increase in the productivity of a fast freezer with a cascade refrigerating machine due to a decrease in the time of low-temperature treatment and much lower energy expenditures on the driver of a cascade refrigerating machine makes it more economically attractive for the fast freezing of fruits and berries.

REFERENCES

1. *Dietary Reference Intakes. Applications in Dietary Assessment* (National Academy Press, Washington, DC, 2000).
2. *Microbial Hazard Identification in Fresh Fruit and Vegetables,* ed. by James, J. (John Wiley & Sons, Dublin, 2006).
3. *Handbook of Food Preservation,* ed. by Rahman, M.S. (CRC Press, Boca Raton, FL, 2007).
4. Almási, E., Erdélyi, L., and Sáray, T., *Élelmiszerek gyorsfagyasztása* (Mezőgazdasági Kiadó, Budapest, 1977).
5. Rasshchepkin, A.N., Korotkiy, I.A., and Korotkaya, E.V., Vliyanie rezhimov nizkotemperaturnoi obrabotki na kachestvennye pokazateli yagod chernoi smorodiny (Effect of low-temperature treatment regimes on the qualitative characteristics of blackcurrant berries), *Tekhnika i tekhnologiya pishchevykh proizvodstv* (Food Processing: Techniques and Technologies), 2014. №1. P. 101–105.
6. Golyand, M.M., Malevannyi, B.N., Pechatnikov, M.Z., and Plotnikov, V.T., *Sbornik primerov raschetov i laboratornykh rabot po kursu "Kholodil'noe tekhnologicheskoe oborudovanie"* (Collection of Calculation Examples and Laboratory Works on the Course in Refrigeration Process Equipment) (Legkaya i pishchevaya promyshlennost', Moscow, 1981).
7. Baranenko, A.V., Kutsakova, V.E., Borzenko, E.I., and Frolov, S.V., *Primery i zadachi po kholodil'noi tekhnologii pishchevykh produktov. Ch. 3. Teplofizicheskie osnovy* (Examples and Problems on Food Refrigeration Technology, Part 3. Thermophysical Principles) (KolosS, Moscow, 2004).
8. Lykov, A.V., *Teoriya teploprovodnosti* (Theory of Heat Conductivity) (Vysshaya shkola, Moscow, 1967).
9. Rogov, I.A., Kutsakova, V.E., Filippov, V.I., and Frolov, S.V., *Konservirovanie pishchevykh produktov kholodom* (Refrigeration Preservation of Food Products) (KolosS, Moscow, 1999).
10. Korotkiy, I.A., *Mashiny nizkotemperaturnoi tekhniki. Chast' I* (Low-Temperature Engineering Machines. Part I) (KemTIPP, Kemerovo, 2004).
11. Korotkiy, I.A., *Mashiny nizkotemperaturnoi tekhniki. Chast' II* (Low-Temperature Engineering Machines. Part II) (KemTIPP, Kemerovo, 2008).

A STUDY OF PROPERTIES OF STRUCTURE-STABILIZING AGENTS FOR PRODUCTS BASED ON DAIRY RAW MATERIALS

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Abstract: An important strategic objective of the food industry is to meet the demands of all categories of the population in high-quality, biologically valuable, and safe food. At present, structured products based on dairy raw materials are commonly used. Food materials used to generate the necessary rheological properties or modify the existing properties for food systems that adjust or form the consistency of food products are referred to as structure-stabilizing agents. Both individual ingredients and complex mixtures thereof can be structure-stabilizing agents. The use of these agents contributes to an increase in the density and to the formation of a specific structure of the food product, which is retained even after heat treatment. In this study, the composition and properties of structure-stabilizing agents for products based on dairy raw materials have been analyzed. The following characteristics have been selected to examine: bulk density, viscosity, the fraction of undissolved residue, microvoid content, specific volume, specific surface area, and characteristic diameter. The dynamics of structural changes that occur during the interaction with the solvent have been analyzed from micrographs. The weight fraction of chemical elements (oxygen, nitrogen, carbon, sodium, chlorine) has been determined via analyzing the spectrophotometric profile.

Keywords: structure-stabilizing agents, modification, structure, dairy products, spectrophotometric analysis

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INTRODUCTION

At present, the production of structured products based on dairy raw materials is one of the most dynamically developing industrial sectors [6]. The quality of dairy products, along with other parameters, is determined by their structure and consistency, which depend on the proper implementation of the production process. The development of modern technological prin-ciples of food production is associated with the search for universal methods for impacting multicomponent aqueous systems; one of these methods is the use of food structure-stabilizing agents [7]. The role of structure-stabilizing agents is to provide a good consistency, which does not change during storage and exhibits stability during the process implementation.

The production of stabilized products based on dairy raw materials is characterized by the structure formation owing to the coagulation of casein at the isoelectric point [8]. In this case, the stabilizing agents used in food production must hinder the separation of the product and the isolation of whey; when using fillers and additives, structure-stabilizing agents must provide the uniformity of their distribution over the volume during packing and subsequent storage. The use of structure-stabilizing agents can provide the formation of a gel structure at pH values other than the isoelectric point [1]. Moreover, using structure-stabilizing agents, it is possible to targetedly adjust the structural–mechanical and physicochemical properties, organoleptic parameters, and quality of the end product [3].

Stabilizing systems are commonly used in the dairy industry [9]. Among the currently known structure-stabilizing agents, anionic polysaccharides—both natural (pectin, agar, agaroid, pyrophosphate) and artificial (oxidized starch)—are extensively employed. Alginates, cellulose derivatives, carboxymethyl cellulose (CMC), and various gums enjoy wide application abroad [4]. Stabilizing agents are promising because their composition and properties are constantly being improved. The use of stabilizing agents improves the quality of products (particularly consistency) and increases the yield and the shelf life [2].

Examination of the structure formation laws in dairy systems is of significant theoretical and practical importance because it provides the formation of disperse systems with desired structural–mechanical properties.

The described studies were conducted in conjunction with A.N. Arkhipov and A.V. Pozdnyakova.

OBJECTS AND METHODS OF RESEARCH

To examine the composition of the structure-stabilizing agents, a JEOL JED-2300 analysis station was used; the electron probe microanalysis conducted with this instrument yielded spectrometric profiles that can be used to determine the chemical composition of the structure-stabilizing agents [5].

The objects of research were structure-stabilizing agents with varying bulk density, which are presented in Table 1.

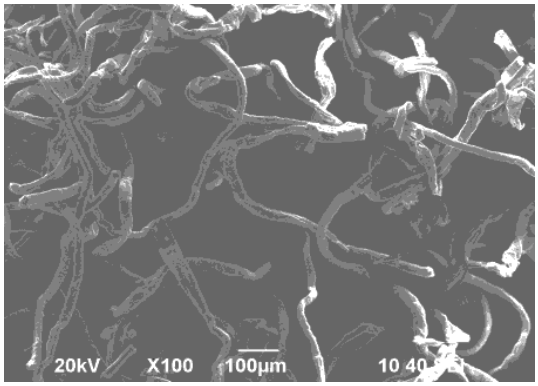
Table 1. Bulk density of the studied structure-stabilizing agents

Structure-stabilizing agent	Bulk density, g/dm^3
CMC (carboxymethyl cellulose) Akucell 3265	450
Konjac gum	540
Pectin ARA 105	580
CMC Akucell 2785	680
Xanthan gum	830

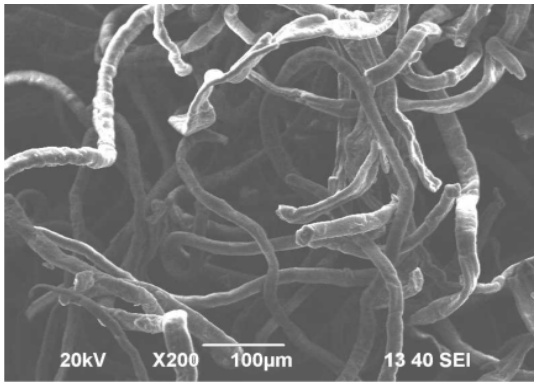
RESULTS AND DISCUSSION

CMC Akucell 3265

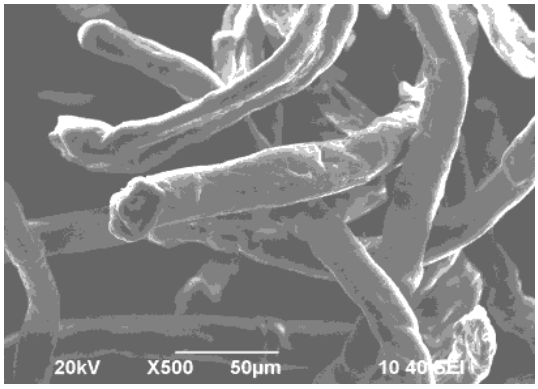
Figure 1 shows micrographs of CMC Akucell 3265 at a magnification of 100, 200, and 500×.



(a)



(b)



(c)

Fig. 1. Microstructure of CMC Akucell 3265 at a magnification of (a) 100, (b) 200, and (c) 500×.

The micrographs in Fig. 1 show that the structure of CMC Akucell 3265 is composed of dispersed elements in the form of elongated fibers with a rough surface and a diameter of about 20–30 µm. CMC Akucell 3265 has the lowest bulk density of all the studied structure-stabilizing agents; it is 450 g/dm^3.

CMC is a salt of a weak carboxylic acid produced through a reaction between sodium monochloroacetate and alkaline cellulose according to the following schemes:

$$\zeta\text{-OH}+\text{NaOH}\rightarrow\zeta\text{-ONa}+\text{H}_2\text{O}$$
$$\zeta\text{-Ona}+\text{Cl-CH}_2\text{-COONa}\rightarrow\zeta\text{-O-CH}_2\text{-COONa}+\text{NaCl}$$

To prepare this structure-stabilizing agent, α-cellulose derived from plant fibers is exposed to a strong alkaline solution and chloroacetic acid; the resulting sodium chloride is washed out. The composition of CMC comprises sodium chloride, sodium glycolate, and unsubstituted cellulose.

Each anhydropyranose unit of the CMC molecule contains three OH groups capable of reacting with sodium monochloroacetate. The substitution of all three OH groups is theoretically possible (the degree of substitution is three); however, the degree of substitution in real CMC samples is 0.4–1.2. At a degree of substitution of 0.5 and 0.8, the pK values of the carboxyl groups are 4.0 and 4.4, respectively. At a pH level of 7.0 and 5.0, about 90 and 10% of the carboxyl groups are in a salt form. CMC is an ionogenic cellulose ester; therefore, its stabilizing effect depends on salt concentration and other properties of the medium.

The highest viscosity of equiconcentrated CMC solutions is observed at a pH of 6–8 (Fig. 2).

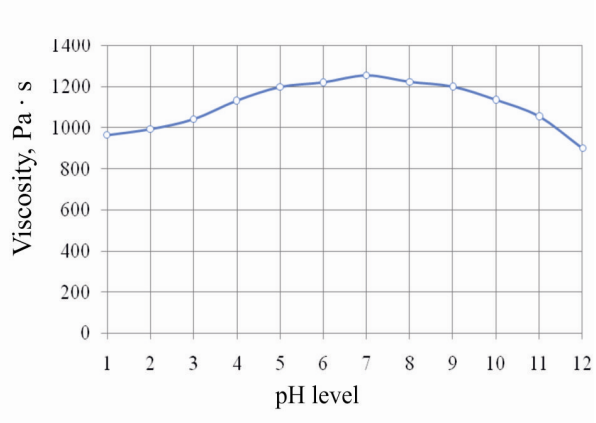
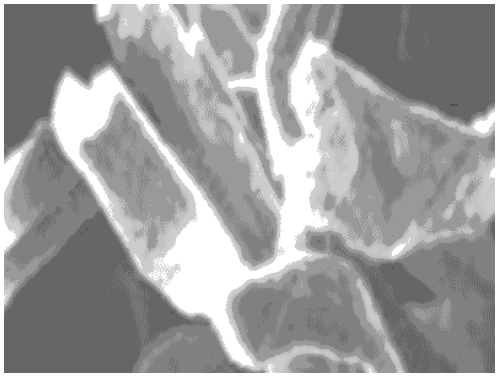
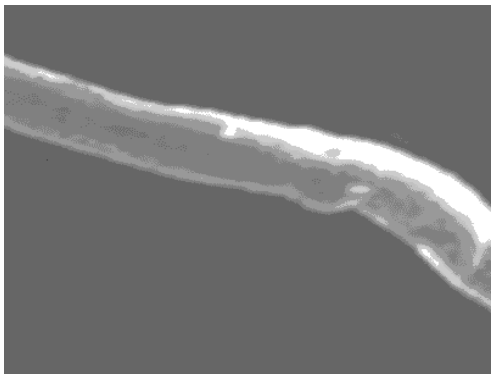


Fig. 2. Dependence of the viscosity of equiconcentrated CMC solutions on pH value.

Most probably, in this pH range, the macromolecules undergo unfolding owing to the electrostatic repulsion of the ionized carboxyl groups. At low pH values, the ionization of the carboxyl groups in an acid form is suppressed, while at high pH values, the repulsive forces decrease because of the presence of a large amount of sodium ions. This fact suggests that the CMC macromolecule is folded at extreme pH levels and unfolded in an optimum pH range of 6–9 (Fig. 3).



(a)



(b)

Fig. 3. (a) Folded and (b) unfolded CMC macromolecule at a magnification of 5000×.

The solubility and processing properties of CMC are affected by the chain length and the degree of substitution and neutralization. Thus, in the presence of 1% CMC in an aqueous solution, the viscosity varies from 20 to 3500 Pa • s. CMC exhibits high solubility in water and alkalis; it is poorly soluble in glycerol and acids and almost insoluble in organic solvents (Fig. 4).

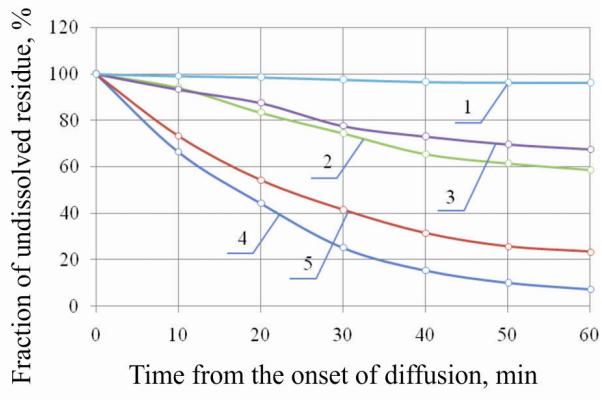


Fig. 4. Dependence of the undissolved residue of CMC on the time of treatment in (*1*) organic solvents, (*2*) acids, (*3*) glycerol, (*4*) water, and (*5*) alkali.

The solubility of CMC can be improved by using more active solvents, one of which is a diluted solution of sodium hydroxide, which makes it possible to increase the degree of substitution to values close to 1.2.

Figure 5 shows the spectrometric profile of the composition of CMC Akucell 3265. The derived profile exhibits three characteristic peaks corresponding to carbon, oxygen, and nitrogen and two plateaus attributed to nitrogen and chlorine.

Table 2 lists the component composition of CMC Akucell 3265.

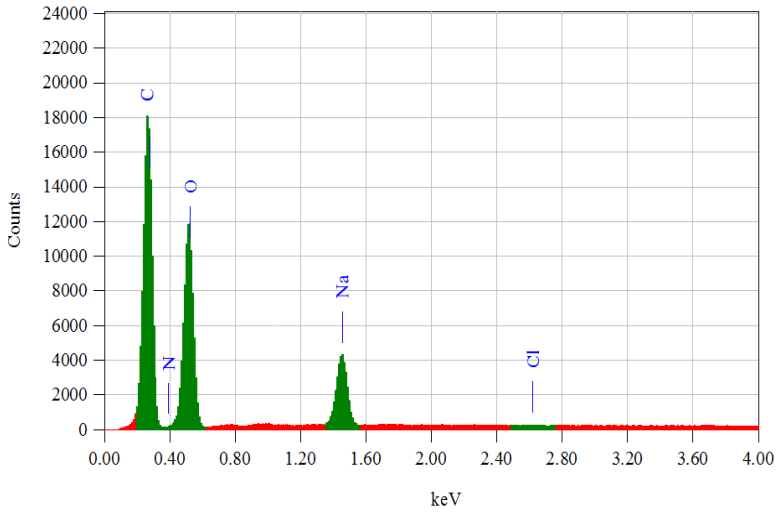


Fig. 5. Spectrometric profile of the composition of CMC Akucell 3265.

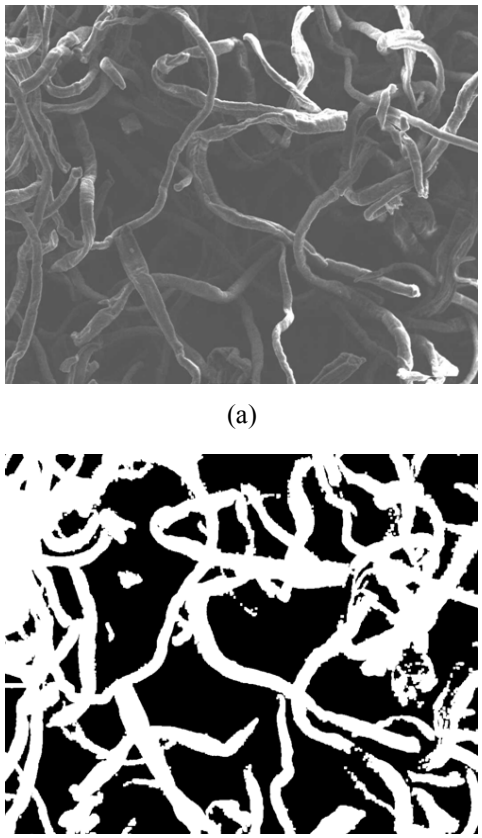
Table 2. Component composition of CMC Akucell 3265

Element	Relative content, wt %
Carbon	31.15 ± 0.93
Nitrogen	21.90 ± 0.65
Oxygen	42.78 ± 1.28
Sodium	4.01 ± 0.12
Chlorine	0.07 ± 0.002

The results show that oxygen is the dominant component of CMC Akutsel 3265 (42.78%). Chlorine content is the lowest (0.07%).

The microvoid content in CMC Akucell 3265 was determined using the micrograph shown in Fig. 1a. In this case, it was necessary to filter the background elements, which was implemented by enhancing the contrast and manually correcting the mask. The results of determina-

tion of the microvoid content in CMC Akucell 3265 are shown in Fig 6.



(a)

(b)

Fig. 6. Results of determination of the microvoid content in CMC Akucell 3265: (a) a micrograph at a magnification of 100×, (b) a mask of the micrograph shown in panel (a).

According to the histogram, the microvoid content in CMC Akucell 3265 was 51.24 ± 2.2%.

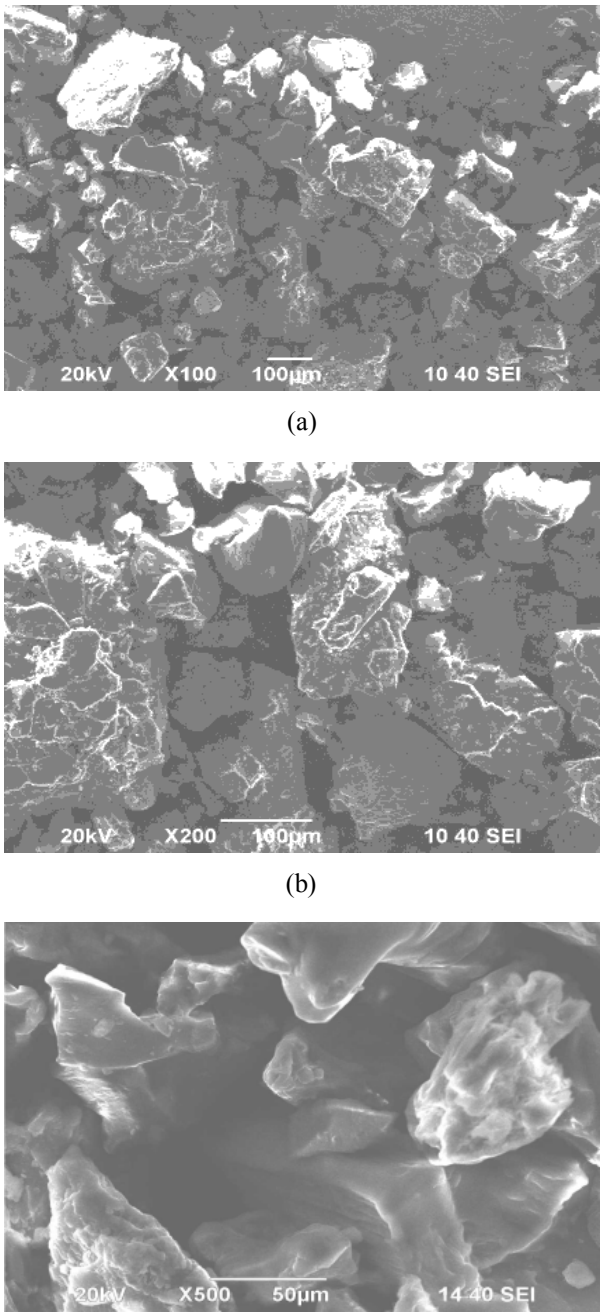
Thus, the microstructure of CMC Akucell 3265 is characterized by a low bulk density of 450 g/dm3; its elements have the form of elongated fibers with a diameter of 20–30 μm. The chemical composition of CMC Akucell 3265 comprises carbon, nitrogen, oxygen, sodium, and chlorine. The microvoid content in the studied structure-stabilizing agent was 51.24 ± 2.2%.

Konjac Gum

Figure 7 shows the microstructure of konjac gum at a magnification of 100, 200, and 500×. The bulk density of this structure-stabilizing agent is 540 g/dm3. It is evident from Fig. 7a that the microstructure of konjac gum is composed of large and small irregularly shaped granules, the size of which varies from 10 to 250–300 μm. The microstructure comprises granules with both a rough crystalline surface and a smooth surface (Fig. 7c).

Dispersion of konjac gum in hot and cold water results in the formation of highly viscous solutions with a pH of 4–7 (Table 3).

As the konjac gum concentration increases, the pH level of the aqueous solution decreases. The solubility increases during stirring and heating. This structure-stabilizing agent is composed of D-mannose and D-glucose monomers linked by a β-glycosidic bond.



(a)

(b)

(c)

Fig. 7. Microstructure of konjac gum at a magnification of (a) 100, (b) 200, and (c) 500×.

Table 3. pH level of an aqueous solution of konjac gum

Weight fraction of konjac gum in the solution	pH
0.5	6.7
1.0	5.9
1.5	5.4
2.0	4.7
2.5	4.1

The solubility of konjac gum in hot water is higher than in cold water, as evident from the dependence of the fraction of dry residue of konjac gum in an aqueous solution derived after 60-min diffusion at different temperatures (Fig. 8).

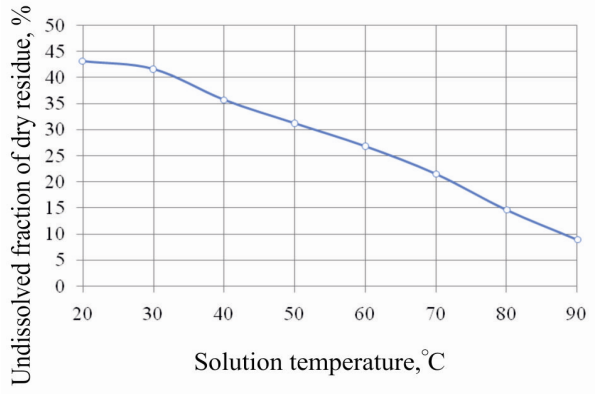


Fig. 8. Undissolved fraction of the dry residue of konjac gum in an aqueous solution in 60 min after the onset of diffusion at varying temperature.

This structure-stabilizing agent is insoluble in organic solvents. A distinctive feature of this structure-stabilizing agent is a high level of viscosity even at low concentrations. The main component in the composition of this material is chitosamin, which is responsible for the dissolution of the structure-stabilizing agent particles in water. In addition, the composition of konjac gum comprises alcohol, which is involved in the precipitation and drying of glucomannan.

In the food industry, konjac gum is commonly used as a fat substitute in the production of fat-free and low-fat meat products. Furthermore, it contributes to the formation of a desired consistency in the production of jellies, puddings, beverages, yogurts, etc.

Figure 9 shows a spectrometric profile for determining the composition of konjac gum; the component composition is listed in Table 4.

Konjac gum contains carbon, nitrogen, oxygen, potassium, and sodium. The content of carbon and oxygen is similar to that observed in CMC 4500-6000. Konjac gum differs from the previously discussed structure-stabilizing agents in the presence of potassium and the absence of sodium.

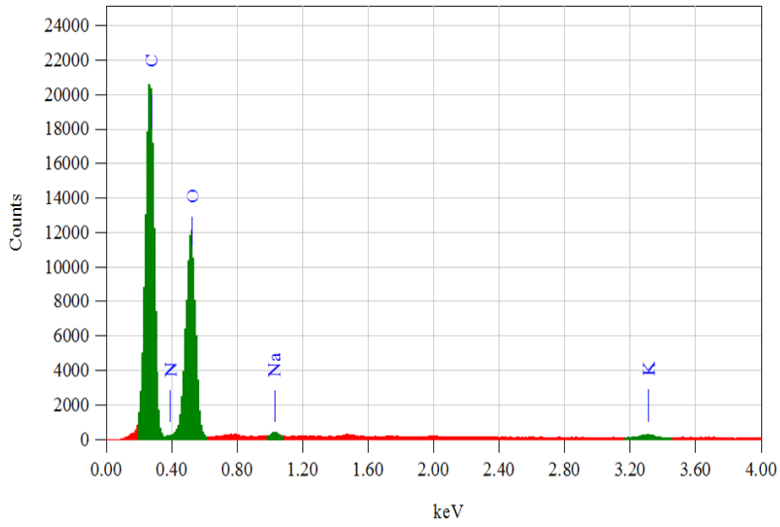


Fig. 9. Spectrometric profile of the component composition of konjac gum.

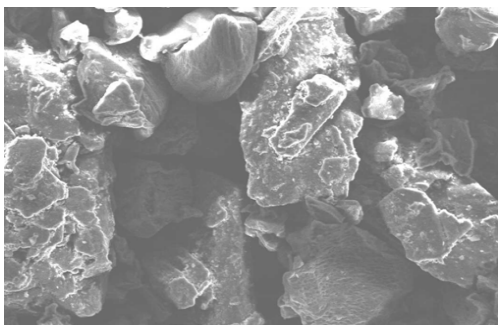
Table 4. Component composition of konjac gum

Element	Relative content, wt %
Carbon	29.79 ± 0.89
Nitrogen	22.29 ± 0.67
Oxygen	47.73 ± 1.43
Potassium	0.17 ± 0.005
Sodium	0.02 ± 0.001

The microvoid content in konjac gum was determined using a micrograph recorded at a magnification of 200× (Fig. 10). The microvoid content was 33.87 ± 1.1%. In this case, it was necessary to enhance the image contrast in order to filter the background particles.

Thus, the elements of konjac gum have the form of irregularly shaped granules with a size of 10–300 μm. The component composition of konjac gum comprises carbon, nitrogen, oxygen, potassium, and sodium.

According to the results of mathematical processing, the microvoid content in konjac gum is 33.87 ± 1.1%.



(a)

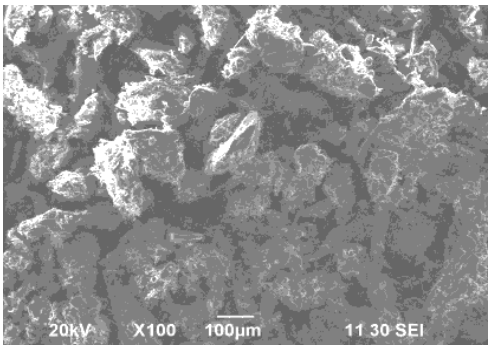
Fig. 10. *Begin.* Results of determination of the microvoid content in konjac gum: (a) a micrograph at a magnification of 200×.

(b)

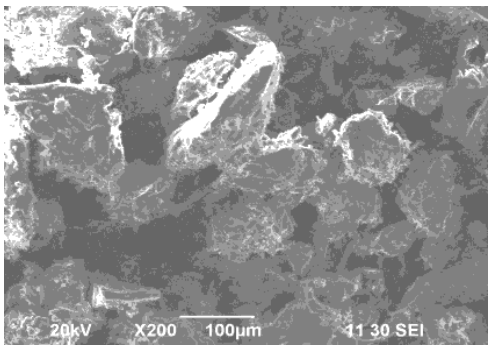
Fig. 10. *End*. Results of determination of the microvoid content in konjac gum: (b) a mask of the micrograph shown in panel (a).

Pectin ARA 105

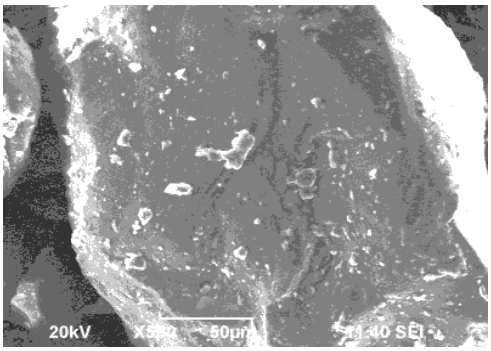
Figure 11 shows micrographs of pectin ARA 105 at a magnification of 100, 200, and 500×.



(a)



(b)



(c)

Fig. 11. Microstructure of pectin ARA 105 at a magnification of (a) 100, (b) 200, and (c) 500×.

The micrographs suggest that the structure of elements of pectin ARA 105 is composed of irregularly shaped dispersed particles with a size of 20–250 μm; in some regions, large granules with a size higher than 300 μm are observed (Fig. 11a). Crystalline clusters are present on the surface of the granules (Fig. 11c). The cluster size can vary from a few micrometers to 30–40 μm (Fig. 11b). The bulk density of pectin ARA 105 is 580 g/dm^3.

The main structural element of the macromolecules of pectin as a polysaccharide of plant tissues is galactopyranosyl uronic acid, which comprises L-D-galacturon. Pectin also contains neutral carbohydrates, such as galactose and arabinose. The pattern of distribution of ester groups in the pectin macromolecule has an effect on some physicochemical properties, such as gelling ability, solubility, and surface activity. The thermodynamic flexibility of pectin macromolecules is significantly affected by the degree of esterification. The water solubility of pectin can be improved by decreasing the molecular weight and increasing the degree of esterification (Fig. 12).

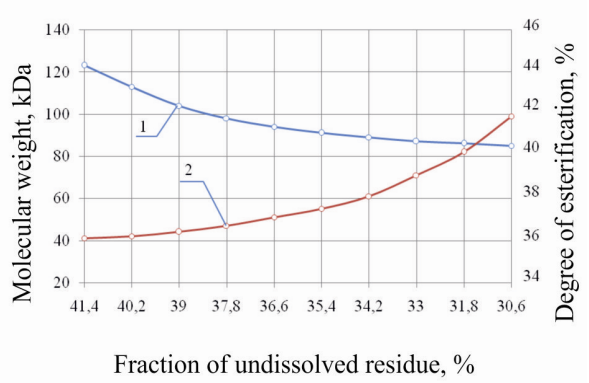


Fig. 12. Effect of (*1*) the molecular weight and (*2*) the degree of esterification on the fraction of the undissolved residue of pectin ARA 105 in an aqueous solution in 30 min after the onset of diffusion.

Pectin exhibits the highest stability at a pH of 3–4 (Fig. 13). A deviation of pH to one side from this range leads to a decrease in the charge density of the pectin macromolecules; this phenomenon is most pronounced at temperatures above 50°C.

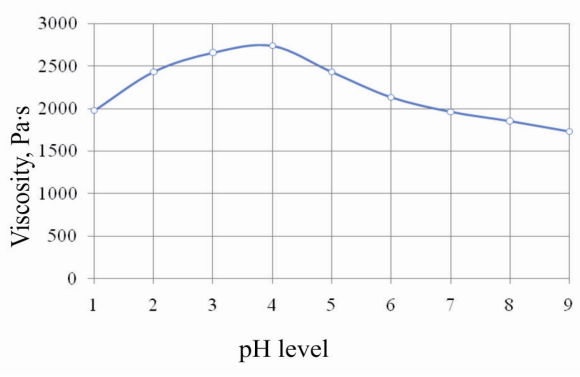


Fig. 13. Dependence of the viscosity of a pectin ARA 105 solution on pH level.

Pectins with a high degree of esterification exhibit gelling properties in an acid medium in the presence of sucrose, whereas pectins with a low degree of esterification are capable of gelling in the presence of salts of polyvalent metals. The widespread use of pectins as structure-stabilizing agents in the confectionery and-pharmaceutical industries is associated with their physiological inertness and high gelling properties.

Figure 14 shows a spectrometric profile for determining the component composition of pectin ARA 105. The profile exhibits three peaks corresponding to carbon, oxygen, and sodium and a plateau attributed to nitrogen.

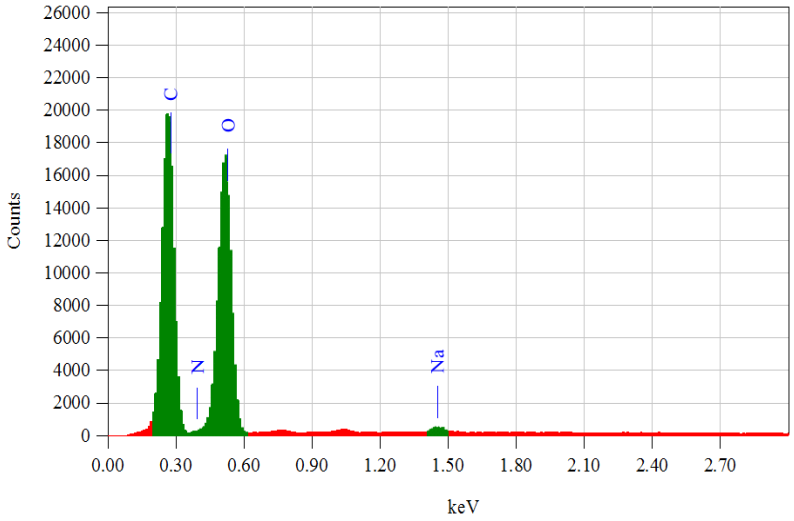


Fig. 14. Spectrometric profile of the component composition of pectin ARA 105.

The component composition of pectin ARA 105 is listed in Table 5. The content of carbon and nitrogen is at almost the same level as in konjac gum. The composition of pectin ARA 105, in common with CMC, comprises sodium and chlorine; the amount of these elements is 3.28 and 0.12%, respectively.

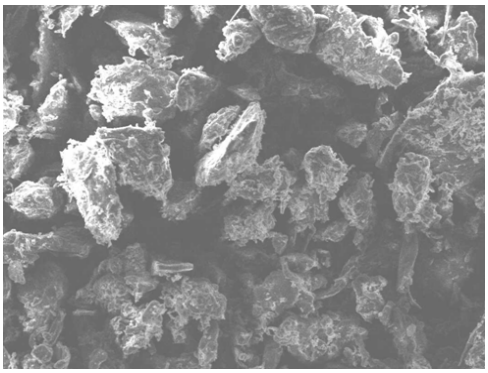
Table 5. Component composition of pectin ARA 105

Element	Relative content, wt %
Carbon	29.04 ± 0.87
Nitrogen	22.70 ± 0.69
Oxygen	44.86 ± 1.34
Sodium	3.28 ± 0.10
Chlorine	0.12 ± 0.004

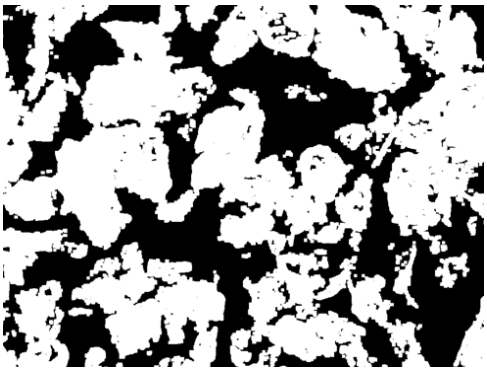
The micrograph of the structure of pectin ARA 105, which is shown in Fig. 15a, was used to determine the microvoid content, while the micrograph shown in Fig. 15c was used to prepare a mask of the disperse entities on the surface of the elements of the structure-stabilizing agent. The respective micrographs and resulting masks are shown in Fig. 15.

The microvoid content in pectin ARA 105 was 39.60 ± 1.2%, while the amount of disperse entities on the surface of the elements of the structure-stabilizing agent was 12.69 ± 0.7%. It should also be noted that, to prepare the mask shown in Fig. 15d, it was necessary to enhance the image contrast and manually correct certain areas of the mask because the required elements in the micrograph had a nonuniform color over the entire surface.

Thus, the microstructure of pectin 105 ARA is characterized by irregularly shaped dispersed particles with a size of 20–250 μm and large granules with a size of higher than 300 μm. Crystalline clusters are present on these elements. In addition to carbon, nitrogen, and oxygen, pectin ARA 105 contains sodium and chlorine. The content of microvoids and disperse entities on the surface of the elements is 39.60 and 12.69%, respectively.

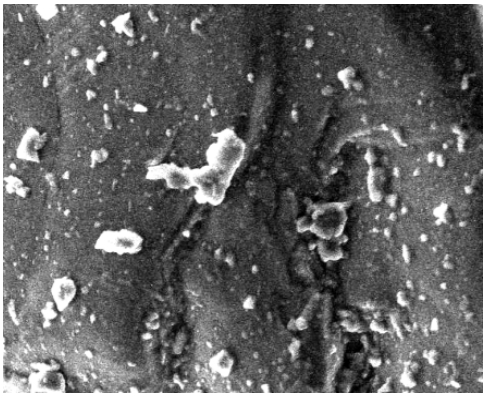


(a)

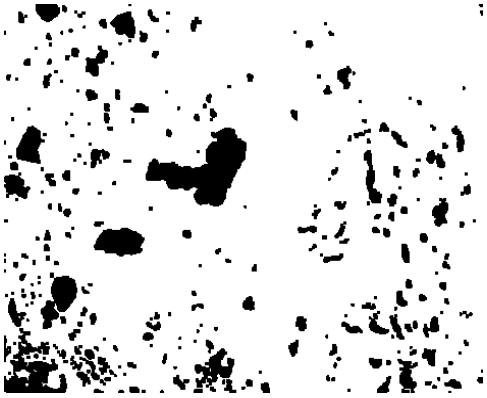


(b)

Fig. 15. *Begin.* Results of determination of (a, b) the microvoid content and (c, d) disperse entities of pectin ARA 105: (a) a micrograph at a magnification of 100×, (b) a mask of the micrograph shown in panel (a).



(c)



(d)

Fig. 15. *End.* Results of determination of (a, b) the microvoid content and (c, d) disperse entities of pectin ARA 105: (c) a fragment of the micrograph, and (d) a mask of the micrograph shown in panel (c).

CMC Akucell 2785

Figure 16 shows micrographs of CMC Akucell 2785 at a magnification of 100, 200, and 500×.

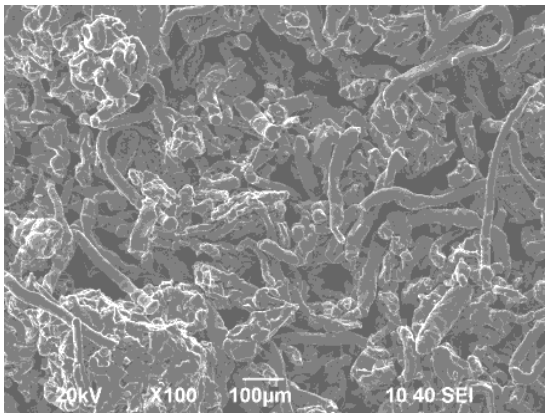
The microstructure of CMC Akucell 2785 is similar to that of structure-stabilizing agents CMC 4500-6000 and CMC 6000-9000. The microstructure of CMC Akucell 2785 is characterized by the closest packing of dispersed elements of all the studied CMC samples; these elements have the form not only of characteristic fibers, but also more rounded irregularly shaped particles with a rough surface (Fig. 16c) and a size of 30–70 μm, while the diameter of the fibers is about 40–60 μm. The structure also comprises large clusters of elements (Fig. 16a). The bulk density of CMC Akucell 2785 is 680 g/dm^3.

Table 6 lists the component composition of CMC Akucell 2785.

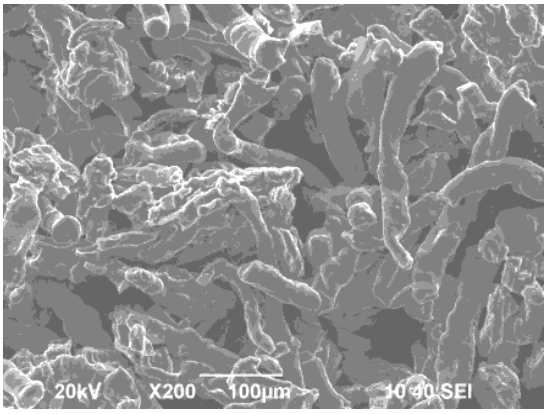
Table 6. Component composition of CMC Akucell 2785

Element	Relative content, wt %
Carbon	30.89 ± 0.93
Nitrogen	22.68 ± 0.68
Oxygen	42.28 ± 1.26
Sodium	4.13 ± 0.12
Chlorine	0.02 ± 0.001

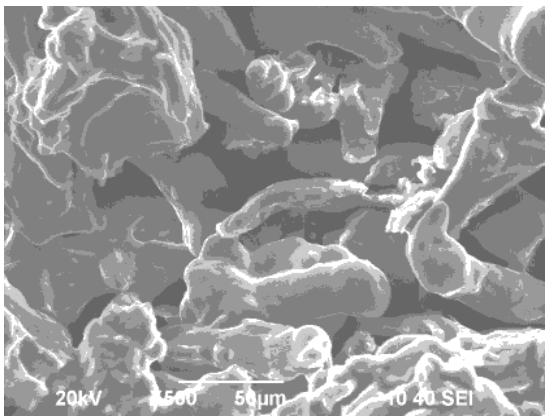
Comparative analysis of the component composition of the CMC Akucell 2785 and CMC Akucell 3265 shows a similarity in chemical composition. Compared to the previous types of CMC, these structure-stabilizing agents are characterized by a higher content of carbon and nitrogen and a lower content of oxygen, sodium, and chlorine.



(a)



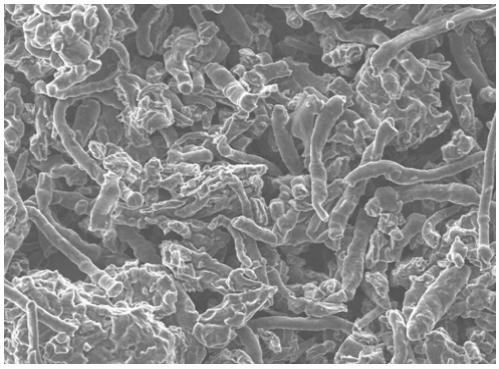
(b)



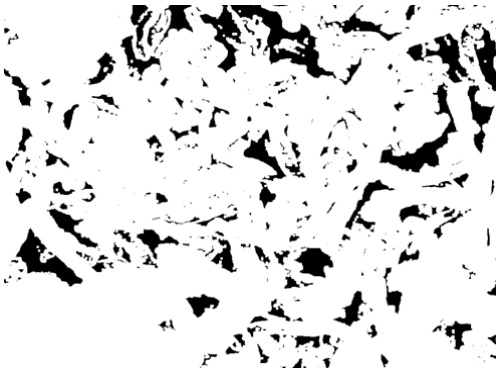
(c)

Fig. 16. Microstructure of CMC Akucell 2785 at a magnification of (a) 100, (b) 200, and (c) 500×.

Figure 17 shows photographs of microvoids in CMC Akucell 2785. The mask was prepared using color marking, reducing the boundaries of the marked areas by 1 pixel, and manually editing individual elements. The microvoid content was 11.43 ± 0.5%.



(a)



(b)

Fig. 17. Results of determination of the microvoid content in CMC Akucell 2785: (a) a micrograph at a magnification of 100× and (b) a mask of the micrograph shown in panel (a).

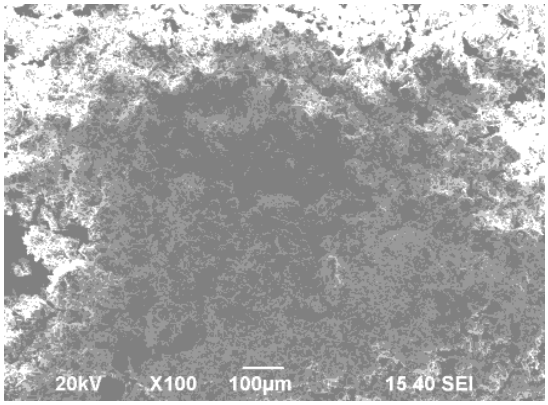
To summarize the data, we can conclude that the structure of CMC Akucell 2785 comprises elements in the form of fibers with a rough surface and a diameter of about 40–60 μm and rounded particles with a size of 30–70 μm. The component composition includes carbon, nitrogen, oxygen, sodium, and chlorine. The microvoid content in CMC Akucell 2785 is 11.43 ± 0.5%.

Xanthan Gum

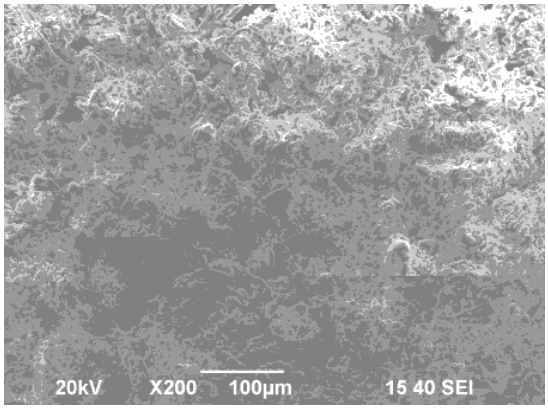
Micrographs of xanthan gum at a magnification of 100, 200, and 500× are shown in Fig. 18.

Xanthan gum is characterized by the highest bulk density of all the studied structure-stabilizing agents: it is 830 g/dm^3. Xanthan gum has a densely spaced highly dispersed structure. Most of the granules are elongated; their size is 5–40 μm (Fig. 18c). This particle size provides a rapid formation of a highly viscous solution in both hot and cold food systems and contributes to high quality of foods. Some elements of xanthan gum form conglomerates. In addition, the structure contains irregularly shaped microvoids (Fig. 18a).

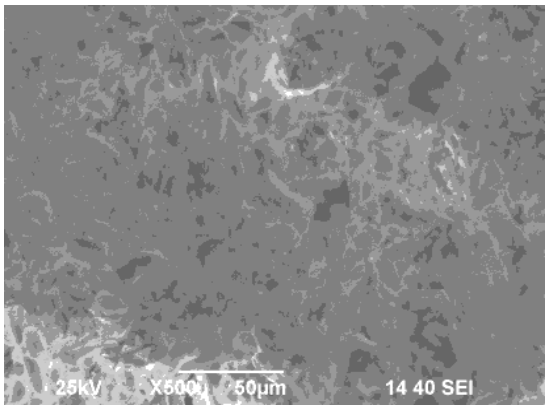
Xanthan gum is produced by the fermentation of sucrose or glucose using the *Xanthomonas compestris* bacteria, which secrete a slimy substance on the outer surface of the cell walls to protect them from viruses and drying up. After fermentation, the culture medium is pasteurized, precipitated with alcohol, and purified by microfiltration to obtain a white powder.



(a)



(b)



(c)

Fig. 18. Microstructure of xanthan gum at a magnification of (a) 100, (b) 200, and (c) 500×.

The stabilizing effect of xanthan gum does not depend on the presence of salts, acids, and heat or mechanical impact. The viscosity of the resulting xanthan gum system does not vary at a pH of 2–12 (Fig. 19).

Xanthan gum is a microbial biopolymer composed of α-D-mannose, β-D-glucose, and α-D-glucuronic acid in an approximate ratio of 3 : 3 : 2. The adsorption of water by xanthan molecules leads to the formation of a three-dimensional network of double helices linked by intermolecular hydrogen bonds. The structure of the resulting substance is similar to that of a gel; however, it exhibits a higher mobility. Under significant mecha-

nical impacts, the networks are destroyed and the double helices elongate in the direction of effort, while the viscosity sharply decreases. After the impact, the three-dimensional network of macromolecules is restored and the viscosity returns to the initial value.

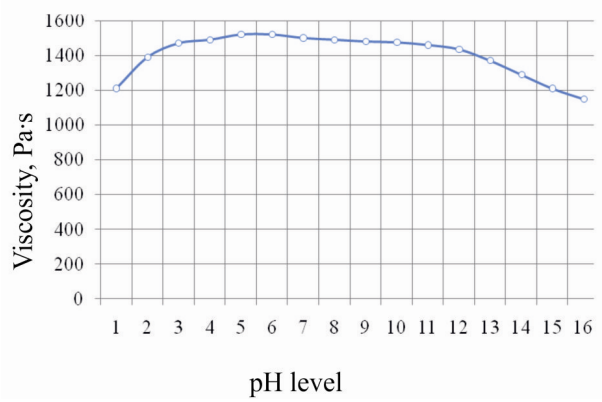


Fig. 19. Dependence of the viscosity of a xanthan gum solution on pH level.

Table 7 lists the component composition of xanthan gum.

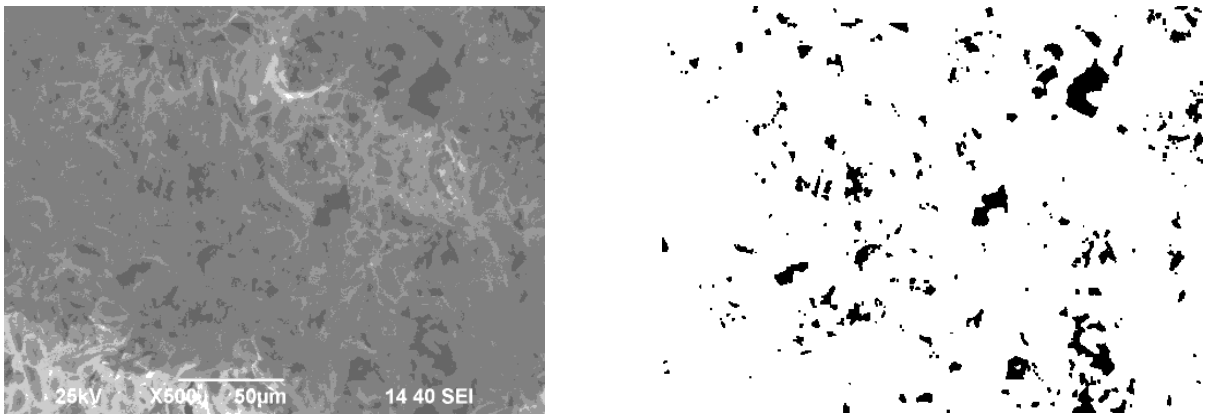
Table 7. Component composition of xanthan gum

Element	Relative content, wt %
Carbon	30.08 ± 0.90
Nitrogen	23.42 ± 0.70
Oxygen	46.38 ± 1.39
Potassium	0.12 ± 0.004

Xanthan gum also comprises carbon, nitrogen, oxygen, and potassium.

The microvoid content in xanthan gum was determined using a micrograph with a magnification of 500× because it provides the highest degree of accuracy. The results are shown in Fig. 20. The microvoid content in the structure of xanthan gum was 6.51 ± 0.3%.

Thus, the microstructure of xanthan gum is composed of closely spaced elongated granules with a size of 5–40 μm. The component composition comprises carbon, nitrogen, oxygen, and potassium; the percentage ratio of these chemical elements is similar to that of konjac gum. According to the results of mathematical processing, the microvoid content in xanthan gum is 6.51 ± 0.3%.



(a) (b)

Fig. 20. Results of determination of the microvoid content in xanthan gum: (a) a micrograph at a magnification of 500× and (b) a mask of the micrograph shown in panel (a).

CONCLUSIONS

The main properties of structure-stabilizing agents with different bulk density have been discussed on the basis of analysis of their micrographs. It has been found that characteristics of the studied structure-stabilizing agents differ in the size, shape, and packing density of the particles. Characteristic particle sizes of each of the structure-stabilizing agents have been shown. The composition of the structure-stabilizing agents has been examined using an electron microscope. Spectrometric profiles of the chemical composition have been derived; the ratio of the main components in the structure-stabilizing agents has been determined. Some similar features with respect to the presence of particular chemical elements and their ratio in the composition of structure-stabilizing agents of one type (CMC, gum, and pectin) have been revealed. The mathematical processing of micrographs of the structure-stabilizing agents has been conducted; masks for determining the microvoid content in the particle structure of the studied structure-stabilizing agents have been prepared.

REFERENCES

1. Arkhipov, A.N., Masunov, N.A., and Pozdnyakova, A.V., Issledovanie mikrostruktury i komponentnogo sostava pishchevykh stabilizatorov (A study of the microstructure and component composition of food stabilizers), *Vestn. Krasnoyarsk. Gos. Agrarn. Univ.* (Bull. Krasnoyarks State Agrar. Univ.), 2012. № 1. P. 178.

2. Arkhipov, A.N. and Pozdnyakova, A.V., Issledovanie vliyanya massovoi doli stabilizatora KMC AKUTSEL' 3265 na prodolzhitel'nost' skvashivaniya molochnokislymi mikroorganizmami serii "DELVO-YOG" (A study of the effect of the weight fraction of the CMC Akucell 3265 stabilizer on the duration of ripening with lactic microorganisms of the DELVO-YOG series), *Materialy Mezhdunarodnoi konferentsii "Innovatsionnoe razvitie malykh gorodov Rossii: nauchnyi i tekhnologicheskii potentsial"* (Proc. Int. Conf. on Innovative Development of Small Towns in Russia: The Scientific and Technological Potential), Meleuz, 2011, P. 65.
3. Prosekov, A. Yu., *Sovremennye aspekty proizvodstva produktov pitaniya: Monografiya* (Modern Aspects of Food Production: A Monograph) (Kemerov. Tekhnol. Inst. Pishch. Prom-st., Kemerovo, 2005).
4. Arkhipov, A.N., Pozdnyakova, A.V., Krupin, A.V., and Vakanova, M.V., Sravnitel'noe issledovanie mikrostruktury i sostava stabilizatorov rastitel'nogo proiskhozhdeniya (A comparative study of the microstructure and composition of stabilizers of vegetable origin), *Tekh. Tekhnol. Pishch. Proizvod.* (Tech. Technol. Food Prod.), 2011. № 4. P. 14.
5. Prosekov, A. Yu., Babich, O.O., and Sukhikh, S.A., *Sovremennye metody issledovaniya syr'ya i biotekhnologicheskoi produktsii* (Modern Methods for Analyzing Raw Materials and Biotechnology Products) (Kemerov. Tekhnol. Inst. Pishch. Prom-st., Kemerovo, 2013).
6. Monaci, L., Tregoat, V., van Hengel, A.J., et al., Milk allergens, their characteristics and their detection in food: A review, *Eur. Food Res. Technol.,* 2006. V. 223. P. 149.
7. Poonnov, P., Tansakul, A., and Chinnan, M., Artificial neural network modeling for temperature and moisture content prediction in tomato slices undergoing microwave-vacuum drying, *Journ. Food Sci.,* 2007, V. 72. 2007. № 1. P. E42.
8. Pozdnyakova, A.V., Arkhipov, A.N., Kozlova, O.V., and Ostroumov, L.A., Composition and microstructure investigation for the modeling and classification of dietary fiber derived from plants, *Foods Raw Mater.,* 2014. V. 1. № 2. P. 40. doi 10.12737/4129.
9. Wal, J.M., Cow's milk proteins/allergens, *Ann. Allergy Asthma lmmunol.,* 2002. V. 89. P. 3.

ADVANTAGES OF PORCINE BLOOD PLASMA AS A COMPONENT OF FUNCTIONAL DRINKS

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Abstract: The composition and properties of blood plasma obtained from slaughtered farm animals and intended for use in the manufacturing of foods for the prevention of oxygen deficiency (hypoxia) are analyzed in the present paper. The use of aerated functional products (oxygen cocktails) is an efficient approach to hypoxia prevention. Protein-based foaming agents are known to form the most stable foams. Porcine blood plasma is a rich source of high molecular weight proteins. A method of processing of the blood of farm animals using a centrifuge with the separation factor (Fr) of 2000 or lower is described in the present article. Fractional composition of blood proteins from farm animals is reported, the choice of porcine blood plasma as a foaming agent is justified, and data on the content and amino acid compositionof high molecular weight proteins from porcine blood plasma is presented.

Keywords: Oxygen cocktail, foaming agent, blood plasma of slaughtered animals, total protein content, fractional composition of blood proteins

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INTRODUCTION

Human nutrition is undergoing significant changes due to demographic changes in the society and the industrialization of food production that lead to increased imbalance of the major diet components. The newly formed nutrition structure undoubtedly has a negative effect on the body's protective systems, thereby increasing the risk of many diseases.

Unfavorable ecological situation in Russia is another factor contributing to increased probability of diseases, and this motivates the search for procedures increasing the body's resistance to adverse environmental effects. Oxygen deficiency, more commonly known as hypoxia, is an acute problem of the society nowadays, along with micronutrient deficiency. Unbalanced diet, excessive alcohol consumption, sedentary lifestyle, chronic stress, and pollution of the environment are the main causes of this problem.

Many experts in the field of functional food and dietary products consider oxygen cocktails, which have a positive physiological effect due to the presence of pure oxygen and micronutrients added to the cocktail base, promising basic components of oxygen therapy. Saturation of such drinks with oxygen results in the formation of foam consisting of stable microscopic bubbles filled with the gas, with an oxygen content of about 150 cm^3 per serving. The use of syrups containing vitamins and herb extracts enhances the effect of treatment. Convenience of use and better preservation of oxygen in the drink require the foam to be stable during at least 15–20 minutes.

The effect of oxygen therapy is due to the high content of oxygen in the drinks. Intense absorption of oxygen through the mucous membranes of the stomach enables its entry into the blood and intensive oxygenation of tissues, thereby improving cellular metabolism, activating blood circulation, and normalizing metabolic and regenerative processes, as well as reflexes. Absorption of valuable micronutrients increases due to the overall activation of metabolic processes. This contributes to increased micronutrient activity and a stronger physiological effect of both micronutrients and oxygen [1].

Moreover, oxygen cocktails are used to treat insomnia and chronic fatigue. They promote increased stress resistance of the organism and faster recuperation of the brain after excessive mental loading.

The actual functional effect of the cocktail is highly dependent on the foaming agent used to form the oxygenated foam. A foaming agent of high quality enables the production of foam with a high stability and expansion ratio.

Eggwhite- or dairy-based foaming agents are used in the food industry. Extracts of tea, soybean, and cotton seeds have foaming properties similar to those of egg white. Stabilizers, such as casein, alginates, gelatin, and others, are usually added to edible foams to improve the stability of the latter [4, 6, 7].

Selection of a foaming agent by the manufacturer is guided by the specific properties of the former, which underlie its qualitative characteristics. Foaming agents most widely used in the production of aerated foods are listed in Table 1 [8, 9, 10].

The commonly used foaming agents have certain disadvantages: for example, the use of licorice root extract requires the addition of certain essential amino acids in order to ensure the presence of the required amount of all the essential amino acids in the final product.

Table 1. The main foaming agents used in the production of oxygen cocktails

Foaming agent	Advantages	Disadvantages
Most common		
Egg white	Raw material easily available	Risk of microbial contamination
Licorice root extract	Epidemiologically safe product	Unbalanced amino acid composition
Gelatin	Lowcost	Low availability of nutrients
Least common		
Pishchevit complex additive	Balanced composition	Large number of components
Dry albumin (ovalbumin)	Low cost	Risk of microbial contamination
Rosehip extract	Nutritional assets of rosehip berries	Low foaming capacity
By-products of dairy industry (whey, buttermilk, etc.)	Raw material easily available	The product contains fat

The use of egg protein presents a risk of microbiological contamination. Egg protein is an allergen, has an unpleasant taste, and besides, the multiplicity and stability of the foam obtained are low. Low solubility of gelatin makes its use problematic if oxygen cocktails are to be prepared in isolated production units [10,13].

Blood plasma of slaughtered animals is a source of high molecular weight protein compounds with a high foaming potential, and therefore it is a promising component of oxygen cocktails [3, 14, 15].

The aim of the present work was to investigate the physico-chemical parameters and protein composition of swine blood plasma and to provide a justification for its use in oxygen cocktail production.

OBJECTS AND METHODS OF RESEARCH

Swine blood plasma obtained by fractionation on a CM-50 laboratory centrifuge was used in the present study. Selection of the optimal value of the separation factor providing for the minimal duration of the process without erythrocyte hemolysis was a principal stage in the development of the separation technique. The separation factor was determined according to the formula proposed by A.I.Samburskii [11]:

$$Fr = 11.18 \cdot 10^{-7} \cdot r \cdot n^2, \quad (1)$$

where r is the radius of rotation for the substance, mm; n is the speed of centrifuge rotation, rpm.

The mass fraction of moisture in the blood plasma was determined using a reference method in accordance with GOST (State Standard) R 51479-99. The method is based on drying of a sample mixed with sand to a constant weight at a temperature of (103 ± 2°C). The total protein content in the raw material was determined using a Rapid N Cube protein nitrogen analyzer that implements the Dumas method. Encapsulated samples were used for the protein assay; the accuracy of the analysis was 0.5%. Total protein content was calculated by multiplying the total nitrogen content by a conversion factor, which equals 6.36 for blood proteins.

Molecular weight distribution of proteins and peptides was assessed by polyacrylamide gel electrophoresis (PAGE) according to the procedure of Laemmli. Polyacrylamide gel plates were prepared, the electrophoresis chamber was filled with electrode buffer solution (0.066 M Tris, 0.19 M glycine, 0.1% SDS), and samples were loaded into each well of the gel. Samples were prepared for analysis by mixing 20 µl of the protein solution, 10 µl of sample buffer, and 10 µl of distilled water, vortexing, and boiling for 5 min. Protein separation was controlled visually after the device was turned on. The starting current was 50 ± 0.1 mA and later it was increased to 75 ± 0.2 mA. After electrophoresis the gel was rinsed and sequentially incubated in a fixative solution, a washing solution, and a staining solution (all incubations were performed at 80 ± 2°C for 10 minutes). The gel was subsequently destained in distilled water at a temperature of 25 ± 2°C, examined, and photographed using a TCP-20M UV transilluminator (VilberLourmat, the United States) at a wavelength of 312 nm. A gel documenting system (Vitran-Photo) was used to save and process the data.

An automatic amino acid analyzer Aracus PMA GmbH conforming to the EU Directives 98/64 and 2000/45 was used for amino acid analysis. The functioning of this device is based on cation exchange separation of amino acids in a stepwise pH gradient and postcolumn derivatization with ninhydrin. Protein samples were subjected to hydrolysis by enzymes or a strong acid (6 M hydrochloric acid, 110 ± 5°C, (4-24) ± 0.05 h) prior to analysis.

RESULTS AND DISCUSSION

Properties required of the foaming agents have to be considered in detail prior to the analysis of blood plasma used as a raw material for the production of foaming agents. Surfactant properties of foaming agents and the basic characteristics conferred to the foam by this agent, namely, expansion factor, degree of dispersion, and stability, are the most important characteristics to be considered. These parameters vary considerably between the foaming agents currently used for the production of oxygen cocktails.

Analysis of published data [12, 16] showed that plasma proteins have a high nutritional value due to their composition, and therefore the use of foaming agents derived from plasma would allow for enrichment of the product by essential amino acids. Qualitative and quantitative composition of plasma proteins is illustrated by Table 2.

Table 2. Amino acid composition of plasma proteins

Essential amino acids	Amino acid content, % of total		
	Fibrinogen	Globulins	Albumins
Phenyl-alanine	7.0	3.8	6.2
Tryptophan	3.5	2.3	0.6
Arginine	6.7	5.2	6.2
Histidine	2.3	3.5	3.8
Lysine	9.0	6.2	12.4
Methionine	2.6	1.0	1.3
Threonine	7.9	8.4	6.5
Leucine	14.3	18.7	13.7
Isoleucine	5.0		2.9
Valine	3.9	5.5	0.5

As shown in Table 2, plasma proteins contain all the essential amino acids and are rich in leucine/isoleucine, lysine, and threonine. Fibrinogen is rich in phenylalanine. These data are indicative of the high biological value of plasma and imply its advantages over similar raw materials used for the production of foaming agents. Comparative analysis of the reported values of protein content in blood plasma of various farm animals (Table 3) showed that porcine blood plasma has higher protein content than other samples [12, 15].

Table 3. Fractional composition of blood plasma proteins of different animals

Animal species	Protein content in blood plasma, %			
	albu-mins	globu-lins	fibrino-gen	Total protein
Cattle	3.61	2.9	0.6	7.11
Small cattle	3.83	3.0	0.46	7.29
Swine	4.42	2.96	0.65	8.05

This is especially true for albumin, which is present in porcine blood plasma at markedly higher concentrations than in the blood plasma of other farm animals. Globulin and fibrinogen content in swine blood plasma is similar to those in the blood plasma of large and small cattle. Since the blood of small cattle is not used as a raw material in food production, porcine blood plasma was chosen as the research object.

The curve characterizing the dependence of the separation factor on the speed of the centrifuge rotor was constructed in the present study; it is shown in Fig. 1.

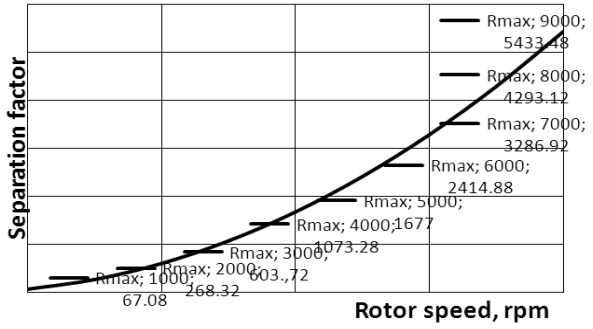

Fig. 1. Separation factor values for different rotation speeds (centrifuge CM - 50).

Separation of erythrocytes and plasma was not complete at low separation factor values (1000 and lower), and therefore the content of residual iron in the plasma was high (1.1–8.0 mg%), while at separation factor values of 1500÷2000 the iron content was consistently low, not exceeding 0.5 mg%. Centrifugation at higher separation factor values led to erythrocyte hemolysis (erythrocyte membrane disruption and hemoglobin release into the plasma), which is undesirable. Erythrocyte hemolysis caused the plasma to acquire a reddish hue, while its characteristic color is yellow, similar to the color of straw.

Thus, the minimum duration of the fractionation not resulting in hemolysis equals 6 minutes for porcine blood and corresponds to a separation factor of 2000. The separation parameters and centrifuge rotation speed for whole blood fractionation can be selected using the curve shown in Figure 1.

Quantification of protein content in the plasma allows for an overall assessment of the foaming potential of the plasma. It is necessary to determine the moisture content of blood plasma, because a foaming agent used for the production of oxygen cocktail must be supplied in a dry powdered form.

Comparison of the results of the present study and the previously reported values [12] for moisture content and total protein content of the plasma is presented in Table 4.

Table 4. Total protein concentration and moisture content in blood plasma

Parameter	Previously reported value [12]	Result of the present study
Total protein, %	8.1	9.5
Moisture content by weight, %	91.8	90.5

The nitrogen compounds present in the plasma are not limited to the known proteins (albumins, globulins, and fibrinogen), since plasma also contains amino acids, ammonia, the products of purine and pyrimidine nucleotide metabolism, etc., and this can account for the discrepancy between the total protein content values obtained in the present study and a previous study [12].

Moreover, small amounts of formed elements can be present in plasma obtained by whole blood fractionation. Leukocytes contain a lot of protein, and therefore their presence affects the total protein content readouts; consequently, the analysis of fraction composition of plasma proteins is obviously necessary for a comprehensive assessment of plasma as a protein-containing raw material. Separation of proteins by polyacrylamide gel electrophoresis was used for this purpose and provided an overall image of the fractional composition of the plasma proteins (Fig. 2).

The results of electrophoretic separation of proteins are shown in Fig. 2, with Roti-MarkStandard marker in lane M and blood plasma proteins in lane B. The use of protein markers is required for the assessment of molecular weights of the proteins. Molecular weights and concentrations of porcine blood plasma proteins are listed in Table 5.

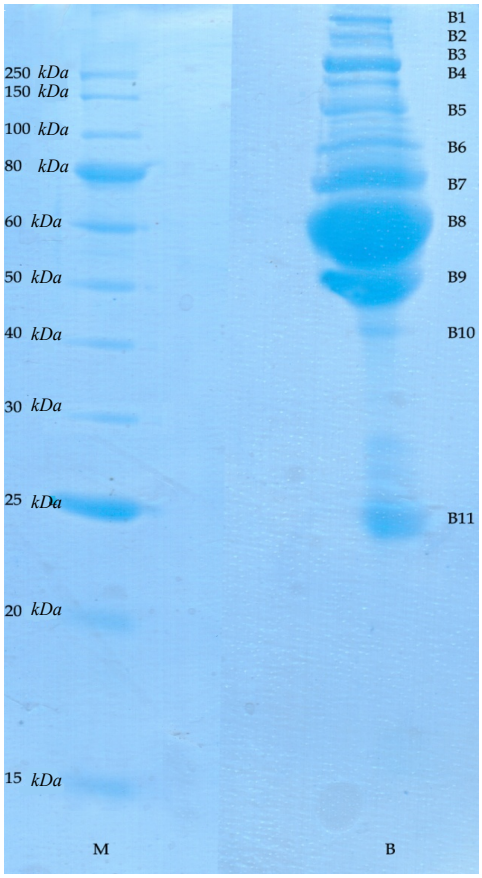

Fig. 2. Polyacrylamide gel electrophoresis (12% separating gel, 4% stacking gel): M - protein markers; B –blood plasma.

Table 5. Actual values of concentration and molecular weight for all protein fractions of pig blood plasma

Band number	Molecular weight, kDa	Content,% of the total protein
B1	854.23	1.94
B2	596.60	2.54
B3	311.80	2.76
B4	208.93	2.42
B5	127.88	2.75
B6	94.24	3.12
B7	77.40	6.39
B8	58.95	46.83
B9	49.54	17.17
B10	41.71	4.99
B11	24.47	9.09

The results of electrophoresis demonstrate the presence of a large variety of proteins in porcine blood plasma; the molecular weights of the proteins range from 24 to 855 kDa. Protein concentration is the highest (64.04%) for the combination of bands B8 and B9 (50÷60 kDa). This range corresponds to albumin, which is expected to be the predominant protein in blood plasma. Albumin accounts for about 80% of the osmotic pressure of plasma due to the relatively low molecular weight and high concentration of this protein.

The region corresponding to the protein fibrinogen (300 ÷350 kDa) contains a band with the molecular weight of 311.8 kDa and a concentration of 2.76%. Four types of globulins exist, namely, α_1– globulin, α_2– globulin, β – globulin, and γ – globulin. The concentrations of these fractions vary, and their molecular weights range from 20 to 200 kDa. Polymorphism is characteristic of many plasma proteins, such as albumin, α1-antitrypsin, haptoglobin, transferrin, ceruloplasmin, α2-macroglobulin, and immunoglobulins. Almost all plasma proteins, with the exception of albumin, are glycoproteins.

Thus, eleven protein fractions with molecular weights ranging from high (596.6 kDa and 854.23 kDa) to low (24.47 kDa) were detected in porcine blood plasma by polyacrylamide gel electrophoresis. The large number of compounds with a high molecular weight (208.93 kDa, 311.8 kDa, 596.6 kDa, and 854.23 kDa) accounts for the high foaming capacity of plasma.

All blood proteins, except hemoglobin, are of high biological value, since they contain all the essential amino acids. Amino acid composition of porcine blood plasma proteins is presented in Table 6 and Fig. 3.

Table 6. Amino acid composition of porcine blood plasma proteins

Amino acid	Content, g/100 gprotein,
Valine	7.5±0.75
Isoleucine	1.8±0.18
Leucine	9.9±0.99
Lysine	9.5±0.95
Methionine + cystine	3.0±0.3
Threonine	4.0±0.4
Tryptophan	1.5±0.15
Phenylalanine + tyrosine	8.9±0.89
Alanine	8.5±0.85
Arginine	4.5±0.45
Aspartic acid	9.0±0.9
Histidine	5.7±0.57
Glutamicacid	8.4±0.84
Glycine	4.6±0.46
Hydroxyproline	1.0±0.1
Proline	3.3±0.33
Serine	8.9±0.89

All proteinogenic amino acids were detected in porcine blood plasma proteins, however, some amino acids were present in the plasma protein hydrolyzates at low concentrations, and others (namely, valine, leucine, lysine, phenylalanine + tyrosine group, alanine, aspartic acid, glutamic acid, and serine) were present in the samples at high concentrations ranging from 7.5 to 9.9 g per 100 g protein.

The ratio of the contents of essential amino acids in a protein is a most important characteristic of its biological value. The biological value of pig blood plasma proteins was inferred from the content of essential amino acids in them, and the amino acid score was calculated and compared to the reference values proposed by FAO/ WHO (Table 7).

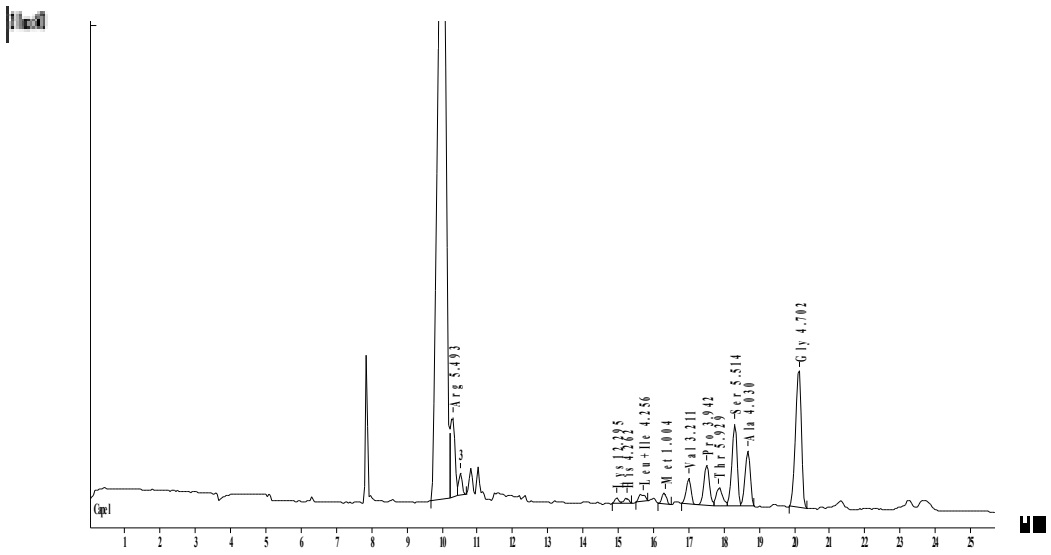

Fig. 3. Amino acid composition of proteins of pig blood plasma.

Table 7. Comparison of the content of essential amino acids in pig blood plasma to reference values

Amino acid	FAO/WHO reference scale		Porcine blood plasma	
	A, g/100 g protein	S	A, mg/100 g protein	S
Valine	5.0	1.00	7.5	1.50
Isoleucine	4.4	1.00	1.8	2.44
Leucine	7.0	1.00	9.9	1.41
Lysine	5.5	1.00	9.5	1.72
Methionine + cystine	3.5	1.00	3.0	0.85
Threonine	4.0	1.00	4.0	1.0
Phenylalanine + tyrosine	6.0	1.00	8.9	1.48

As shown in Table 7, methionine and cystine are the limiting components in porcine blood plasma proteins, but the amino acid scores for porcine blood plasma proteins are generally higher than the reference levels provided by the FAO / WHO, and therefore the biological value of plasma proteins can be regarded as sufficiently high.

As the results of the present study are summarized, the following advantages of blood plasma as a raw material for foaming agents used in oxygen cocktails become evident:

1. Use of blood plasma from slaughtered animals increases the functional value of functional beverages, supplementing them with a complex of all essential amino acids.

2. High foaming capacity of blood plasma is due to the presence of a large number of high-molecular weight compounds which can act as surfactants.

3. The technological application of plasma rather than whole blood will ensure the absence of an unpleasant "ferrous" side-taste, and therefore the taste of the oxygen cocktail will not be compromised.

The next stage of research will involve direct assessment of the foaming properties of blood plasma and a comparative investigation of the qualitative characteristics of the foam based on blood plasma or the currently used analogues.

The problem of resource shortage, that affected only few branches of industry in the early twenty-first century, is expected to aggravate from decade to decade, and therefore the application of non-conventional secondary raw materials for the production of functional foods is relevant and timely.

REFERENCES

1. *Biokhimiya: Ucheb. dlya vuzov* (Biochemistry: A University Textbook), ed. by Severin, E.S., 2003.
2. Danilova, N.S. *Fiziko–khimicheskiye osnovy proizvodstva myasa i myasoproduktov* (Physico-Chemical Basis of Meat Processing)(KoloS, Moscow, 2007).
3. Kudryashov, L.S., *Pererabotka i primeneniye krovi zhivotnykh* (Processing and use of animal blood), *Myasnaya industriya* (Meat Industry), 2010. № 9.P. 28.

4. Nepovinnykh, N.V., Grosheva, V.N., and Ptichkina, N.M., Ispol'zovanie biopolimerov dlya stabilizatsii belkovoi kislorodnoi peny (The use of biopolymers for protein oxygen foam stabilization),*VestnikVGUIT*(Bulletin of Voronezh Engineering University), 2013. № 4.P. 197.
5. Izgaryshev, A.V., Prosekov, A.Yu, and Kriger O.V., *Ustroistvo dlya pererabotki krovi sel'skokhozyaistvennykh zhivotnykh i ego primenenie* (Apparatus for processing of blood of farm animals and its application), Patent (RF) № 2484639 (2013).
6. Kut'ev, A.A. *Individual'noe sredstvo dlya prigotovleniya kislorodnogo kokteilya i ego gazovyi ballon* (Individual device for preparing oxygen cocktails and a gas tank thereof), Patent (RF) № 24950 (2002).
7. Svetlova, E.L. *Smes' dlya kislorodnogo kokteilya i sposob prigotovleniya kislorodnogo kokteilya* (Mixture for oxygen cocktail and method of oxygen cocktail preparation), Patent (RF) № 2150856 (2000).
8. Popov, V.G., Butina, E.A., and Gerasimenko E.O., *Razrabotka novykh vidov funktsional'nykh pishchevykh produktov s zadannymi fiziologicheski–aktivnymi svoystvami* (Development of new functional foods with predetermined physiologically active properties), *Novye tekhnologii* (New Technologies), 2009. № 4. P. 25.
9. Rodionova, N.S. (et al.) *Vliyanie kompleksnoi pishchevoi dobavki "Pishchevit" na protsess penoobrazovaniya* (The effect of the complex food additive Pischevit on the foaming process), *Khranenie i pererabotka sel'khozsyr'ya* (Storage and processing of agricultural raw materials), 2012. № 2.P.27.
10. Rodionova, N.S., Pashchenko, L.P., and Klimova, E.A., *Svoistva razlichnykh penoobrazovatelei v tekhnologii kislorodnykh kokteilei* (Properties of different foaming agents used inoxygen cocktail technology), *Pivo i napitki* (Beer and Beverages), 2009. № 5. P. 20.
11. Samburskii, A.I., Laboratornye tsentrifugi. *Klassifikatsii i rekomendatsii po ispol'zovaniyu* (Laboratory centrifuges. Classification and recommendations for use), *Medtekhnika i medizdeliya* (Medical equipment and medical products), 2008. № 3 (46). P.28.
12. Chechetkin, A.V., Golovatskii, I.D., Kaliman, P.A., and Voronyanskii, V.I. *Biokhimiya zhivotnykh* (Animal Biochemistry) (Vysshaya shkola, Moscow, 1982).
13. Emoto, M., *Gelatinous food product and process for preparing the same*: P 6458395 USA A 23L1/29.
14. Georgikas, S.A., et al. The use of blood plasma proteins as an additive in cooked meat, in *32nd European Meeting of Meat Research Workers* (Ghent, Belgium, 1986). V.2. P.345.
15. Caldironi, H.A. and Ockermann, H.W. Incorporation of blood proteins into sausage. *J. Food Sci*, 1982. № 47. P. 405.
16. Mare, J. and Vancikova, O. Changing the sensory properties of blood for food purposes, *Prumysl Potravin*, 1978. № 29. P. 369.

TECHNOLOGICAL FEATURES OF THE USE OF WILD-GROWING RAW MATERIALS IN THE PRODUCTION OF SOUR-MILK BEVERAGES

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Abstract: This paper reviews the data on the development of the technologies of sour-milk beverages with the use of wild-growing raw materials. The general principles of the creation of preventive sour-milk beverages and the principal requirements to wild-growing raw materials used to enrich their composition are formulated. Tavolga syrup based on herbs, such as megasea (*Bergénia crassifólia*), meadowsweet (*Filipéndula ulmária*), and peppermint (*Méntha piperíta*), and also whey extract and syrup based on balm (*Melissa officinalis*) are used in the work. The main regularities of the formation of organoleptic, physicochemical, mechanostructural, and probiotic properties of the sour-milk products with the use of aqueous and whey extracts and syrups based on wild-growing raw materials are shown. The prospects and possibility of the application of curd whey, which extracts nutrients from plant raw material, in the production of enriched sour-milk products is demonstrated. The content of milk whey in a sour-milk product is from 5 to 30%. The use of Tavolga syrup excludes the addition of colorants, flavors, and stabilizers, reduces the energy expenditures due to a decrease in the baking time, inhibits the accumulation of lactic acid by starter cultures, and also suppresses the growth of opportunistic microflora during the storage of sour-milk beverages. The technologies of Tavolga phyto-fermented baked milk and phytoyoghurt and Melissa and Lesnoi kefir beverages are described, and the nutritional value of the new sour-milk beverages is shown, including their vitamin composition. The developed technologies of the listed sour-milk beverages are competitive, as confirmed by their relevance, scientific validity, and engineering, social, and economic profitability.

Keywords: sour-milk beverages, phytoyoghurt, phyto-fermented baked milk, kefir beverage, wild-growing raw materials, megasea, meadowsweet, peppermint, balm, aqueous and whey extracts and syrups of wild-growing raw materials

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INTRODUCTION

The food consumption pattern and nutritional status of population are among the most important development indicators of a country. The importance of the nutritional status as a national health forming factor is confirmed by the adoption of the Food Security Doctrine of the Russian Federation, which classifies some real food consumption indicators as food security estimation criteria. One of the main ways to solve this problem is to provide dairy self-sufficiency [1].

In recent years, the food consumption pattern of population, including the population of Russia, is observed to be unbalanced by proteins, fats, and carbohydrates and deficient in complete proteins and polyunsaturated fatty acids at excess fats and carbohydrates. The nutrition ration of most peoples is observed to be deficient in polyunsaturated fatty acids (omega-3 and omega-6), soluble and insoluble dietary fibers, vitamins (group B, E, etc.), a wide spectrum of vitamin-like natural substances, macronutirents, and micronutrients.

Relying on the principles of evidence-based medicine, absolutely new data on the biological role of so-called minor bioactive substances for a human were obtained. This first of all concerns such bioactive substances as [2, 3]:

– Different groups of flavanoids (flavanols and their glycosides (quercetin, campherol, rutin, etc.), flavons (luteolin, apigenin, etc.), flavonons (naringenin, hesperidin, etc.), dihydroflavanols, proanthocyanidin, catechins, etc.), the physiological functions of which are very multifarious and important for decreasing the risk of many currently widespread diseases [4];
– Indoles, one of the most important function of which is the regulation of the activity of first- and second-phase xenobiotic metabolizing enzymes and the protective role with respect to some cancer pathology forms;
– Organic acids (succinic, malic, hydroxycitric, and other acids); and
– Phenols (hydroquinone, arbutin, hydroxycinnamic acids, etc.), which have a specific biological effect on the various functions of individual metabolic systems and an organism as a whole.

Among the bioactive substances of plant raw materials with a regulative effect on many body functions are phytositosterols, isoflavons, isothiocyonates, glucomannans, polyfructans, inulin, chlorophyll, caffeine, and many others.

The data accumulated in the field of nutrition science indicate that the need of an organism for all the nutrition and minor bioactive substances required for its survival can not adequately be satisfied via

traditional nutrition under contemporary human life conditions. A considerable increase in the consumption of traditional food with the purpose of elevating the amount of micronutrients in it will lead to an equally abrupt increase in the consumption of fats and carbohydrates and, consequently, to obesity and associated diseases. The problem of nutrition unbalance with respect to micronutrients can be solved via the targeted enrichment of food products by health-beneficial ingredients.

The deficiency of these nutrients and bioactive components in the ration lead to different disfunctions in the immune system of adult population and a decrease in the resistance of an organism to adverse environmental factors. This provokes the growth of the disease (infective, allergic, and cancerous) incidence rate and decreases the efficiency of therapeutic measures.

One of the objective reasons of the worsening of actual nutrition is the consumption of canned, refined, cured, and preserved food products. The rapid development of new technologies for the processing of raw materials and the production and preservation of food products has led to a considerable decrease in the share of native food products in the ration of a contemporary human. Severe technological regimes of processing and preservation deprive food of important bioactive substances, to the consumption of which our organism adapted for several thousands of years [5, 6]. Unfortunately, the share of such products in the nutrition of population continuously decreases.

The insufficient awareness of population on the rational nutrition requirements to the nutritional value of individual food products, food preparation techniques, which provide the preservation of essential nutrients, nutrition regime, etc., should be mentioned among the subjective reasons worsening the qualitative adequacy of nutrition.

Such a nutrition situation has a pronounced negative effect on the health and workability of population and requires nutrition rationalization measures.

In this connection, contemporary nutrition science assigns an important role to therapeutic and preventive food products alongside with the organization of adequate, rational, and safe nutrition [7, 8]. The development of therapeutic and preventive nutrition is required even now, as there are almost no absolutely healthy peoples, many of which work or live in an adverse environment. It must be pathogenetically substantiated therapeutic and preventive individual nutrition, which would take into account the state of health, the existence of a pathology (acute of chronic diseases), energy expenditures, the occupational factor, the state of a living environment, climate, genetic disposition, ethnic cuisine features, and also negative pathology risk factors. However complicated the problem of the development of pathogenetically substantiated therapeutic and preventive individual nutrition may currently seem, life urgently requires its solution, as the efficiency of health-improving measures depends on this factor [7].

In the therapy of patients suffering from different diseases, great priority is currently assigned to the improvement of the efficiency of their nutrition, in particular, by elevating the bioactivity of food products.

An important element of balanced healthy nutrition is functional food products. They are designed for the nutrition of all the major groups of healthy population in the structure of traditional rations, but contain health-beneficial food ingredients, which can produce a favorable effect on one or several organism's functions. Functional products play a great part in the optimization of population nutrition rations as remedies for the prevention and early correction of different diseases. The systematic consumption of functional products may increase the role of a ration as a health-maintaining factor [9].

In recent years, food industry manifests a great interest in medicinal plants of native regions, as they contain bioactive substances, such as alkaloids, glycosides, saponins, essential and fatty oils, vitamins, organic acids, and many others. This complicated spectrum of components at naturally dosed ratios in combination with therapy promotes metabolism, stimulates an organism as a whole, normalizes the state of its internal environment, and increases the resistivity of an organism to adverse factors. At the same time, wild-growing plants are more beneficial sources of plant raw materials from the environmental viewpoint than the traditionally used plants cultivated with the application of fertilizers and pesticides.

The positive properties of many plants (in particular, medicinal, essential oil, spicy-aromatic plants) are due to their ability to activate the enzyme systems and energy supply of an organism.

The structure of the bioactive substances of a plant cell has much in common with the components formed in animal and human cells. For this reason, they are easily digested and biochemically destructed in a human organism.

The value of wild-growing raw materials, including medicinal plants, consists in that they contain bioactive substances in the combination, which is difficult to reproduce artificially. The components extracted from plants have not often the same therapeutic effect as the plants themselves. The use of wild-growing raw materials in the production of food products enables the creation of targeted and preventive functional foods, such as tonic, antistress, diabetic, and radioprotective products and foods improving the functioning of the immune, cardiovascular, and gastrointestinal systems and other organs.

Hence, on the one hand, the evidences of the important role of bioactive substances in maintaining the life of a human organism have been obtained on the basis of broad-scale epidemiological studies and, on the other hand, their deficit in the nutrition of a contemporary human has been revealed. Among different groups of foods, dairy products are of great interest. The enrichment of dairy products with minor bioactive substances contained in wild-growing herbal raw materials may be considered as one of the alternative ways to satisfy the deficit of these micro-nutrients in the food of great masses of population.

One of the approaches in population nutrition policy is the use of the native raw material sources of consumer living regions. This increases the economic efficiency of food enterprises, decreases the primecost, and enriches the ration of population with necessary macro- and microelements, vitamins, and other non-synthetic components, the deficit of which has been registered in certain regions.

Taking into account the urgency of the matter, the scientific and practical principles of the production of dairy products with the use of wild-growing raw materials of the Siberian region have been developed in the Kemerovo Institute of Food Science and Technology. A particular stage of the work was the development of the technology of functional sour-milk products enriched with the bioactive substances of wild-growing raw materials.

REVIEW OF SOUR-MILK PRODUCTS TECHNOLOGIES WITH THE USE OF WILD-GROWING RAW MATERIALS OF THE SIBERIAN REGION

The creation of new sour-milk products was based on the principles developed for dairy products with a complex raw material composition, such as

– The food safety of new enriched dairy products;
– The biological and nutritional adequacy of new products;
– High organoleptic characteristics; and
– A sufficient content of vitamins, minerals, and other bioactive substances.

In this case, the following principal requirements to wild-growing raw materials for the enrichment of dairy products were taken into consideration:

– The resources of such plant raw materials must be easily renewable, and plants must not be registered in the Red Book;
– Used plants must be safe by the characteristics, which are commonly accepted in the world practice, such as toxic elements, pesticides, mycotoxins, sanitary-significant microorganisms, radionuclides, etc.;
– Applied medicinal plants must be approved for use by the Ministry of Healthcare, as the use of uncertified medicinal plants with a pronounced pharmacological effect (hellebore, false helleborine) may be harmful to human health;
– Plant raw materials must not contain any components decreasing the digestion of valuable substances of milk raw materials, such as antienzymes, antivitamins, and demineralizing substances; and
– Medicinal plants must not contain great amount of components with a pronounced pharmacological activity, such as cardiac glycosides, biogenic amines, some alkaloids, etc.

The consideration of the dose of wild-growing raw materials requires the following approach. It is necessary, first, to provide the presence of substances, which are vitally important for an organism, in a new product, second, to exclude the occurrence of its medicinal properties and, third, to provide its safety by the criterion of the content of specific pharmacologically active components [10]. The dose of used plant raw materials must satisfy the need of an organism in declared pharmacologically active components by no less than 10% and no more than 50–60% of a single therapeutic dose in the use of a given plant as a medicinal remedy.

The technologies of the Tavolga, Melissa, and Lesnoi sour-milk beverages with the use of wild-growing and introduced raw materials of the Siberian region were developed in compliance with the above stated concepts. Extracts and syrups of wild-growing raw materials were used to enrich the composition of the sour-milk beverages.

The technology of the sour-milk beverages, namely, Tavolga phytoyoghurt and phyto-fermented baked milk, was developed with the use of syrup based on wild-growing herbs, such as megasea, meadowsweet, and peppermint. The performed studies have shown their considerable effect on the formation of organoleptic, physicochemical, probiotic, and rheological properties of the sour-milk beverages with long-term pasteurization regimes and an increased level of protein.

The introduction of wild-growing plant syrups into the sour-milk beverages leads to the loss of their viscosity. The negative effect of a syrup dose can be neutralized by increasing the mass fraction of dry skim solids in a product.

In the process of storage, the sour-milk beverages on the basis of Tavolga syrup acquire the character of a condensation or irreversibly destroying structure, so bonds become more fragile and weak after the stirring of a blob, and the viscosity of a product decreases. The highest degree of syneresis was observed for samples with a low mass fraction of dry skim solids and fat and a high dose of syrup. This indicates a decrease in the moisture-retaining ability of milk blobs upon the introduction of Tavolga wild-growing herb syrups. The above listed factors smooth away this negative effect. The formation of acid during the storage of the sour-milk beverages with syrups was observed to be slightly reduced in comparison with control samples.

The rational process parameters in the production of the sour-milk beverages on the basis of Tavolga syrup are the following:

– Syrup dose, 4.5–5.0%; blob acidity before the introduction of syrup, 65–70°T; baking regime, 1.5 h at a temperature of 97 ± 2°C for phyto-fermented baked milk;
– Syrup dose, 5%; blob acidity before the introduction of syrup, 75 – 80°T for phytoyoghurt.

The use of Tavolga syrup

– Excludes the introduction of colorants, flavors, stabilizers, and sweeteners due to pronounced brown color, rich flavor, and bactericidal properties, performing the functions of the above listed ingredients in the production of phytoyoghurt;
– Reduces the expenditures of heat and energy resources due to a decrease in the baking time (from 3–4 to 1.5 h) and also the time of the entire technological cycle in the production of phyto-fermented baked milk; and
– Produces an inhibiting effect on the accumulation of lactic acid by starter cultures (*Str. salv. subsp. thermophilus* and *Lbc. delbr. subsp. bulgaricus*) and also suppresses the growth of opportunistic microflora du-ring the storage of the sour-milk beverages.

The technology of the Lesnoi kefir and Melissa beverages was developed with the use of whey extract and syrup based on balm raw materials, respectively.

The technology of the production of whey extracts was substantiated and described in the works [11, 12]. Relying on the performed studies, the possibility and advisability of the use of aqueous and whey extracts and syrups of wild-growing raw materials in the production of sour-milk products were substantiated.

The effect of the dose of balm extract and the type and dose of a stabilizer on the formation of the rheological characteristics and organoleptic properties of the beverages were studied. The optimal balm extract dose was established to be 30% of the mixture mass, and locust bean gum in the amount of 0.4% was selected as a stabilizer.

The effects of the balm syrup dose, the acidity regulator type, and the syrup introduction temperature on the formation of the rheological and organoleptic properties of the kefir beverages were studied. The optimal parameters were established to be the following: balm syrup dose, 10%; introduction temperature, 16°C; a mixture of lactic and citric acids at a ratio of 1 : 1 was selected as an acidity regulator.

The effect of different individual and mixed sweeteners on the organoleptic properties of the kefir beverages with balm extract was studied, and their sugar equivalents were calculated. Relying on these studies, stevioside was recommended as a sweetener for the kefir beverage with balm extract.

In accordance with the results of studies, the technology of the sour-milk beverages, such as Tavolga phyto-fermented baked milk, Tavolga phytoyoghurt, and Melissa kefir beverage, by the vat method was proposed. The name Tavolga of the sour-milk phyto-beverages was agreed with OOO Medicina, nauka, tekhnika (Novokuznetsk, Kemerovo oblast), a syrup producer.

The sour-milk beverages on the basis of wild growing raw materials are produced with the use of
– Milk, which corresponds to GOST R (Russian State Standard) 52054-2003 and is no worse than second-rate quality;
– Dry skim milk corresponding to GOST R (Russian State Standard) 52791;
– Tavolga syrup based on wild-growing herbs, such as megasea, meadowsweet, and peppermint (TU (Technical Specifications) 9185-021- 02068315-97);
– Whey obtained in the production of cottage cheese by ultrafiltration or curd whey ultrafiltrate; and
– Dry balm raw materials.

All the raw materials and fillers must be approved for use by the institutions of the Hygienic and Epidemiological Service of the Russian Federation and correspond to the hygienic requirements stated in "Federal Law. Technical Specifications on Milk and Dairy Products" to the quality and safety of food raw materials and products.

The technological process of the production of Tavolga phyto-fermented baked milk and phytoyoghurt includes the following operations:
– Acceptance and quality control of milk;
– Cooling;
– Normalization of the mass fraction of fat for phyto-fermented baked milk and fat and protein for phytoyoghurt;
– Exposure for an hour and stirring for phytoyoghurt;
– Purification of the normalized mixture;
– Homogenization of the mixture;
– Pasteurization;
– Baking for phyto-fermented baked milk;
– Cooling to an inoculation temperature;
– Inoculation and souring;
– Stirring and cooling;
– Addition of syrup based on wild-growing herbs and stirring;
– Bottling, packing, and labeling; and
– Cooling.

The technological process of the production of Tavolga phyto-fermented baked milk is performed as follows. The raw materials selected by quality are normalized by the mass fraction of fat. The mixture is further heated to a temperature of 43 ± 2°C, purified on centrifugal milk purifiers, homogenized at a pressure of 15.0 ± 2.5 MPa and a temperature of 45–85°C, pasteu-rized at a temperature of 97 ± 2°C, and allowed to stand at this temperature for 1.5 h. In the course of ba-king, the mixture is stirred 1–2 times to prevent the formation of skins. After baking, the mixture is cooled to an inoculation temperature of 40 ± 2°C. The mixture for phyto-fermented baked milk in inoculated with the symbiotic starter consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* cultures with the addition of the specially prepared starter on the basis of *Lactobacillus acidophilus* strains in the amount of 20% of the total starter volume.

The starter is introduced into the mixture in the amount of 3–5% of the mixture volume. It is allowed to use lyophilized and freeze-dried direct vat set (DVS) cultures containing the above listed set of lactic starter strains. The inoculated mixture is stirred for 15 min. The termination of souring is determined by the formation of a rather strong blob and the attainment of an acidity of 68–70°T. The process of souring lasts from 3 to 4 h. After the termination of souring, the mixture is cooled to a temperature of 25–27°C for 20–50 min by supplying glacial water into the interwall space of the vat. The blob is then stirred for 10–15 min, thereupon Tavolga syrup with a temperature of 20–25°C is introduced. The product is carefully stirred for 10–15 min and delivered to bottling. The packed product is cooled in a cooling chamber to 4 ± 2°C.

The principal properties of the finished product are given in Tables 1 and 2.

Table 1. Organoleptic properties of Tavolga phyto-fermented baked milk

Property	Characterization
Appearance and consistence	A homogeneous mass with a destroyed blob and a viscosity inherent to this blob. The separation of whey on the surface in the amount of no more than 3% of the product volume is admitted.
Taste and smell	A pure moderately sweet sour-milk product without foreign taints and smells with a specific filler (Tavolga syrup) flavor.
Color	Pronounced cream and uniform over the entire mass.

Table 2. Physicochemical properties of Tavolga phyto-fermented baked milk

Property	Standard for phyto-fermented baked milk		
Fat, wt %, no less	1.0	2.5	4.0
Acidity, °T	70 – 110	70 – 110	70 – 110
Temperature of release from an enterprise, °C	4 ± 2	4 ± 2	4 ± 2
Phosphatase	n/a		

The organization of the technological process of Tavolga phytoyoghurt consists in the following.

Selected raw materials are normalized by the mass fraction of fat and solids. The fat-normalized mixture is then normalized by the mass fraction of solids via the addition of dry whole or skim milk at a temperature of 40–60°C (depending on the design of a used apparatus). The normalized mixture is carefully stirred for 10–15 min. The amount of milk components must correspond to the recipe.

The normalized mixture heated to 43 ± 2°C is purified on centrifugal purifiers, homogenized at a temperature of 45–85°C and a pressure of 15 ± 2.5 MPa. The mixture is then pasteurized at a temperature of 92 ± 2°C or 87 ± 2°C and allowed to stand for 2–8 or 10–15 min, respectively. After heat treatment, the normalized mixture is cooled to a temperature of 40–43°C. The storage of the non-inoculated mixture is not admitted. The inoculation and souring of the mixture is performed in vats equipped with a cooling jacket and agitators providing the uniform stirring of the soured mixture and finished blob.

The density of the mixture before inoculation must be no less than 1.042 g/cm^3. The mixture is inoculated immediately after cooling with the symbiotic starter consisting of pure *Streptococcus thermophilus* and *Lactobacillus bulgaricus* cultures. The starter is prepared in compliance with the instruction on the application and preparation of starters. The mass fraction of the starter is 3–5% with respect to the volume of the normalized mixture. The use of lyophilic freeze-dried DVS cultures containing the strains of the above listed lactic bacteria is admitted. The starter amount and the inoculation temperature are specified in compliance with the application instruction. The starter is introduced into the vat together with the normalized and pasteurized mixture and stirred for 30 min.

The souring of the mixture is performed at a temperature of 40–43°C for 3–6 h. The termination of souring is determined by the formation of a strong blob and the attainment of an acidity, which must not exceed 85°T. After the termination of souring, glacial water is delivered into the interwall space for 30–60 min, thereupon stirring is performed for 15–30 min. Syrup based on wild-growing herbs is introduced into the product partially cooled to a temperature of 25–27°C. The syrup temperature must be 20–25°C. Stirring is performed for 10–15 min, thereupon the finished product is delivered to packing. The packed product is additionally cooled in a cooling chamber to 4 ± 2°C. The principal properties of the finished product are given in Tables 3 and 4.

Table 3. Organoleptic properties of Tavolga phytoyoghurt

Property	Characterization
Appearance and consistence	A homogeneous mass with a destroyed blob and a viscosity inherent to this blob. The separation of whey on the surface in the amount of no more than 3% of the product volume is admitted.
Taste and smell	A pure moderately sweet sour-milk product without foreign taints and smells with a specific filler (Tavolga syrup) flavor.
Color	Pronounced cream and uniform over the entire mass.

Table 4. Physicochemical properties of Tavolga phytoyoghurt

Property	Standard for phytoyoghurt		
Fat, wt %, no less	1.5	2.5	3.5
Milk protein, wt %, no less	3.9	3.6	3.5
Dry skim milk solids, wt %, no less	11.0	10.0	9.5
Titrable acidity, °T	75–140	75–140	75–140
Total sugar on invert sugar basis, wt %, no less	7.1	6.6	5.1
Temperature of release from an enterprise, °C	4 ± 2	4 ± 2	4 ± 2
Phosphatase	n/a		

Melissa and Lesnoi kefir beverages are produced with whey balm syrup and extract, respectively. The technological process of the production of kefir beverages is begun with the preparation of extract or syrup. The whey obtained in the production of cottage cheese by ultrafiltration or the curd whey purified from proteins is immediately cooled to 4 ± 2°C and stored for no longer than 12 h before processing. It is further pasteurized at a temperature of 85 ± 3°C, balm is added in the amount of 10% of the whey mass, and extraction

is performed for 30 min. Then pulp is separated out. The obtained extract is used in the production of Lesnoi beverage in compliance with the process chart or in the preparation of syrup. For this purpose, granulated sugar is introduced into the extract at a ratio of 1 : 1, and the resulting mixture is then stirred, heated to a boiling temperature without exposure, and cooled to a temperature of 60–65°C, thereupon the acidity regulator (lactic and citric acids) is added to pH 4.3–4.5.

The obtained syrup is cooled to 14–16°C (syrup introduction temperature) or to 4 ± 2°C in the case of long-term storage. The process chart of the production of Lesnoi kefir beverage is shown in Fig. 1.

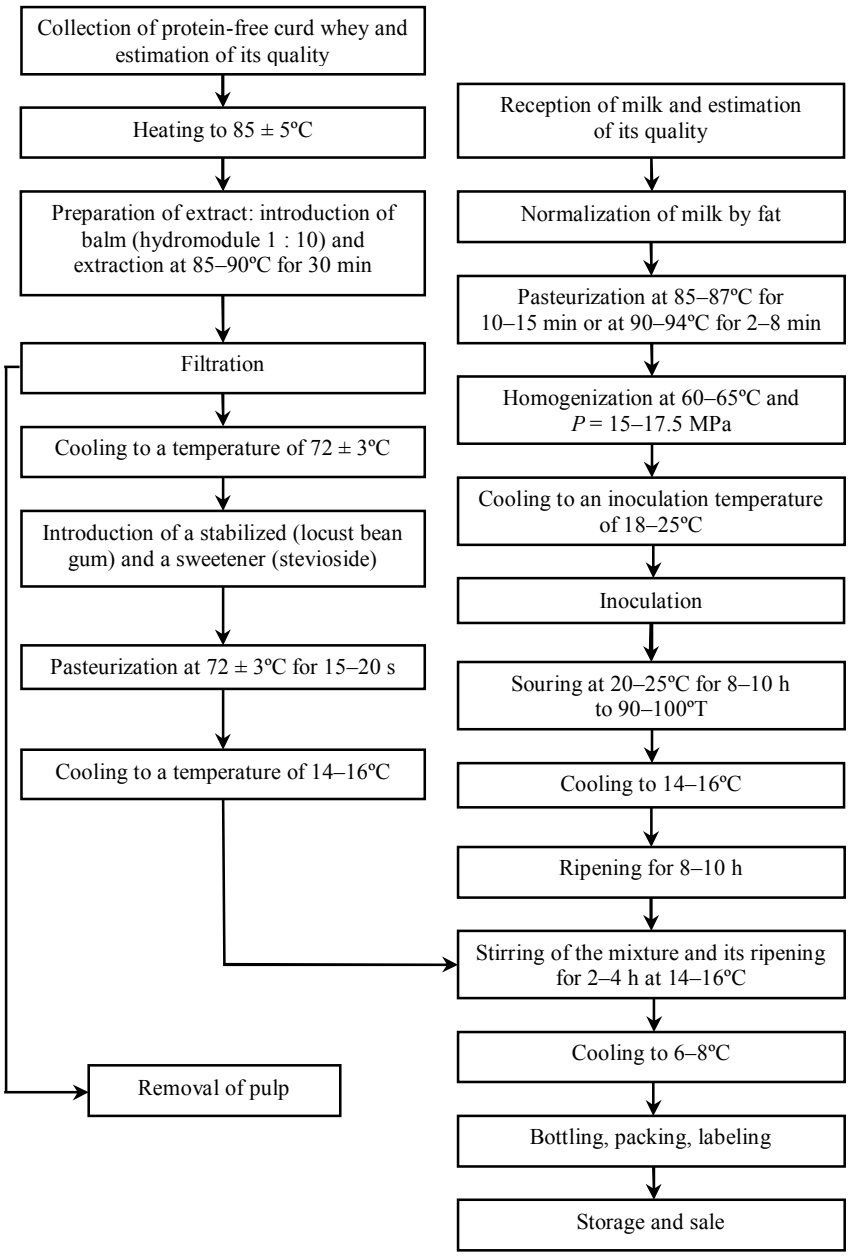

Fig. 1. Process chart for the production of Lesnoi kefir beverage.

Milk of no worse than second-rate quality with an acidity of no more than 19°T and a density of no more than 1027 kg/m³ is used for the production of the sour-milk beverages. The milk is normalized by the fat mass fraction. The normalized mixture is subjected to heat treatment.

After pasteurization and homogenization, the mixture is cooled to an inoculation temperature of 20–25°C and then delivered to an inoculation vat. A starter in the amount of 3–5% of the inoculated mixture mass is introduced into the cooled mixture.

After the termination of souring, the product is immediately cooled to a temperature of 14–16°C to perform ripening. The ripening time is 8–10 h, thereafter balm syrup is added, the mixture is stirred, and ripe-ning is continued for 2–4 h. The finished product is cooled to a temperature of 6–8°C, bottled, packed, labeled, and delivered to a cooling chamber. Thereafter the process is considered to be completed, and the product is ready for sale. The organoleptic properties of Melissa and Lesnoi kefir beverages are given in Table 5.

The nutrition and energetic value of the sour-milk beverages are given in Table 6.

The use of functional ingredients (syrups and extracts of wild-growing raw materials) has allowed the enrichment of the sour-milk beverages with tannins, bioflavonoids, carotenoids, vitamin C, and tocopherols (Table 7).

Table 5. Organoleptic properties of Melissa and Lesnoi kefir beverages

Property	Standard
Structure and consistence	Homogeneous, with a destroyed blob in the vat method. The separation of whey in the amount of no more than 2% of the product volume and the formation of gases in the form of isolated bubbles due to the growth of normal microflora are admitted.
Taste and smell	Sour-milk, refreshing, with light lemon-mint flavor and taste. Taste is moderately sweet, slightly spicy.
Color	Light-brown, homogeneous over the entire mass, typical for the filler.

Table 6. Nutrition and energetic value of the sour-milk beverages

Product	Content of components per 100 g of product, g				Energetic value, kcal
	protein	fat	carbohydrates	ashes	
Tavolga phyto-fermented baked milk	3.2	1.0	6.7	0.8	51
	3.1	2.5	6.6	0.8	63
	3.0	4.0	6.6	0.8	76
Tavolga phytoyoghurt	3.9	1.5	7.5	0.7	60
	3.6	2.5	6.0	0.7	66
	3.5	3.5	6.8	0.6	74
Melissa kefir beverage	2.7	2.5	8.7	0.7	68
Lesnoi kefir beverage	2.3	1.0	4.1	0.7	34

Table 7. Vitamin composition of the sour-milk beverages with wild-growing raw materials

Components	Tavolga phyto-fermented baked milk	Tavolga phytoyoghurt	Melissa kefir beverage	Lesnoi kefir beverage
Tannins, mg/100 g	150	150	0.78	2.5
Bioflavonoids, mg/100 g	14	14	1.4	6.0
Vitamins, mg/100 g A (retinol)	0.03	0.03	0.03	0.03
B-carotin	0.2	0.1	0.37	1.45
B_1 (thiamine)	0.05	0.06	0.34	0.35
B_2 (riboflavin)	0.20	0.30	0.20	0.40
B_3 (pantothenic acid)	0.15	0.15	0.14	0.55
B_6 (pyridoxine)	0.06	0.05	0.05	0.22
B_{12} (cobalamin), μg/100 g	0.30	0.43	0.14	0.54
C (ascorbic acid), mg/100 g	7.3	5.8	7.5	13
E (tocopherols)	0.03	0.03	0.03	0.11

Among tannins are arbutin and bergenin, which are able to bond metal ions and withdraw them from an organism and have radioprotective, anti-inflammatory, and bactericidal properties.

Bioflavonoids have P-vitamin activity and an antihypertonic and capillarotonic effect. Many studies show that the preparations created on the basis of different groups of flavonoids are highly efficient antineoplastic remedies, have antioxidant properties, and decrease the risk of cardiovascular diseases [13, 14].

Due to the presence of the above listed components, Tavolga sour-milk beverages may be classified as products, the consumption of which will favor the prevention of the mentioned diseases.

Lesnoi kefir beverage is characterized by a higher content of vitamins in comparison with Melissa sour-milk beverage. This is explained by the use of the

enriching component in the form of extract and its higher dose. The content of bioactive substances in extracts is nearly two times higher than in syrups.

The sour-milk beverages on the basis of syrups and extracts of wild-growing raw materials have a well-balances composition of basic minerals. They have an increased content of potassium, calcium, iron, and zinc in comparison with traditional sour-milk beverages. The new sour-milk beverages are especially rich in iron (0.6–1.2 mg/100 g). For comparison, traditional kefir contains 0.08–0.10 mg/100 g of iron.

The sell-by period of the beverages of developed assortment is from 5 to 8 days. During this period, the products are ensured to have high organoleptic characteristics with a content of living lactobacteria of no less than 10^7 CFU/g at the end of the use-by period. The use of Tavolga syrup in the production of sour-milk beverages suppresses the growth of opportunistic microflora in the process of storage, thus increasing the sell-by periods of the products.

The developed technologies of the mentioned sour-milk beverages are competitive, as confirmed by their relevance, scientific validity, and engineering, social, and economic profitability.

REFERENCES

1. Zobkova, Z.S., Prodovol'stvennaya bezopasnost' Rossii: odin iz glavnykh putei—samoobespechennost' tsel'nomolochnymi produktami (Food safety of Russia: one of the principal ways is to provide self-sufficiency in whole milk products), *Molochnaya promyshlennost'* (Dairy Industry), 2012. №5. P. 40–44.
2. Shatnyuk, L.N., Pishchevye mikroingredienty v sozdanii produktov zdorovogo pitaniya (Food microingredients in the creation of functional nutrition products), *Pishchevye ingredienty: syr'e i dobavki* (Food Ingredients: Raw Materials and Additives), 2005. № 2. P. 18–22.
3. Shatnyuk, L.N., Kodentsova, V.M., and Vrzhesinskaya, O.A., Ispol'zovanie innovatsionnykh ingredientov v molochnoi industrii: nauchnoe obosnovanie i prakticheskii opyt (Innovative ingredients in dairy industry: scientific substantiation and practical experience), *Pishchevaya industriya* (Dairy Industry), 2012, V. 2. № 11. P. 32.
4. Tutel'yan, V.A. and Lashneva, N.V., Biologicheski aktivnye veshchestva rastitel'nogo proiskhozhdeniya. Flavonoly i flavony: rasprostranennost', pishchevye istochniki, potreblenie (Bioactive components of plant origin. Flavonols and flavones: abundance, food sources, and consumption), *Voprosy pitaniya* (Nutrition Problems), 2013. V. 82. № 1. P. 4–22.
5. Poznyakovskii, V.M., Chelnakova, N.G., Kuznetsova, O.S., and Gavrilov, A.F., Krizis pitaniya sovremennogo cheloveka. Voprosy kachestva i bezopasnosti pishchevykh produktov (Food crisis of a contemporary human. Food quality and safety problems), *Izvestiya vuzov. Pishchevaya tekhnologiy*a (Transactions of Higher Education Institutions. Food Technology), 2004. № 1. P. 6–7.
6. Usenko, N.I., Poznyakovskii, V.M., and Olovyanishnikov, A.G., Mify o kachestve (osobennosti sovremennogo promyshlennogo proizvodstva pishchevoi produktsii) (Myths about quality (some peculiarities in the contemporary industrial production of food products)), *EKO. Vserossiiskii ekonomicheskii zhurnal* (ECO. All-Russian Economic Journal), 2013. № 6. P. 133–148.
7. Dotsenko, V.A., Lechebno-profilakticheskoe pitanie (Therapeutic and preventive nutrition), *Voprosy pitaniya* (Nutrition Problems), 2001. №1. P. 21–25.
8. Tutel'yan, V.A., Sharafetdinov, Kh.Kh., Pogozheva, A.V., and Plotnikova, O.A., Analiz normativno-metodicheskoi bazy po organizatsii lechebnogo pitaniya v meditsinskikh organizatsiyakh (Analysis of the technical standard basis for the organization of therapeutic nutrition in medical institutions), *Voprosy pitaniya* (Nutrition Problems), 2013. V. 82. № 3. P. 19–29.
9. Kochetkova, A.A., Kolesnov, A.Yu., and Khurshudyan, S.A., Tekhnicheskii komitet po standartizatsii "Funktsional'nye pishchevye produkty" i sozdanie sistemy tekhnicheskikh norm dlya otrasli funktsional'nykh pishchevykh produktov RF (Technical standardization committee "Functional Food Products" and creation of the system of technical standards for the industry of functional food products in the Russian Federation), *Pishchevye ingredienty: syr'e i dobavki* (Food Ingredients: Raw Materials and Additives), 2008. №2. P. 52–54.
10. Tutel'yan, V.A. and Sukhanov, B.P., Biologicheski aktivnye dobavki k pishche: sovremennye podkhody k obespecheniyu kachestva i bezopasnosti (Bioactive food additives: modern approaches to the provision of quality and safety), *Voprosy pitaniya* (Nutrition Problems), 2008, № 4, P. 4–15.
11. Lupinskaya, S.M., Vasil'eva, O.G., and Gralevskaya, I.V., Tekhnologicheskie parametry ekstragirovaniya BAV is dikorastushchego syr'ya tvorozhnoi syvorotkoi (Process parameters of the extraction of bioactive substances from wild-growing raw materials with curd whey), *Tekhnika i tekhnologiya pishchevykh proizvodstv* (Food Processing: Techniques and Technologies), 2012. №4. P. 23–26.
12. Lupinskaya, S.M., Orekhova, S.V., Vasil'eva, O.G., and Gralevskaya, I.V., Razrabotka tekhnologii syvorotochnogo ekstrakta i siropa na osnove dikorastushchego syr'ya (Development of the technology of whey extract and syrup on the basis of wild-growing raw materials), *Tekhnika i tekhnologiya pishchevykh proizvodstv* (Food Processing: Techniques and Technologies), 2012. №2. P. 66–69.
13. Andersen, O.M. and Markham, K.R., *Flavonoids: Chemistry, Biochemistry and Applications* (Taylor & Francis, Boca Raton, FL, 2006).
14. Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P., and Piatti, E., Honey flavonoids as protection agents against oxidative damage to human red blood cells, *Food Chem.,* 2007. V. 104. № 4. P. 1635–1640.

RESEARCH AND DEVELOPMENT OF A PEPTIDE COMPLEX TECHNOLOGY

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Abstract: Results of biotechnological research on controlled hydrolysis of casein and production of peptide complexes are summarized in the present article. Selection of processing parameters and optimization of the conditions of enzymatic hydrolysis of milk proteins allowed for the development of a resource-efficient technology for the production of peptide complexes. Enzymatic hydrolysis of peptide bonds R_1-CONH-R_2 + H_2O → R_1COOH + NH_2R_2 was considered using the example of casein and a range of proteolytic enzymes (trypsin and chymotrypsin) belonging to the hydrolase class. Enzyme solution (0.1% m/v) was added to each protein solution so that the final enzyme-substrate ratio was 1:25, 1:50, or 1:100. The enzyme-substrate ratio of 1:50 was shown to be optimal, and the recommended temperature and pH values were 50±1°C and 7.50±0.01, respectively. The degree of hydrolysis is one of the parameters characterizing the overall changes in the amino acid composition of proteins. Therefore, a time period of 12.00±0.05 h was chosen as the optimal duration of the hydrolysis process. Further research was focused on the analysis of peptide profiles using MALDI-TOF MS based on identification of peptide sequences. The studies have shown that casein hydrolysates are rich in biologically active peptide complexes. The detection of such complexes in hydrolysates of casein was the main result of the study. For example, the peptide β-casokinin (amino acid sequence Ala-Val-Pro-Tyr-Pro-Gln-Arg) is an inhibitor of angiotensin-converting enzyme.

Keywords: casein, enzyme preparations, peptides, prevention, hypertensive disease, chronic cardiac insufficiency

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INTRODUCTION

According to a report by the All-Russian Healthcare Organization, the mortality from cardiovascular disease in Russia is the highest in the world. Deaths caused by cardiovascular diseases account for 57% of total mortality in Russia, with 1.3 million people dying from these diseases every year. Coronary heart disease and hypertension remain the most common diseases of the cardiovascular system. High blood pressure remains unnoticed very often due to the lack of clinical manifestations, and therefore it is a risk factor for the development of coronary heart disease, congestive heart failure, stroke, and disturbances of the function of kidneys and other organs [1].

The development of technology for the manufacturing of therapeutics and functional foods intended for the prevention of cardiovascular disease is of especial importance. Functional foods of plant and animal origin play an important role in the prevention of disease. Functional foods are systematically used in the form of special diets capable both of preventing disturbances of physiological functions and metabolic processes in the body and of improving human health due to the presence of essential nutrients. These food products play a considerable role in the prevention of various diseases. Proper nutrition including nutriceuticals and functional foods enhances immunity and defense capacity of the body and activates anabolic processes, having a general positive effect on health [2].

The development and implementation of food for persons who are prone to or suffering from hypertension is currently in progress in Russia. Notably, foods enriched in peptides, individual amino acids, or fatty acids, as well as dairy products obtained using selected strains of lactic acid bacteria, and other products of this type are entering the market of functional foods and dietary supplements [4]. At this, the interest towards the structure and function of low molecular weight peptides produced by controlled enzymatic hydrolysis of high-quality proteins fulfilling a range of specific biological functions in the body is increasing.

A large number of biologically active peptides that are either naturally present in food or formed during enzymatic or chemical (acid) hydrolysis of the dietary proteins was identified during the past decade. Natural processing of dietary protein in the digestive tract (and preceding fermentation in the case of pre-fermented foods) enables the release of these peptides from the proteins and their functioning as independent regulatory units with hormone-like activity. A considerable number of such peptides possessing a wide range of biological functions has been identified already [3, 4].

Raw materials of animal origin, namely, milk and dairy products, are among the sources of biologically active peptides. However, such peptides can be isolated from a variety of foods such as egg, fish, shellfish, grains (rice, wheat, buckwheat, barley, and maize), soybeans, and radishes. Functional proteins from milk have an exceptionally broad range of biological activity. Bioactive peptides can be formed from both

casein (α_s, β, k, and κ-casein) and whey proteins (lactoferrin and immunoglobulin, α-lactalbumin, and β-lactog-lobulin) [5]. Use of dietary products enriched with peptides derived from milk proteins and capable of blocking the conversion of the inactive peptide angiotensin I into the active compound angiotensin II is an efficient and safe way of lowering blood pressure. Nitric oxide activates the enzyme guanylate cyclase and promotes the formation of cyclic guanosine monophosphate, which has vasodilatory activity due to its effect on smooth muscle cells [1]. Supplementation of food with functional whey casoplatelins possessing an antithrombotic effect reduces the risk of complications in patients suffering from hypertension and hypercholesterolemia [7].

Technology of peptide isolation from natural products is crucial. The structure and the biological properties of the peptides formed depend on physico-chemical parameters of the hydrolysis. Enzymatic hydrolysis is most often used for the processing of proteins in food industry.

Both casein and whey proteins from cow's milk can undergo hydrolysis. The biological value of whey proteins is higher than that of caseins due to the higher content of essential amino acids cysteine and tryptophan, and therefore whey hydrolysates are considered more physiological than casein hydrolysates. However, formation of small peptides during hydrolysis is among the advantages of casein making it more efficient in the dietary treatment of food allergy [8]. Casein hydrolysates are very often used as additional ingredients in functional foods and children's nutrition.

Various methods are used for casein hydrolysis. Four factors of the highest importance for the production of a casein hydrolysate with desired properties are the following: the degree of hydrolysis, which determines the formation of shorter peptides with lower allergenic capacity; low content of free amino acids, which is considered advantageous if the hydrolysate is added to foodstuffs; reduced bitterness; and a high total yield of peptides and amino acids [11]. Protein hydrolysates produced by enzymatic hydrolysis compensate for protein deficit and maintain the nitrogen balance in patients after gastrointestinal surgery, severe burns, etc. Development of technologies for the production of milk protein hydrolysates is of particular relevance due to the lack of protein preparations and protein hydrolysates of enhanced biological value; the supply of enteral nutrition products and specialized breast milk substitutes based on protein hydrolysates is also insufficient [9].

Peptides isolated from α-, β-, and κ-casein and lactoferrin (such as β-casokinins, casomorphins (caseomorphins), ecsorphin derived from α-casein, caseinophosphopeptides, and lactoferricin) exhibit antihypertensive properties, as well as antimicrobial and antithrombotic activity (Table 1) [10].

Table 1. Examples of biologically active peptides derived from casein hydrolyzates

System of the body	Physiological function	Peptide source	Fragment	Amino acid sequence of the peptides
Cardiovascular system	Antihypertensive	α_{s1}-casein	144–151	Asp-Ala-Tyr-Pro-Ser-Gly-Ala-Trp
		α_{s1}-casein	129–134	Leu-Ala-Tyr-Phe-Tyr-Pro
		α_{s1}-casein	181–186	Thr-Thr-Met-Pro-Leu-Trp
	Antioxidant	κ-casein	117–127	Ala-Arg-His-Pro-His-Pro-His-Leu-Ser-Phe-Met
	Antithrombotic	κ-casein	106–116	Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys
	Hypocholesterinemic	β-casein	99–101	Val-Pro-Pro
		κ-casein	129–131	Ile-Pro-Pro

In view of the above, the aim of the present work was the investigation and development of a technology for the production of biologically active peptide complexes intended to reduce blood pressure and prevent chronic heart failure.

OBJECTS AND METHODS OF RESEARCH

The milk protein casein (GOST 17626-81) was selected as the object of investigation, since it is the most easily accessible and biologically valuable source of protein.

Conventional, standard and original methods of research were used in the present work. Sampling and preparation of samples for the analysis were carried out in accordance with GOST 26809-86 «Milk and dairy products. Acceptance rules, methods for sampling and preparation of samples for analysis» and GOST 9225-84 «Milk and dairy products. Methods for microbiological analysis».

Enzymes capable of cleaving polypeptide chains into short peptides, namely, trypsin (activity 50-60 U/mg protein) and chymotrypsin (activity 40-60 U/mg protein) were chosen for the enzymatic hydrolysis; both enzymes were from Sigma (the United States).

Hydrolysis was performed with constant stirring in a thermostat at optimum temperature (50 ± 1 °C) and pH (7.5 ± 0.01, phosphate buffer) recommended by the manufacturers of the proteolytic enzymes used.

Total nitrogen/protein was determined according to the method of Dumas; the nitrogen formed was registered by the thermal conductivity detector of the «Rapid N cube» nitrogen analyzer. Amino nitrogen was determined spectrophotometrically using 2,4,6-trinit-

robenzene sulfonic acid (TNBS); the method is based on spectrophotometric detection of chromophores generated in the reaction of primary amines with TNBS. The amount of amino nitrogen was determined using a calibration curve constructed with standard dilutions of a known substance. The degree of hydrolysis of the protein was determined as the ratio of amino nitrogen to total nitrogen.

Qualitative and quantitative amino acid analysis was performed using an automatic amino acid analyzer Aracus PMA GmbH. The procedure of amino acid detection is based on separation of molecules on a cation exchanger with elution in a stepwise pH gradient and postcolumn derivatization with ninhydrin.

Molecular weight distribution of peptides and proteins in the obtained hydrolysates was evaluated using electrophoresis according to the procedure of Laemmli. Denaturing polyacrylamide gel (12% separating and 4% stacking gel) with 0.1% SDS-Na was used for the separation of proteins. Electrophoresis was performed at 15 mA in 1×electrode buffer supplemented with 0.1% SDS-Na. The gels were viewed on a UV transilluminator TCP-20M (Vilber Lourmat, the United States) at illumination wavelength of 312 nm. Gel documentation system Vitran-Photo was used for data processing.

Amino acid sequences of the peptides formed were determined by chromatography-mass spectrometry on an Agilent 5975 C system: the MALDI-TOF approach involving separation of ions by mass/charge ratio was used.

Identification of peptide sequences was accomplished by searching the NCBI (http://www.ncbi.nlm.nih.gov/) and SwissProt (http://web.expasy.org) databases.

RESULTS AND DISCUSSION

Development of technology for the production of peptide complexes possessing biological activity involves controlled hydrolysis of casein by enzyme preparations to achieve the predetermined degree of hydrolysis and subsequent separation of peptides. Selection of processing parameters and optimization of the conditions of the enzymatic hydrolysis of casein enables the development of a resource-efficient technology for the production of peptide complexes. The optimal enzyme - substrate ratio was chosen and the effect of fractional composition of the proteins on hydrolysis efficiency was investigated at the present stage of research. The final enzyme-substrate ratio after addition of 0.1 % solution of the enzyme to the protein solution was 1:25 in the first series of experiments, 1:50 in the second series of experiments, and 1:100 in the third series of experiments. The results of these experiments are shown in Fig. 1 and Table 2.

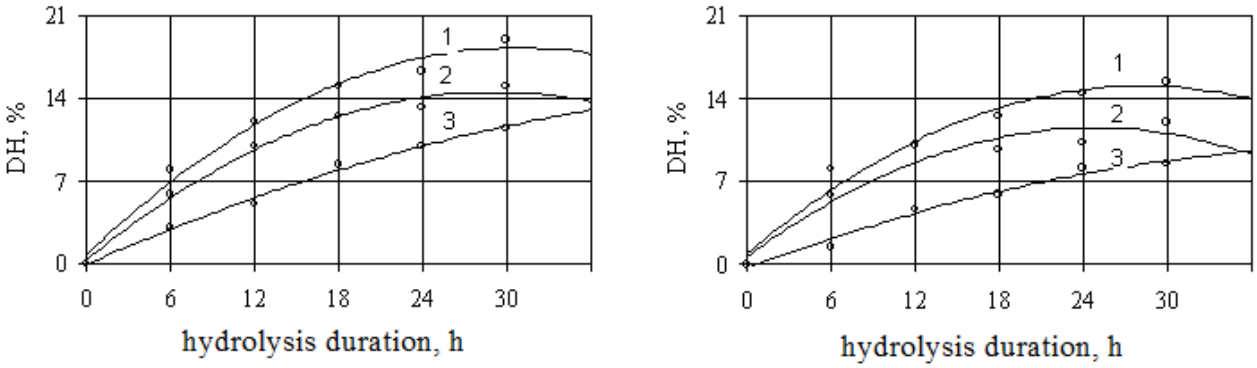

Fig. 1. Dependence of the degree of hydrolysis on process duration. Enzyme - substrate ratio: 1 - 1:25; 2 - 1:50; 3 - 1:100 ((a) trypsin treatment, (b) chymotrypsin treatment).

Table 2. Basic parameters of hydrolysates obtained by treatment with proteolytic enzymes

Parameter	Control	Enzyme-substrate ratio at hydrolysis duration, h								
		1:25			1:50			1:100		
		6.00± 0.05	12.00± 0.05	24.00± 0.05	6.00± 0.05	12.00± 0.05	24.00± 0.05	6.00± 0.05	12.00± 0.05	24.00± 0.05
Trypsin										
Degree of hydrolysis, %	0	3.00± 0.18	5.00± 0.30	10.10± 0.61	5.80± 0.45	10.00± 0.60	12.50± 0.75	8.00± 0.48	12.00± 0.72	16.30± 0.98
pH	7.50	7.40	7.25	7.18	7.38	7.36	7.29	7.42	7.35	7.24
Chymotrypsin										
Degree of hydrolysis, %	0	1.50± 0.09	4.70± 0.28	8.12± 0.79	5.82± 0.35	10.36± 0.62	11.22± 0.67	8.56± 0.51	10.17± 0.61	14.40± 0.86
pH	7.5	7.12	7.22	7.06	7.50	7.28	7.20	7.30	7.29	7.26

High efficiency of trypsin used at an enzyme - substrate ratio of 1:25 was demonstrated in the present study: the degree of hydrolysis exceeded 3% after 6.00 ± 0.05 h of treatment, and by twelve hours of treatment it was above 5%. When the enzyme-substrate ratio was 1:50, the degree of hydrolysis equaled 5.80 ± 0.45 and 10.0 ± 0.60%, respectively, for the two timepoints mentioned above. The degree of hydrolysis at an enzyme - substrate ratio of 1: 100 was significantly higher than in the other experiments and amounted to 12.0 ± 0.72% after six hours of fermentation. The active acidity of the mixture was found to change only slightly at different enzyme-substrate ratios; during the whole treatment process, it remained within a range of 7.0-8.0, which is optimal for the enzyme activity. A similar course of enzymatic hydrolysis was observed in experiments with chymotrypsin. The reaction rate was lower at enzyme - substrate ratios of 1:25 or 1:100, and therefore liberation of the peptides from the polypeptide chain was suppressed.

Changes in the degree of hydrolysis were correlated with changes in the molecular weight of the hydrolysate related to formation of low molecular weight peptides. An attempt to determine the molecular weight distribution of the peptides formed at different time points for each enzyme-substrate ratio was undertaken in order to fully understand the patterns of hydrolysis. Electrophoresis was used to study the kinetics of hydrolysis and assess the molecular weight distribution of the fragments found in the hydrolysates. Native casein with a molecular weight of 28 kDa was used as a control. The molecular weight distribution of the peptides produced by enzymatic hydrolysis of casein by trypsin and chymotrypsin are illustrated by Fig. 2 and Table 3; the molecular weight distribution was assessed using electrophoresis in polyacrylamide gel.

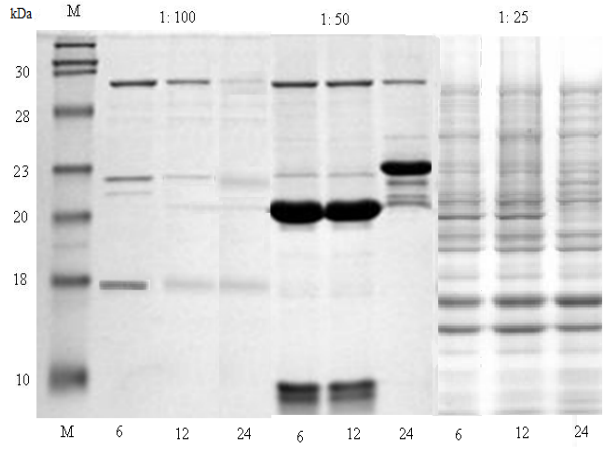

Fig. 2. Polyacrylamide gel electrophoresis of tryptic hydrolysates of casein (enzyme-substrate ratio 1:25, 1:50, or 1:100; treatment duration 6.00 ± 0.05, 12 ± 0.05, and 24 ± 0.05 h).

Redistribution of peptide fractions was shown to occur during hydrolysis by trypsin and chymotrypsin at all the enzyme-substrate ratios used. As the duration of the fermentation process increased, the amount of peptide structures with a molecular mass of 30 kDa decreased and that of low-molecular peptides and free amino acids increased. An additional estimate of the properties of the hydrolysate obtained was based on the analysis of the dynamics of free amino acid accumulation in the hydrolysate produced under various conditions. Amino acids constituted about 25.8% of the hydrolysate, the content of lysine, leucine, arginine, serine, and tyrosine being the highest.

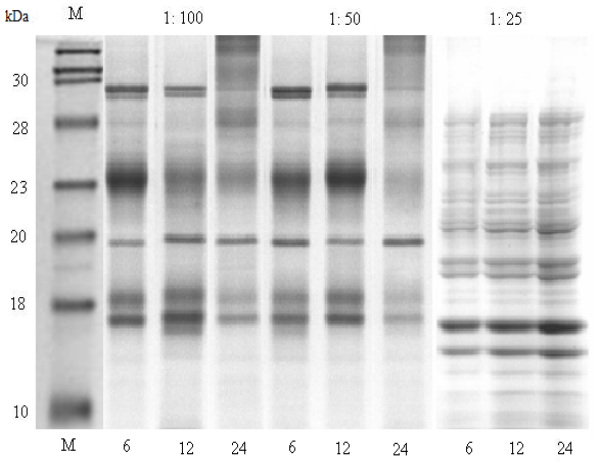

Fig. 3. Polyacrylamide gel electrophoresis of chymotryptic hydrolysates of casein (enzyme-substrate ratio 1:25, 1:50, or 1:100; treatment duration 6.00 ± 0.05, 12 ± 0.05, and 24 ± 0.05 h).

The results of the present study allow for the conclusion that accumulation of amino acids formed due to the action of proteolytic enzymes occurs along with the cleavage of intact substrate molecules resulting in formation of peptide products already present in the mixture. Despite the high specificity of the proteolytic enzymes used, they are capable of cleaving a polypeptide chain into low molecular weight peptides and free amino acids. The optimal parameters of the hydrolysis reaction were selected according to the results of the experiments described above. The optimal parameters for an enzyme system consisting of trypsin and chymotrypsin were the following: temperature 50 ± 1°C, enzyme-substrate ratio of 1:50, and process duration of 12.00 ± 0.05 hours.

Analysis of peptide profiles with MALDI-TOF MS-based peptide identification was the next step of the study. Peptides of many different types were detected in casein hydrolysates by MALDI-TOF mass spectrometry. Multicomponent composition of the samples studied was demonstrated using analysis of the mass spectra. The chromatogram of the tryptic hydrolysate of casein is shown in Fig. 4 and the peaks are listed in Table 3. The chromatogram of the hydrolysate formed after chymotrypsin treatment is shown in Fig. 5 and the peaks are listed in Table 4.

Analysis of the peptide profiles indicates that all investigated hydrolysate fractions contain both short and long peptides, which consist of two or more amino acid residues. Identification of peptide sequences obtained was performed by searching the NCBI (http://www.ncbi.nlm.nih.gov/) and SwissProt (http://web.expasy.org) databases. The results of the comparative studies are shown in Table 5.

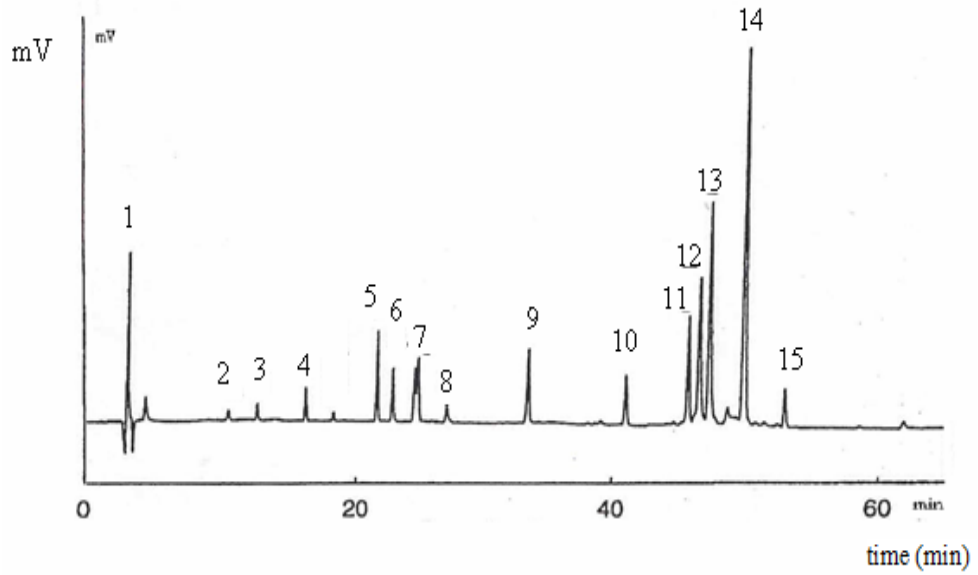

Fig. 4. Chromatogram of the tryptic hydrolyzate of casein (the location of the peptide fragments within the complete protein sequence is indicated by Latin letters).

Table 3. Characteristics of the peptide profiles generated by tryptic hydrolysis of casein

Peak number	Molecular weight, Da	Location in the polypeptide chain	Amino acid sequence
1	3803	1–25	RELEELNVPGEIVESLSSSEESITR
2	615	26–28	INK
3	98	29	K
4	390	30–32	IEK
5	1463	33–48	FQSEEQQTEDELQDK
6	4451	49–97	IHPFAQTQSLVYPFPGPIHNSLPQNIPPLTPVVVPPFLQPEVMGVSK
7	180	98–99	VK
8	664	100–105	EAMAPK
9	233	106–107	HK
10	690	108–113	EMPFPK
11	2107	184–202	DMPILYQEPVLGPVR
12	589	170–176	VLPVPQK
13	687	177–183	AVPYPQR
14	5172	114–169	YPVEPFTESQSLTLTDVENLHLPLPLLQSWMHQPHQPLPPWVMFP PQSVLSLSQSK
15	585	203–209	GPFPIIV

Note. A – alanine; D – aspartic acid; E – glutamic acid; G – glycine; H – histidine; I – isoleucine ; L – leucine; K – lysine; N – asparagine ; Q – glutamine ; P – proline; S – serine; T – threonine; W – tryptophan ; Y – tyrosine ; V – valine.

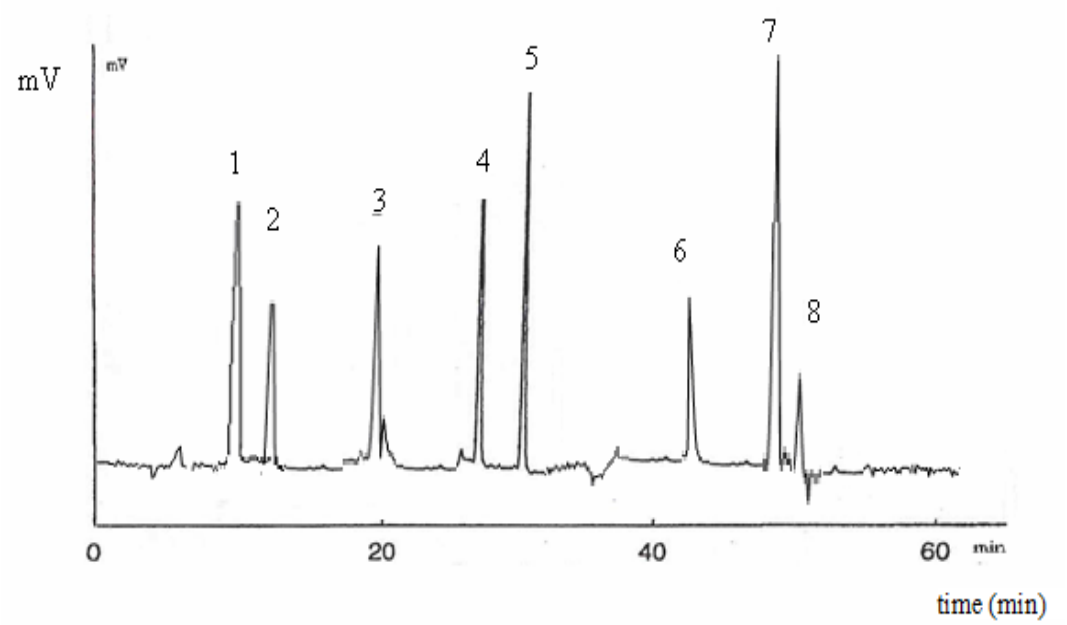

Fig. 5. Chromatogram of casein hydrolysate produced by chymotrypsin treatment (the location of peptide fragments within the complete protein sequence is indicated by Latin letters).

Table 4. Characteristics of the peptide profiles generated by hydrolysis of casein with chymotrypsin

Peak number	Molecular weight, Da	Location in the polypeptide chain	Amino acid sequence
1	3803	1–25	RELEELNVPGEIVESLSSSEESITR
2	1802	26–32	INKKIEKF
3	2329	33–51	QSEEQQQTEDELQDKIHPF
4	3754	52–86	AQTQSLVYPFPGPIPNSLPQ NIPPLTQTPVVVPPF
5	3668	87–118	LQPEVMGVSKVKEAMAPKHK EMPFPKYPVEPF
6	2734	119–142	TESQSLTLTDVENLHLPLPL LQSW
7	5286	143–189	MHQPHQPLPPTVMFPPQSVL SLSQSKVLPVPQKAVPYPQR DMPIQAF
8	1717	190–209	QEPVLGPVRGPFPIIV

Note. A – alanine; D – aspartic acid; E – glutamic acid; G – glycine; H – histidine; I – isoleucine ; L – leucine; K – lysine; N – asparagine ; Q – glutamine ; P – proline; S – serine; T – threonine; W – tripotofan ; Y – tyrosine ; V – valine.

Table 5. Peptides identified in the hydrolysates investigated

Fragment	Enzyme used	Amino acid sequence of the peptide	Name	Function
1–25	trypsin, chymotrypsin	Arg-Glu-Leu-Glu-Glu-Leu-Asn-Val-Pro-Gly-Glu-Ile-Val-Glu-Ser(P)-Leu-Ser(P)Ser(P)-Ser(P)-Glu-Glu-Ser-Ile-Thr-Arg	phosphopeptide	mineral absorption stimulant
177–183	trypsin	Ala-Val-Pro-Tyr-Pro-Gln-Arg	β-casokinin	inhibitor of angiotensin converting enzyme

CONCLUSIONS

Peptide complexes with different molecular weight and amino acid sequence were detected in casein hydrolysates during the present study. Investigation of the controlled hydrolysis enabled us to determine the optimal process parameters and the amounts of the enzymes added. Optimal parameters for enzymatic hydrolysis were the following: temperature 50 ± 1ºC, duration of 6.00 ± 0.5 h, pH 7.5 ± 0.1, and enzyme-substrate ratio of 1:50.

Analysis of peptide profiles with peptide identification by MALDI-TOF mass spectrometry showed that peptide complexes with different molecular weight were formed under optimal conditions of enzymatic hydrolysis. A peptide with the amino acid sequence Ala-Val-Pro-Tyr-Pro-Gln-Arg was reported to possess antihypertensive activity [12, 13]. The amino acid sequence of a component of a peptide complex obtained from β-casein by controlled hydrolysis was identical to that of the peptide mentioned above. Due to its sequence features, the peptide obtained is of interest for research related to its biological activity and its use for lowering blood pressure and prevention of chronic heart failure.

REFERENCES

1. Trifonov, S.V., Resursnoe obespechenie profilaktiki i lecheniya arterial'noi gipertonii v Rossiiskoi Federatsii (Resourcing the prevention and treatment of arterial hypertension in the Russian Federation), *Ekonomika zdravookhraneniya* (Healthcare Economics), 2001. № 11-12.
2. Nilov, D.Yu. and Nekrasova, T.E., Sovremennoe sostoyanie i tendentsii razvitiya rynka funktsional'nykh produktov pitaniya i pishchevykh dobavok (Current state and development trends of the market of functional foods and dietary supplements), *Pishchevye ingrediyenty: Syr'e i dobavki* (Food Ingredients: Raw Materials and Additives), 2005. P. 28.
3. Tutel'yan, V.A., Shabrov, A.V., and Tkachenko, E.I., Ot kontseptsii gosudarstvennoy politiki v oblasti zdorovogo pitaniya naseleniya Rossii k natsional'noy programme zdorovogo pitaniya (From the concept of public policy concerning healthy nutrition of the population of Russia to a national program of healthy nutrition), *Klinicheskoye pitaniye* (Clinical Nutrition), 2004. № 2. P. 2.
4. Rogov, I.A., Antipova, L.V., Dunchenko, N.I., and Zherebtsov, N.A. *Khimiya pishchi: Belki: Struktura, funktsii, rol' v pitanii. V 2-h kn. Kn. 1* (Food chemistry: Proteins: Structure, Function, and Nutritional Role. In 2 volumes. Vol. 1) (Kolos, Moscow, 2000).
5. Anisimov, S.V., Produkty pitaniya kak istochnik biologicheski aktivnykh belkov i peptidov (Food as a source of biologically active proteins and peptides), *Kosmetika i meditsina* (Cosmetics and Medicine), 2007. № 3. P. 5.
6. Tutel'yan, V.A., Khavinson, V.H., and Malinin, V.V., Fiziologicheskaya rol' korotkikh peptidov v pitanii (Physiological role of short peptides in nutrition), *Byull. Eksp. Biol. Med* (Bull. Exp. Biol. Med.), 2003. Vol. 135. № 1. P. 1.
7. Kurbanov, M.G., Razumnikova, I.S., and Prosekov, A.Yu., Belkovye gidrolizaty s biologicheski aktivnymi peptidami (Protein hydrolysates containing bioactive peptides), *Molochnaya promyshlennost'* (Dairy Industry), 2010. № 9. P. 70.
8. Kurbanov, M.G., Babich, O.O., and Prosekov, A.Yu., Napravlennyi gidroliz belkov moloka (Controlled hydrolysis of milk proteins), *Molochnaya promyshlennost'* (Dairy Industry), 2010. № 10. P. 73.
9. Bouhallab, S. and Bouglé, D., Biopeptides of milk: Caseinophosphopeptides and mineral bioavailability, *Reprod. Nutr. Dev.*, 2004. V. 44. P. 493.
10. Borisova, G.V., Bessonova, O.V., and Prosekov, A.Yu., Osobennosti tselenapravlennogo udaleniya gistidina iz kazeina (Special aspects of targeted histidine removal from casein), *Khranenie i pererabotka sel'khozsyr'ya* (Storage and processing of agricultural raw materials), 2011. № 11. P. 28.
11. Dziuba, M., Dziuba, B., and Iwaniak, A., Milk proteins as precursors of bioactive peptides, *Acta Sci. Pol., Technol. Aliment.*, 2009. Vol. 8 (1). P. 71.
12. Korhonen, H. and Pihlanto, A., Bioactive peptides: Production and functionality, *Int. Dairy J.*, 2006. Vol. 16. P. 945.

CURRENT TRENDS IN NONFAT DAIRY PRODUCTION

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Abstract: This article is dedicated to the development of new nonfat dairy products and methods of improving the quality of low-calorie foods. Advantages and drawbacks of the consumer properties of nonfat dairy products are shown. Some carbohydrate and protein fat imitators are characterized. The advantages of protein fat imitators are shown that are able not only to increase the protein content but also to add a creamy flavor to nonfat products. Possible options of using whey protein microparticulates as fat imitators in the production of nonfat dairy products are considered. The research findings are given on the use of whey protein microparticulates in the production of dairy products with higher protein contents: curd products, sweetened condensed milk, and natural cheeses: thermal-acid, soft acid-rennet, and brined.

Keywords: nonfat dairy products, butterfat imitator, whey protein microparticulate, milk-protein product, soft acid-rennet cheese, brined cheese, thermal-acid cheese, curds, condensed milk

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INTRODUCTION

Among the distinctive features of the food patterns of economically developed countries, including the Russian Federation, is a high energy capacity of the food ration. Taking into account decreased physical loads, prevailing trends toward sedentary lifestyles, and the increased share of intellectual work, the excessive consumption of food nutrients, particularly fats and carbohydrates, is becoming a very urgent problem. According to the formula of balanced nutrition, the daily demand of the human organism for fats is 102 g; however, the analysis of the macronutrient status of Russians shows the exceedance of this indicator by more than two times. The World Health Organization registers a firm growth in the number of people with progressing civilizational diseases: obesity, diabetes mellitus, and cardiovascular diseases [1].

Western countries and the United States faced long ago the consequences of overweight, which may lead to hypertension, coronary heart disease, stroke, gall-bladder disease, osteoarthritis, dyspnea, breast cancer, rectal cancer, diabetes, asthma, hormonal disorders, and reproduction problems, to say nothing of psychological stresses and complexes, developing against the worsening outer appearance.

In this context, the reduction of the caloricity of food rations, in particular, by reducing the consumption of fats, is very opportune. The reduction of the caloricity of dairy products at least by 25–50% can largely contribute to the prevention of obesity and other alimentary diseases. In addition, all other components (proteins, vitamins, minerals, and microelements) in low-calorie dairy products should be preserved, consequently, retaining the nutritive value of these products and their health benefits. Developed countries of the world have long ago switched to the preemptive production of low-calorie dairy products, which constitute more than 90% of the total dairy output.

The development of new low-calorie dairy products with considerably reduced fat contents is associated with a number of difficulties, the most problematic of which is the reproduction of the organoleptic properties of traditional full-fat products. Defatted dairy products most often have a number of flaws: too hard, resilient, or coarse consistency, poor taste and aroma; for nonfat curds, it is mealiness and the appearance of curd semolina. The more these flaws are expressed, the less fat content is in the product [2].

A way to improve the organoleptic properties of nonfat dairy products is the use of fat imitators in dairy production.

Fat imitators are substances that create the illusion of fat presence in the mouth when consumed. In addition, they either contain no calories or very few of them.

Of special importance here is the search for effective fat imitators, whether artificial or, which is more preferable, natural food components that preserve to the maximum the sensory properties of nonfat products, mainly, their texture, and simultaneously reduce their energy value [1].

Fat imitators are produced on carbohydrate, protein, and fatty bases or their combinations.

Most widespread as butterfat imitators are vegetable fats and oils. Their use makes it possible to increase the amount of deficient polyunsaturated fatty acids and improve the biological effect indicator. However, the caloricity of products on their basis does not change [1].

Among the most progressive and innovative methods of improving the quality of low-calorie foods, we may distinguish low-calorie fat imitators, namely, carbohydrate- and protein-based.

Carbohydrate fat imitators are made from gums, vegetable gel, modified food starch, or grain fibers. Carbohydrate fat substitutes absorb water and imitate fat volume and structure; they are used in the production of baked goods, prefabricated meat products, spreads, soups, salad dressings, icings, and frozen desserts [3].

In 1990, Cerestar Deutschland GmbH proposed an edible fat substitute, Snowflake-01906, which represented a product of enzymatic cleavage of potato starch with high maltodextrin content. The product had a 50% reduced caloricity compared to that of fats, neutral taste and odor, and a high plasticity [4].

Fat substitutes have been developed with physical and functional properties similar to butterfat: Oatrim [5] is oat maltodextrin, containing 1–12% of soluble beta-glucan particles, and, depending on the oat grain type, Opta Grade [6], which is based on cornstarch.

A new fat substitute based on wheat starch, Gludamin, contains 96.5% of products of the destruction of starch polysaccharides with a certain distribution of molecules by size; its use reduces fat contents in products by 15–40% [7].

L.P. Kleman and J.W. Finley have developed low-calorie fat substitutes containing carboxy/carboxylate esters. These compounds have low caloricity, and their use eliminates problems related to metabolism deterioration [8].

In order to obtain good-quality low-calorie milk-protein products with reduced fat contents, it is necessary to create conditions for the formation of acceptable organoleptic indicators, in particular, the same effect of casein hydrolysis as in fat cheeses and the introduction into the product structure of components that act as separators (breakers) of the structure of protein fibers, that have a water-holding ability, and that are taste carriers in order to replace fat as a taste substance solvent and a lubricating factor [9].

These conditions are, no doubt, met by milk-protein concentrates, obtained by protein microparticulation, the so-called fat imitators.

Protein fat imitators are produced by heating and fine-crushing (microgranulating) of milk and albumen, or whey and egg proteins, and xanthan gums. However, they are neither suitable for baking nor good as deep fat, because proteins denature at a high temperature, their structure destroys, and they lose their ability to imitate fat [3].

The idea of the use of microgranulated proteins as fat imitators was first proposed in 1984. Canadian inventors Norman S. Singer, Shoji Yamamoto, and Joseph Latella have established that spherical particles of 1 μm in size are felt as fat during chewing [10]. Their invention underlay the present industrial production of various brands of fat imitators.

A protein concentrate, Nutrilac-7611, has been developed on the basis of whey protein microparticulation to particle sizes of 1–2 μm, which mimics a butterfat flavor by its organoleptic indicators. Nutrilac-7611 is characterized by hydrophilicity and unlimited swelling, forming high-viscosity colloidal solutions, having the ability of structure formation, and thus ensuring a uniform and creamy consistency of nonfat dairy products [11].

A butterfat substitute technology has been developed on the basis of cheese whey. In order to obtain a whey protein microparticulates (WPMs) of whey proteins with sizes of 1–1.5 μm, the whey is concentrated by ultrafiltration and dispersion. The WPM is characterized by a high biological value, as well as probiotic and hypolipidemic properties; it adjusts and activates the natural habitat of bifid and lactic bacteria in the human organism and can be used to enrich nonfat fermented milk products [12, 13].

The most widespread brands of protein fat imitators are Simplesse®, Dairy-Lo®, K-Blazer®, ULTRA-BAKE™, and ULTRA-FREEZE™[14].

The Dairy-Lo® technology is reduced to the controlled thermal denaturation of milk proteins. K-Blazer® resembles Simplesse®, but it is produced differently. ULTRA-BAKE™ is made from vegetable proteins. ULTRA-FREEZE™ is designed for frozen desserts [15, 16].

Microparticulated whey proteins may be used to solve the following problems:

– to replace casein in products, reducing the cost of raw materials;
– to replace fat in dairy products;
– to improve the organoleptic properties of dairy products;
– to develop new products of sport and functional nutriation; and
– to produce microgels for milk desserts and ice cream.

Unlike European countries, Russia raises more often the question of using sour whey concentrate, since, having a characteristic unpleasant taste, it cannot be added directly to dairy products. In this case, the microparticulation process comes to the rescue.

The microparticulation process is based on the thermal treatment of whey proteins α-lactalbumin and β-lactoglobulin. On exposure to temperature, whey proteins are denatured and aggregated. Then the obtained suspension is treated mechanically to form whey protein agglomerates that are similar to fat globules.

At present the main producers of microparticulation equipment are:

– the Kizel'mash Group of Companies, which markets the Export+ units for the production of microparticulates with a dry matter content of up to 50%, which are recommended for the production of low-fat yoghurts, desserts, sauces, mayonnaises, ice creams, and sport/functional products [17];
– the Alpma Co., which manufactures equipment for the production of the Alpma CreamoProt whey protein concentrate, which contains about 8–10% of crude protein as denatured, spherical proteinaceous matrices in dry matter and which is recommended for use in the production of various dairy products, including cheeses [18];
– the Tetra Pak Co., which manufactures the Tetra Therm Micro Part module for the microparticulation of the Simplesse®-100 whey proteins, made from the dry concentrate of whey protein by dissolving it in water, thermal treatment on a tubular pasteurizer, and homogenization. Simplesse®-100 disperses easily, dissolves quickly without using special equipment, and acts as a surrogate disperse phase, replacing fat droplets and simulating the spreading fatty consistency of a product [19].

The composition and nutritive value of Simplesse®-100 do not differ from the usual whey protein concentrates. Its production technology does not use substances that can change its origin. The properties of Simplesse®-100 are given in Table 1.

Table 1. Properties of the Simplesse®-100 denatured whey protein concentrate [158]

Product characteristics	Value
Chemical properties	
Protein content, mass %	53.0
Fat content, no more than, mass %	4.5
Lactose content, mass %	36.0
Moisture content, no more than, mass %	4.0
Organoleptic properties	
Outward appearance	Power
Color	from white to cream-white
Taste	a light flavor of boiled milk
Technical properties	
Dosage, %	0.5–5.0
Marking	whey protein concentrate; milk protein
Shelf life	no less than 18 months in a dry and cool place

Simplesse®-100 [20] is a multifunctional food additive, which does not differ in its composition and nutritive value from a regular whey protein concentrate. This additive disperses easily and dissolves quickly without using special equipment. The Simplesse®-100 powder is introduced into the formula mix with a sufficient amount of water; the contents of dry substances should be at least 40%. In fatty systems, the powder should be hydrated in the water phase until fat or oil is introduced.

The microgranulated protein Simplesse®-100 acts as a surrogate disperse phase, replacing fat droplets, which traditionally function as a disperse phase, and stimulates the spreading fatty consistency of products. This ability of a fat substitute is predetermined by the size and shape of component particles [9].

The Kemerovo Institute of Food Science and Technology conducts a series of scientific studies dedicated to the development of new low-fat dairy technologies that use whey protein microparticulates.

OBJECTIVE

The objective of this work is to study the technological specifics of microparticulated milk-protein concentrates in improving the biological value of new products, as well as their organoleptic and rheological properties.

The solution of the set objective makes it possible to create new-generation dietary dairy products.

During the development of new milk-protein technologies, we took into account the following requirements:

– the maximum use of milk's protein fraction;
– the exclusion of butterfat from the product composition without degrading its consumer properties;
– the improvement of the organoleptic and structural–mechanical properties of a product by the introduction of whey protein concentrates;
– the improvement of the biological value of a product by the introduction of whey proteins; and
– the minimization of production costs.

The working hypothesis of the research conducted involved the following assumptions:
– the protein content increased in the defatted milk mix owing to the introduction of WPMs into it, making it possible to increase the output of the finished product per unit of raw material and improving the rheological properties of the final products; and
– the dose of milk-clotting enzymes decreased during the coagulation of the defatted milk mix with WPMs in the manufacture of protein milk products (cheeses and curds).

RESULTS AND DISCUSSION

Positive results were obtained by postgraduate student S.V. Manylov using the Simplesse®-100 whey protein microparticulate to produce nonfat curds and thermal-acid cheeses [9].

The author has established the following:
– the rational doses of the used concentrate that make it possible to reproduce to the maximum the properties of analogs with traditional fat contents: for the production of the *Antaeus* cheese, the dose of the denatured whey protein concentrate (DWPC) is (1.1 ± 0.02)%, and, for the *Antaeus* curds, (1.0 ± 0.05)%;
– the optimal technological regimes for nonfat products;
– the organoleptic, physicochemical, and rheological properties of new products;
– safety indicators and guaranteed shelf lives of these products; and
– draft technical documents for the production of the *Antaeus* thermal-acid cheese and the *Antaeus* defatted curds, which have the organoleptic indicators given in Table 2. The physicochemical indicators of the new products are given in Table 3.

Postgraduate student E.E. Rumyantseva proposed using microparticulated whey protein to produce nonfat sweetened condensed milk. The author's studies helped develop the technology of a product called SportMilk.

In order to establish the optimal WPM dose, the organoleptic assessment of various nonfat sweetened condensed milk samples was conducted, and the changes in their taste, odor, and consistency over 30 days were analyzed.

The characteristics of the organoleptic indicators of preserved milk products (taste, odor, consistency, outer appearance, and color) depending on the dose of whey protein microparticulates during storage are given in Figs. 1–3. Nonfat sweetened condensed milk was used as the control option. Whole sweetened condensed milk served as the marker of the reference organoleptic indicators.

Table 2. Organoleptic properties of nonfat milk-protein products

Indicator	Characteristic	
	Antaeus nonfat cheese	*Antaeus* defatted curds
Outer appearance	The rind is wrinkled, with cloth traces or smooth, without a thick subrind layer	A homogeneous mass with an even, glazed surface, without noticeable protein clots
Taste and flavor	Clear, slightly sourish, with an expressed flavor and odor of pasteurization, with a slight flavor of whey proteins	Clear, lactic, without foreign flavors and odors, with an expressed flavor and odor of pasteurization
Consistency	The dough is sufficiently compact, homogeneous	Homogeneous, slightly spreading. Minor whey separations are admissible
Pattern	Irregularly shaped eyes, the absence of eyes is admissible	–
Test color	White, uniform throughout its mass	White, uniform throughout its mass

Table 3. Physicochemical indicators of nonfat milk-protein products

Indicator	Value	
	Antaeus nonfat cheese	*Antaeus* defatted curds
Moisture content, mass %, no more than	77	73
Protein content, mass %, no more than	18	20
Fat content, mass %, no more than	0.25	0.5
Common salt content, mass %	1.6-2.0	-

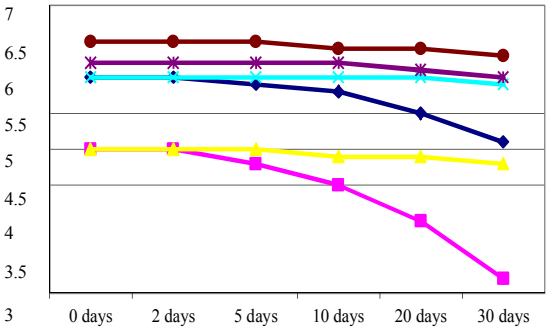

Fig. 1. Taste and odor of sweetened preserved milk products, depending on the WPM dose and storage duration.

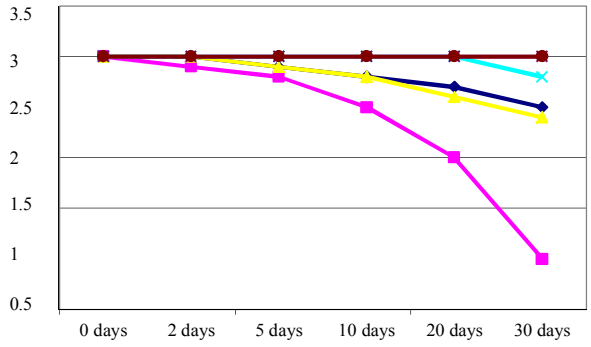

Fig. 2. Consistency and outer appearance of sweetened preserves, depending on the WPM dose and storage duration.

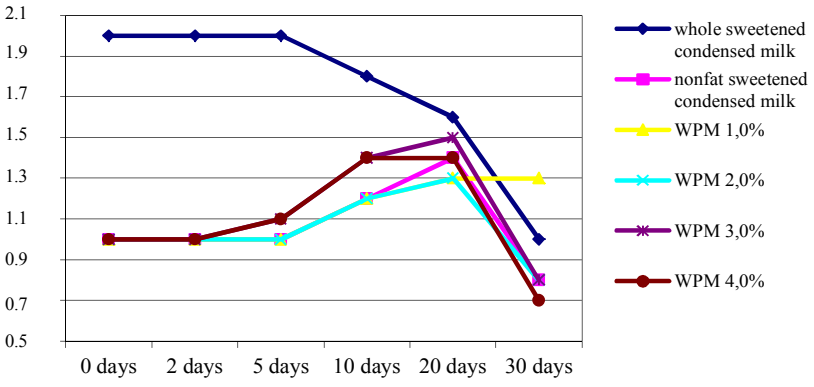

Fig. 3. Color of sweetened milk preserves, depending on the WPM dose and storage duration.

The introduction of Simplesse®-100 made it possible to improve the taste profile of condensed milk, since, during the production of this WPM, the whey proteins are thermally denatured and the sulfhydryl groups are released. In addition, the concentrate acquires a specific taste of "boiled milk" or a pasteurization flavor. This flavor is preserved in nonfat dairy products produced using Simplesse®-100 and is intensified during repasteurization. This is especially important in the production of nonfat preserved milk products, because they are characterized by the insufficiently expressed odor, taste, and aroma.

The main taste defect, which is intensified during storage, is a caramelization flavor, which is associated with a higher content of whey proteins.

The introduction of Simplesse®-100 improved the consistency of preserved milk products and prevented product solidification owing to the strong hydrophilic properties of a whey substitute of fat. However, at a WPM dose of 6.5% and higher, an insignificant amount of protein deposited on the bottom of a jar, slightly impairing the consumer properties of the product.

The color during the storage of milk preserves changed significantly; darkening was noticed ten days after. The addition of Simplesse®-100 made this effect come the earlier the larger the dose was introduced. This made it possible to mimic the color characteristic of whole condensed milk or condensed cream.

The studies conducted by the author resulted in proving the possibility to use the Simplesse®-100 WPM as a butterfat imitator in the production of nonfat sweetened condensed milk with high organoleptic properties, close to whole sweetened condensed milk. The optimal WPM dose of 2.0% in the finished product was established.

In the work by D.A. Smirnov, it was proposed to use whey protein microparticulates in the production of nonfat soft acid–rennet and brined cheeses in order to improve their organoleptic characteristics:

– the recommended doses for the introduction of WPMs were established: for the production of soft acid–rennet cheese, the dose is (0.5 ± 0.1)%, and for brined cheese, (0.6 ± 0.1)%;

– the specifics of the coagulation of defatted milk mixes with WPMs were investigated by rennin and Fromase, and it was established that (0.5 ± 0.2) g of rennin for 1000 kg of the mix were required for the coagulation of defatted mixes with WPMs;

– the optimal technological parameters of the manufacture of new products were established; and

– a draft technical documentation was developed for the production of the *Ichigo* soft acid–rennet cheese and the *Fort* brined cheese, which have organoleptic indicators given in Figs. 4 and 5.

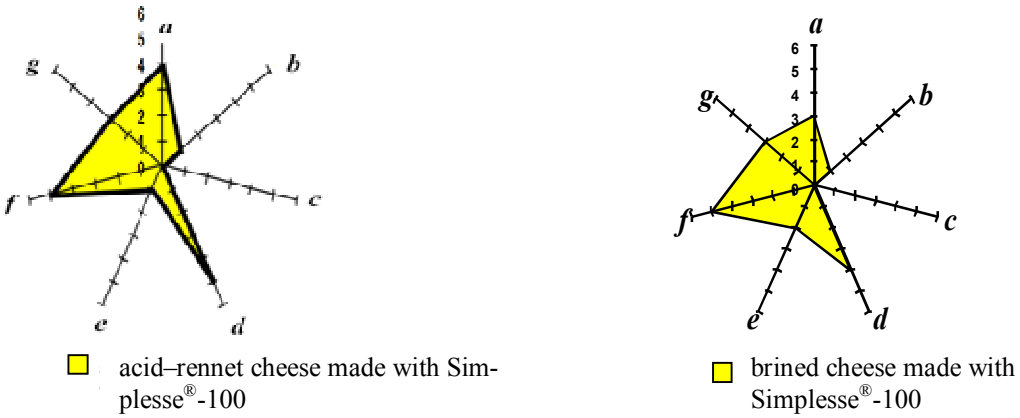

Fig. 4. Profilograms of the outer appearance, pattern, and consistency of cheeses, where: *(a)* surface insularity; *(b)* pimple surface; *(c)* moisture in the cross section; *(d)* cheese-dough density; *(e)* cheese-dough openness; *(f)* creaminess; and *(g)* cream tint.

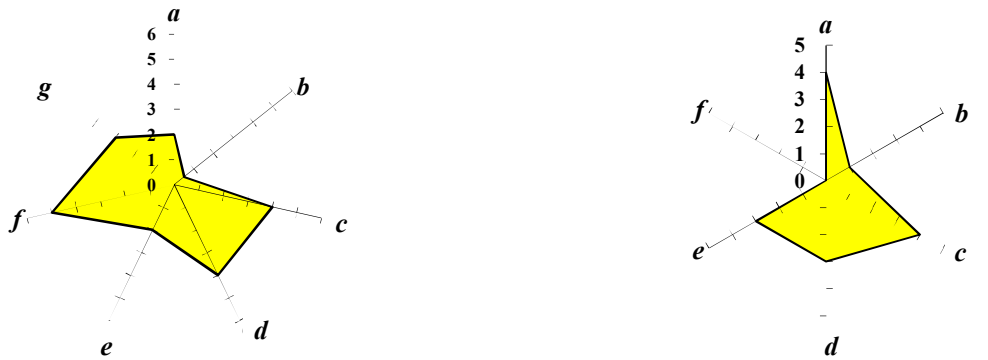

Fig. 5. Profilograms of cheese tastes and odors, where: *(a)* sour taste; *(b)* foreign taste; *(c)* clear milky taste and odor; *(d)* pasteurization flavor; *(e)* spicy flavor; and *(f)* watery flavor.

Characterizing the outer appearance, pattern, and consistency, we may conclude that cheeses made with the Simplesse®-100 whey protein concentrate (WPC) meet all the necessary requirements: their surface was insulated quite well with no signs of pimpling; the cheese dough was sufficiently compact, oozing no moisture when cut, and with a creamy consistency.

The assessment of the taste and odor of (both brined and acid–rennet) cheeses produced with WPCs is quite high: no unwanted flavors were detected in them, and at the same time the cheeses had the pleasant flavors of certain spiciness and pasteurization.

The physicochemical indicators of the new products are given in Table 4.

Table 4. Physicochemical indicators of cheese

Indicator	Indicator value	
	Soft acid–rennet cheese	Brined cheese
Moisture content, mass%, no more than	67.0	50.0
Fat content in dry matter, mass %, no more than	1.0	1.0
Common salt content, mass %	1.6–2.0	2.0–5.0

The findings show the practicability of using microparticulated whey proteins in the production of nonfat dairy products.

Summarizing the above, we may conclude that the use of microparticulated whey protein concentrates in nonfat protein dairy products is quite advisable: they positively affect the product output, increase the product's biological value, and improve its organoleptic indicators.

REFERENCES

1. Mel'nikova, E.I. and Stanislavskaya, E.B., Whey protein microparticulates as butterfat imitators in food production, *Fundamental'nye issled*. (Basic Res.), 2009. № 7. P. 22–23.
2. Shidlovskaya, V.P., *Organolepticheskie svoistva moloka i molochnykh productov. Spravochnik* (Organoleptic Properties of Milk and Dairy Products: Handbook) (Kolos, Moscow, 2000). 280 p.
3. Smirnova, I.A., Shtrigul', V.K., and Smirnov, D.A., Studying milk protein properties in order to form protein imitators of fat, in *Actual'nye problemy tekhniki i technologii pererabotki moloka: Sbornik nauchnykh trudov s mezhdunarodnym uchastiem* (Topical Problems of Milk Processing Equipment and Technology: A Collection of Works with International Participation) (Barnaul, 2012). No. 9. P. 215–222.
4. Pszezola, D.E., Carbohydrate-based ingredient performs like fat for use in a variety of food applications, *Food Technol.,* 1991. V. 45. № 8. P. 262–263, 276.
5. Kucner, J.F., Oatrim – a new fat replacer, *Food Ingredients Eur. Conf. Proc., Paris, 8–10 Oct., 1991* (Maarssen, 1991). P. 168–172.
6. Akoch, C.C., Fat replacers, *Food Technol.,* 1998. V. 52. № 3. P. 47–53.
7. Fat substitution gains pace, *Food Ingredients and Process.,* 1992. Feb. p. 28.
8. US Patent № 4830787.
9. Manylov, S.V., The study of the effect of denatured whey proteins on the properties of low-calorie milk-protein products, *Cand. Sci. (Eng.) Dissertation* (Moscow, 2009). 125 p.
10. Singer, N. S., and Moser, R.H., Microparticulated proteins as fat substitutes, in Altschul, A.M., *Low Calorie Foods Handbook*, Ed. by Dekker, M. (New York, 1993). Chap. 9.
11. Mel'nikova, E.I., Ponomarev, A.N., and Popova, E.E., Milk proteins in ice cream technology, *Molochnaya prom-st'* (Dairy Industry), 2012. № 12. P. 64–65.
12. Mel'nikova, E.I., Stanislavskaya, E.B., Podgornyi, N.A., and Chunosova, E.V., A symbiotic product based on whey protein microparticulates, *Syrodelie maslodelie* (Cheesemaking Buttermaking), 2010. № 6. P. 26–27.
13. Ponomarev, A.N., Mel'nikova, E.I., and Losev, A.N., Microparticulated whey proteins in the symbiotic product technology, *Molochnaya prom-st'* (Dairy Industry), 2013. № 7. P. 62–63.
14. Gershoff, S.N., Nutrition evaluation of dietary fat substitutes. *Nutrition Reviews,* 1995. № 11. http://www/findarticles.com/p/articles/mi_ga3624/is_199511.html.
15. Clareto, S.S. et al., Influence of a protein concentrate used as a fat substitute on the quality of cheese bread, *Brazilian Archives Biol. Technol.*, 2006. V. 49. № 6. P. 1019–1025.
16. Dietary fat replacers. http://www.andrews.edu/NUFS/resource.html.
17. Baranov, S.A., Microparticulation units in modern milk production, *Pererabotka moloka* (Milk Processing), 2012. № 8 (153). P. 14.
18. Al'pma Co.'s complex whey processing technologies, *Syrodelie maslodelie* (Cheesemaking Buttermaking), 2012. № 4. P. 48.
19. Dorman, E.S., Fat substitute. http://www.madehow.com/Volume-2/inde.html.
20. US Patent № 4734287.

TECHNOLOGY OF ALCOHOL OXIDASE PRODUCTION FROM YEAST *CANDIDA BOIDINII* FOR USE IN FUNCTIONAL FOODS INTENDED FOR WITHDRAWAL SYNDROME ALLEVIATION

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Abstract: The present article considers the increasing popularity of beverages containing ethyl alcohol and fermentation products with the population of Russia. Statistical data show that alcoholic beverages with ethanol concentration exceeding 40 vol. % account for the largest share of the market. The development of a procedure for the production of alcohol oxidase from the yeast Candida boidinii is reported; the enzyme is intended for use in the manufacturing of functional foods for withdrawal syndrome alleviation. A procedure for the disruption of cell walls of the yeast Candida boidinii in a planetary ball mill PM 400 and methods for the removal of ballast substances reducing the catalytic efficiency and the specific activity of the enzyme preparation are presented.

Keywords: withdrawal syndrome, foods, beverages, alcohol oxidase, catalase, technology, purification

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INTRODUCTION

The popularity of drinks containing ethanol and fermentation products with the population of Russia has been increasing recently. According to statistical data, alcoholic beverages with ethanol concentration above 40 vol. % account for the largest share of the market, while beer accounts for 25% only, and the share of sparkling wine and wine-based beverages is even lower. However, a trend towards a decrease in the popularity of vodka, whiskey, and cognac is evident, and the demand for low-alcohol beverages, such as beer, cocktails, and effervescent beverages, is growing rapidly [1, 2].

Increased alcohol consumption has an obvious negative impact going beyond the deterioration of physical and psychological health. Heavy alcohol use affects the welfare and health of people in the immediate environment of the drinker and has a negative impact on the society as a whole.

The state policy concerning alcohol production and consumption has always been a major factor affecting the consumption of alcohol by the population of Russia. All the documented increases in alcohol consumption and changes of consumption patterns towards increased use of strong beverages were related to actions of the state. Attempts to reduce regulation and relegate responsibility for alcohol production and consumption or increase profits from these processes have always led to increases in alcohol consumption and the share of strong beverages [3].

Alcoholic beverages are known to mankind since ancient times: wine was consumed in ancient Egypt (7-3 centuries BC), and both wine and beer were common in medieval Russia. Rye (vodka produced from grain) appeared in Russia in the mid-15th century, and illegal distillation of alcohol was widespread by the 1800s already. Statistical data show that the level of alcohol consumption in Russia is among the highest in the world. The annual consumption of ethyl alcohol per capita was 9.3 liters in 1992, while in the early 21st century it reached 15-18 l, a value significantly higher than those for the European countries.

Ethanol is used during medical procedures to sanitize the skin and sterilize surgical and medical instruments, because it induces denaturation of cytoplasmic proteins of various pathogens. The chemical structure and pharmacological properties of ethyl alcohol allow for classifying it as a central nervous system depressant. A general depression of physiological and emotional processes (withdrawal syndrome or hangover) is a consequence of alcohol consumption. The main symptoms of withdrawal syndrome are high blood pressure, chills, headache, hand tremors, loss of appetite, dry mouth, and depression. Withdrawal syndrome usually develops within a few hours after consumption of alcoholic beverages and lasts up to 2–3 days. An adverse consequence of withdrawal syndrome is repeated consumption of alcohol, which provides for a certain relief, but ultimately leads to poisoning of the organism and development of alcohol dependence [4].

Ingested ethanol is rapidly absorbed by diffusion in the stomach; the maximum concentration of alcohol in the blood is attained after 60–90 min. Notably, the rate of absorption varies and is affected by a multitude of different factors. The liver is the main site of ethanol metabolism, but the stomach epithelium can contribute to this process as well. Ethanol is dehydrogenated by alcohol dehydrogenase to form ethanal (acetaldehyde), which is subsequently converted to acetate by aldehyde dehydrogenase [5]. Oxygenase, peroxidase, catalase, and alcohol oxidase also play an important role in the

metabolism of ethanol in a living organism. According to Rumyantsev et al. (1981), consumption of ethanol results in increased concentration of acetaldehyde, which disturbs the functioning of a number of metabolic systems [6].

The quantity and variety of foods and pharmaceuticals intended to reduce the toxic effect of ethanol and its metabolites (acetaldehyde) on a living organism are currently insufficient in Russia, and therefore the development of competitive domestic harmonizing ingredients intended to bind toxic products of ethyl alcohol metabolism and eliminate them from the body is of high relevance and practical importance.

The interaction of chemicals and elements is the material basis of life and technological processes. The rate of biochemical reactions occurring in the body is normally very high due to the catalytic effect of enzymes. Therefore, enzymes and enzyme-based products receive considerable attention nowadays. Enzyme preparations are widely used in chemical, medical, pharmaceutical, and food industries. The world production of enzyme preparations for medical and food purposes increased significantly during the recent years, with more than half of the preparations obtained from microbial sources [7].

The market of enzymes and enzyme preparations for food industry has the highest capacity. Addition of enzyme preparations to the products of food industry offers new opportunities for the use of these products. Firstly, the use of enzymes allows for an increase in production volumes, and since there is a steady demand for foods produced using enzymes, these foods enable successful competition in the market. Secondly, the use of enzyme preparations allows for a considerable improvement of the quality of functional foods. Thirdly, improvement of working conditions in the production facilities becomes possible. Fourthly, the use of enzymes in food processing can contribute to increased food digestibility and nutrient availability, and to improvement of organoleptic properties of the food. Fifthly, the creation of novel foods becomes possible [5].

Enzymes are proteins acting as biological catalysts in living organisms. Enzymes are involved in catabolism and synthesis of vital (essential) substances required for the development and functioning of living organisms. All enzymes in a living organism belong to multienzyme systems that usually contain an enzyme component determining the catalytic activity and bioreaction rate in the whole system [4, 5].

Alcohol oxidase (EC 1.1.3.13) is an enzyme catalyzing the oxidation of ethyl alcohol to form acetic aldehyde, which is further metabolized into acetic acid subsequently decomposed to form water and carbon dioxide. A scheme of ethanol degradation by alcohol oxidase resulting in ethanal formation is shown in Figure 1. The enzyme may catalyze the oxidation of methyl, propyl, isobutyl, and amyl alcohols, ethylene glycol, formic acid, and various aldehydes [8].

The aim of the present work was to analyze the process of cultivation of the yeast *Candida boidinii* on a nutrient medium supplemented with cheese whey and to determine the optimal cultivation parameters for the yeast *Candida boidinii*.

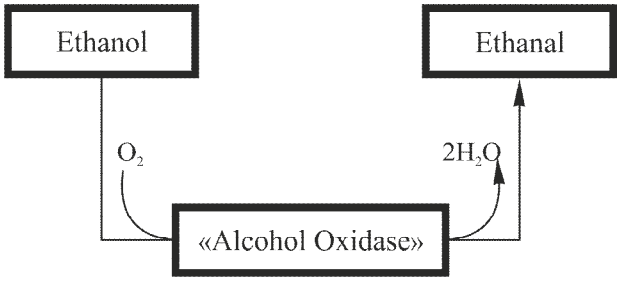

Fig. 1. Reaction of ethanol conversion to ethanal by alcohol oxidase.

OBJECTS AND METHODS OF RESEARCH

Yeast *Candida boidinii*, cheese whey conforming to the Technical Specifications of Belarussia (TU RB) 100098867.131-2001, ethanol (96 vol. %) conforming to GOST (State Standard) 5964-93, cultivation medium containing molasses conforming to GOST (State Standard) 171-81, and yeast autolysate conforming to the Technical Specifications (TU) 9184-003-56588117-08 were used as objects of the present study.

Standard, conventional, and original methods of analysis were used when performing research.

Yeast was grown aseptically under standard cultivation conditions in a batch bioreactor on a molasses medium supplemented with whey. The culture was continuously aerated during the cultivation process. Yeast was separated from the culture fluid by centrifugation and washed with purified water [9].

The specific growth rate was calculated as the yeast growth per hour, per unit of growing biomass.

Yeast biomass was quantitated as the difference between the biomass of microorganisms before and after cultivation.

The content of ash in the yeast biomass was determined by ashing the sample in the presence of an ethanol solution of magnesium acetate, which decomposes easily upon heating, promoting the formation of a large number of pores in the yeast sample.

Fractionation of proteins by molecular weight for further purification was performed using electrophoresis according to the procedure of Laemmli. Electrophoretic fractionation is based on the different mobility of proteins in an external electric field. The sign and magnitude of the electrical charge of a protein molecule are determined by the amino acid composition of the protein, and the mobility of the protein molecule at a given pH, ionic strength, and the strength of the electric current is dependent on its molecular weight and shape of the molecule. If an external electric field is imposed on a protein solution, the charged protein molecules move towards the oppositely charged electrode with a speed proportional to their charge and inversely proportional to the size and the degree of hydration of the particles [10, 11].

Electrophoresis was performed in polyacrylamide gel (PAG) plates immersed in a chamber filled with electrode buffer (0.066 M Tris, 0.19 M glycine, and

0.1% sodium dodecyl sulfate). The solutions to be analyzed were loaded into every well of the gel prepared. Electrophoresis was started at a current of 40 ± 0.2 mA and subsequently the current was increased to 80 ± 0.2 mA. The postelectrophoretic washing and staining of the gel involved consecutive incubations with a fixing solution, a washing solution, and a staining solution; all incubations were performed at 80 ± 2°C for 10 minutes. The gels were then destained in distilled water at a temperature of 25 ± 2°C.

Electrophoregrams were analyzed using an UV transilluminator TCP-20M (Vilber Lourmat, the United States) at a wavelength of 312 nm and a Doc-It LS gel documenting system.

A commercial protein marker kit from SibEnzyme consisting of highly purified recombinant proteins of known molecular weight was used to calibrate the electrophoretic gels. Human serum albumin solutions with known concentrations were used to construct a calibration curve for protein quantification.

Protein concentration in the sample was calculated according to the formula:

$$C = (C_p \cdot C_f)/100, \quad (1)$$

where C_p is the total protein content in the sample, g/100 g and C_f is the relative content of the protein fraction in the total protein, g/100 g protein.

The enzyme activity of alcohol oxidase was determined spectrophotometrically by measuring the decrease of optical density at 340 nm. For this purpose, a precise volume of the liquid under investigation, acetic aldehyde, ethyl alcohol (used as substrate), and phosphate buffer necessary for maintaining the required active acidity of the reaction medium were added into a quartz cuvette using a pipette (dispenser). The changes in optical density were monitored during 1 min. The enzyme activity of alcohol oxidase was calculated according to the formula (2) and expressed as U/mg protein:

$$A = \frac{\Delta E_{340} \cdot 3 \cdot 10^9}{6.22 \cdot 10^6 \cdot t \cdot b}, \quad (2)$$

where ΔE_{340} is the optical density measured at a wavelength of 340 nm; 3 is the sample volume in the spectrophotometric cuvette, ml; 10^9 is the factor for conversion of mol to nanomol; $6.22 \cdot 10^6$ is the molar extinction coefficient; t is the duration of the enzyme activity assay, min; and b is the amount of protein in the sample, mg.

Microfiltration was used to eliminate foreign microflora from the concentrate of the alcohol oxidase preparation. The membranes used were from Merck Millipore and had a pore diameter of 0.22 μm. The filtration was performed at 19 ± 2°C and an underpressure of 0.1–0.3 MPa [12].

Ion exchange chromatography on columns packed with diethylaminoethyl cellulose (DEAE-cellulose) and carboxymethyl cellulose (CM-cellulose) was used to remove extraneous proteins reducing the specific activity of the enzyme from the concentrated alcohol oxidase preparation [13].

The adsorbents were conditioned for ion exchange chromatography according to the following procedure: a suspension of 10 g of adsorbent in dilute hydrochloric acid was prepared and incubated for 1 hour at a temperature of 25 ± 2°C. The adsorbent was subsequently washed with copious amounts of distilled water until the pH of the slurry reached a value of 4.0 ± 0.2. Transfer of the adsorbent to hydrochloric acid and washing with distilled water was repeated several times until a pH value of 7.0 ± 0.2 was reached. Five volumes of sodium hydroxide solution were then added to the sorbent; the suspension was incubated for 1 h at room temperature and rinsed with distilled water until neutral pH was attained.

A chromatographic column was packed with the conditioned ion exchanger and equilibrated with a buffer solution (pH 7.4).

The sample was loaded to the column after almost complete removal of excess eluent; the volume of the sample loaded equaled 3 ml and it was carefully pipetted onto the surface of the sorbent. After the sample was absorbed, the surface was washed twice with 3-ml aliquots of eluent. The elution rate equaled 12 ml/h.

RESULTS AND DISCUSSION

The selection of process parameters of microorganism cultivation in order to increase the yield of the desired product, enable easy separation of the product from the culture fluid and ballast substances, and improve stability during prolonged storage, is an important issue for biotechnological production of biologically active substances used in the manufacturing of functional foods, alcoholic beverages, and soft drinks [9]. Cultivation of the yeast *Candida boidinii* at various process parameters was investigated at the first stage of the present study in order to ensure the maximal yield of alcohol oxidase preparation capable of destroying lower aliphatic alcohols.

Yeast are chemoorganoheterotrophic organisms that use organic compounds as a source of both energy and carbon. They need oxygen to breathe; however, most species are capable of deriving energy from the process of fermentation in the absence of oxygen. Pasteur showed that the presence of oxygen in the culture medium stimulates the breathing of yeasts and the production of alcohol and carbonic acid, which in turn accelerate the growth of the yeast [14].

Yeast cells are quite demanding with regard to cultivation conditions and the composition of the culture medium. Raw materials of vegetable, animal, mineral, and chemical origin were used for the preparation of the yeast culture media. Carbohydrates (sucrose, lactose, and glucose) were used as the energy source in the present study, and the inorganic compound dibasic ammonium phosphate, as well as other ammonium salts, was used as the nitrogen source, notwithstanding the ability of the yeast to metabolize nitrates. The natural components of the culture medium were represented by molasses and yeast autolysate. All the components included in the nutrient medium for yeast cultivation were carefully examined for biochemical suitability. Dairy (cheese) whey was added to the

culture medium for *Candida boidinii* yeast as an additional source of organic and mineral nutrients.

Comparative analysis of quantitative and qualitative composition of organic and inorganic substances found in cheese whey showed that this by-product of dairy industry is not inferior to molasses and can be used as an additional supplement for the cultivation of *Candida boidinii* yeast. Active synthesis of essential amino acids, proteins, and enzymes during the growth of the yeast *Candida boidinii* was detected, and the qualitative and quantitative composition of these substances was shown to depend on the composition and type of culture medium and supplements used in the cultivation of microorganisms.

The duration of yeast cultivation reportedly depends on the mode of cultivation, especially on nutrient, water, and air feed rate, and ranges from 12 to 18 hours [8]. The present study involved the assessment of the effect of culture medium supplementation with whey on the duration of the process. The process was carried out at a temperature of (30 ± 2°C) in a medium with an active acidity of 4.5–5.5 and whey content of 60%. The results of the experiments are shown in Table 1.

Table 1. Kinetics of cultivation of yeast *Candida boidinii*

Parameter	Control (18 h)	Duration of the process, h				
		12	14	16	18	20
Biomass yield, g/g	0.57± ±0.02	0.87± ±0.05	0.93± ±0.05	1.05± ±0.06	1.31± ±0.07	1.20± ±0.06
Yeast biomass accumulation, g/l	18.70± ±1.10	26.98± ±1.62	28.13± ±1.68	31.28± ±1.87	37.30± ±2.24	34.47± ±2.07
Specific growth rate, h^{-1}	0.008± ±0.0006	0.011± ±0.0006	0.013± ±0.0007	0.015± ±0.0007	0.016± ±0.0008	0.016± ±0.0008
Specific activity of alcohol oxidase, U/mg protein	18.51± ±1.11	9.11± ±0.54	18.65± ±1.12	28.41± ±1.70	39.82± ±2.39	39.79± ±2.38
Composition of yeast biomass, %:						
protein content by mass	39.0±2.34	41.5±2.4	42.8±2.5	43.4±2.6	42.0±2.5	34.2±2.0
Ash content in dry matter,%	9.3±0.73	9.2±0.73	9.3±0.73	9.3±0.73	9.3±0.73	9.1±0.5

Most of the intracellular proteins and enzymes are less stable than the extracellular ones due to the absence of disulfide bonds; the reducing properties of the intracellular medium render these bonds unnecessary. Cell wall disruption with the maximal efficiency possible is necessary for enzyme extraction from the cells. A large number of cell wall disruption techniques are currently used in biotechnology. Yeast cell walls are known to have a high strength, and therefore a strong mechanical impact is required to disrupt them: however, destruction of the target products, i.e. the inactivation of the enzyme systems isolated, must be avoided.

Planetary ball mill PM 400 was used in the present study to destroy the cells of yeast *Candida boidinii*. Destruction of the cell walls in the mill is based on vibration of moving parts. The mechanical strength of yeast and bacterial cells is reportedly different, and therefore selection of the correct parameters for cell wall destruction is necessary. The degree of destruction of the cell walls depends on rotor speed, the duration of exposure to centrifugal force, and process temperature.

Figure 2 illustrates the dynamics of degradation of *Candida boidinii* yeast cells.

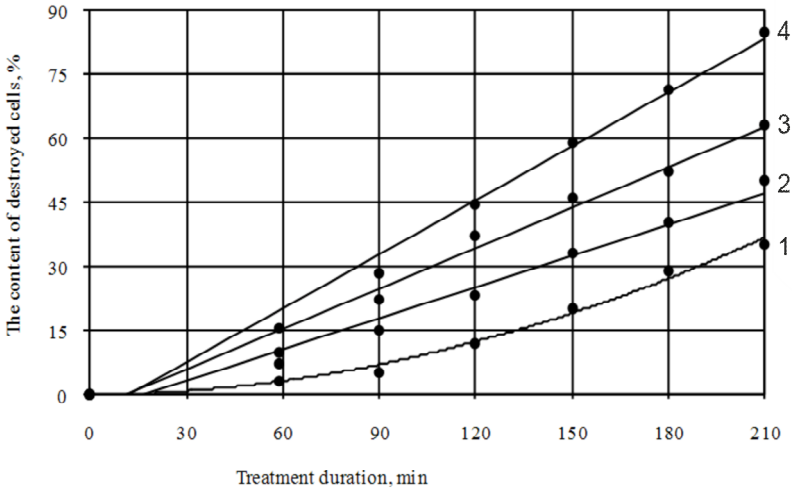

Fig. 2. Dynamics of degradation of yeast *Candida boidinii* cells during treatment with spherical glass beads of approximate size: 1 – 40 mesh; 2 – 60 mesh; 3 – 80 mesh; 4 – 100 mesh.

The use of approximately 60-mesh glass beads resulted in the disruption of 48% of *Candida boidinii* cells, while the use of approximately 80-mesh beads allowed for the increase of this value to 80%, and therefore treatment with approximately 80-mesh glass beads in a planetary mill PM 400 was chosen for cell disruption required for alcohol oxidase production from the yeast *Candida boidinii*.

The structure of the cells is altered and their integrity is lost during the treatment due to the effect of impact and friction forces, as well as Coriolis forces.

Precipitation by organic solvents miscible with water is widely used for the isolation of proteins from raw materials. Adding solvent to the protein extract leads to protein precipitation. The main reasons for this phenomenon are reduction of water activity, protein aggregation induced by the organic solvent, and protein precipitation due to the force of electrostatic attraction being inversely proportional to the dielectric constant of the medium.

The solvent used must be completely miscible with water and have sufficient precipitating capacity; besides, it must not become involved in reactions with proteins. Ethyl and isopropyl alcohols and acetone are most commonly used in protein chemistry for the precipitation of enzymes. Acetone was used in the present study because of its advantages over isopropanol and ethanol, namely, the possibility of using lower concentrations for protein precipitation at low temperatures and higher volatility enabling easy removal of the solvent from the dissolved precipitate under reduced pressure. Consequently, the denaturing effect of acetone is weaker. Dissolved protein precipitates contain residual solvent that must be removed. Removal of solvent and low molecular weight substan-ces is the most important method of concentrating dilute protein solutions; the procedure usually involves the use of semipermeable membranes. The two methods of protein chemistry employing such memb-ranes are dialysis and gel filtration; the former was used in the present study to remove acetone from an alcohol oxidase preparation. The use of special colloi-dal films with ultramicroscopic pores allowed for the retention of high molecular weight protein substances in the concentrate. Ultrafiltration using gas pressure to force the liquid through the membrane was the second method used to concentrate the protein solution. The use of this method resulted in the isolation of two fractions consisting of large macromolecules and smaller macromolecules, respectively. The protein solution was diluted by phosphate buffer several times during concentration because the rate of ultrafiltration decreased concomitantly to the increase of protein concentration in the solution [12, 13].

Microfiltration, which allows for very efficient elimination of suspensions, large colloids, the majority of bacteria and microorganisms, and viruses from solutions, was used to remove pathogenic micro-organisms from the protein concentrate. Microfiltration cartridges produced by Millipore (pore diameter 0.60, 0.40, and 0.22 μm) were used. Microbiological purity of the resulting protein concentrate was evaluated using culture in petri dishes [15, 16]. The results of the experiments are shown in Table 2.

Table 2. Results of microbiological analysis of alcohol oxidase preparations

Parameter	Standard	Pore diameter, μm		
		0.6	0.4	0.22
Coliform bacteria in 0.1g	not allowed	not found		
Pathogenic microorganisms, including *L. monocytogenes* and *Salmonella* spp. in 25.0 g	not allowed	not found		
St. aureus in 1.0 g	not allowed	not found		
Mesophilic aerobic, CFU/g, not more than	$5 \cdot 10^4$	$8 \cdot 10^6$	$9 \cdot 10^4$	$3 \cdot 10^2$
Facultative anaerobic microorganisms, CFU/g, not more than	$1 \cdot 10^4$	$6 \cdot 10^5$	$8 \cdot 10^3$	$0.7 \cdot 10^2$

Analysis of the microbiological purity of the protein concentrate obtained revealed the inefficiency of Millipore microfilters with pore diameter of 0.60 or 0.40 μm, since colonies of microorganisms developed in the samples when these filters were used. Using Millipore microfiltration elements with a pore diameter of 0.22 μm yielded a concentrated enzyme preparation conforming to the microbiological requirements of the State Pharmacopoeia.

Protein molecules are usually selectively adsorbed on solid phases of different types and therefore adsorption techniques, column chromatography in particular, are widely used for the separation of protein macromolecules. The use of these methods often results in the production of proteins having the highest degree of purification, which is equivalent to the maximum possible increase of specific activity in the case of enzymes. Ion exchangers, potassium phosphate (crystalline or in gel form), and a variety of affinity adsorbents designed for certain types of enzymes are the major protein adsorbents.

Two types of ion exchangers, namely, the cation exchanger carboxymethyl cellulose and the anion exchanger diethylaminoethyl cellulose, are used in protein chemistry. DEAE-cellulose, which is used most frequently, has a complex structure and a complex titration curve. The use of ion exchange chromatography on DEAE cellulose allows for enzyme

purification under mild conditions and the preservation of the properties of the native protein.

Electrophoretic analysis of the fractions revealed the presence of a single band with a molecular weight corresponding to that of alcohol oxidase. The enzyme preparation is subsequently immobilized on an edible carrier, usually a polymer, and can be used in the technology of functional foods.

Thus, the process of cultivation of *Candida boidinii* yeast in a nutrient medium supplemented with cheese whey has been investigated in order to increase the activity of yeast enzyme systems. The following cultivation parameters were found to be optimal for *Candida boidinii* yeast: whey concentration 60%, temperature 30 ± 2°C, pH 4.5–5.5, and cultivation time not less than 18 ± 0.5 h. The technology of isolation and purification of alcohol oxidase from *Candida boidinii* yeast for further use of this enzyme in functional food biotechnology has been developed. Destruction of cell walls of *Candida boidinii* yeast in a PM 400 planetary mill filled with glass beads of approximately 80 mesh in size and enzyme precipitation by a nonpolar solvent were shown to be the optimal parameters of enzyme isolation. The optimum parameters for enzyme purification aimed at increasing the catalytic activity comprised the use of a membrane UPM-67 followed by filtration through a Millipore membrane (pore diameter 0.22 μm), and column chromatography on CM-cellulose and DEAE-cellulose.

REFERENCES

1. Report on alcohol and health 2010. World Health Organization. URL: http://www.euro.who.int/__data/assets/pdf_file/0004/128065/e94533.pdf
2. *Sotsial'noe polozhenie i uroven' zhizni naseleniya Rossii: Stat. Sb.* (Social Situation and Living Standards of the Popualtion of Russia: Stat. Comp.) (Rosstat, Moscow, 1997–2010).
3. Krevyakina, E.V., Medvedev: «V bednoy strane p'yanstvo ne pobedit'» (Medvedev: “A poor country cannot defeat alcoholism”), *Komsomol’skaya Pravda*, August 09, 2009.
4. Pleteneva, T. V., *Toksikologicheskaya khimiya* (Toxicological Chemistry) (GEOTAR Media, Moscow, 2005).
5. Pavlishko, H., Gayda, H., and Gonchar, M., Alcohol oxidase and its bioanalytical application, *Vysnyk of L'viv Univ. Biology series*, 2004. № 35. P. 3.
6. Rumyantsev, A.P., Tiunov, L.V., and Ostroumova, I.A., Metabolizm organicheskikh soedinenii opiinogo ryada (Metabolism of a range of organic opioid compounds), *Itogi nauki i tekhniki* (Achievements in Science and Technology) (VINITI, Moscow, 1981). V. 12. P. 65.
7. Gonchar, M., Metabolically engineered methylotrophic yeast cells and enzymes as sensor biorecognition elements, *FEMS Yeast Research*, 2002. V. 2. P. 307.
8. Ashin, V.V. and Trotsenko, Yu.A., Alcohol oxidase of the methylotrophic yeasts: new findings, *J. Mol. Catal. B: Enzym.*, 2000. V. 10. № 1-3. P. 295.
9. *Yeast Biotechnology: Diversity and Applications*, ed. by Satyanarayana, T. and Kunze, G. (2009).
10. Prosekov, A.Yu, Babich, O.O., and Sukhikh, S.A., *Sovremennye metody issledovaniya syr'ya i biotekhnologicheskoi produktsii: Laboratornyi praktikum* (Modern Methods of Analysis of Raw Materials and Biotechnology Products: A Laboratory Guide) (Kemerovo Technological Institute of Food Industry, Kemerovo, 2012).
11. Shugalei, I.V., Garabadzhiu, A.V., and Tselinskii, I.V., *Khimiya belka: uchebnoe posobie* (Study Materials in Protein Chemistry) (Prospekt Nauki, St. Petersburg, 2011).
12. Scopes R, *Metody ochistki belkov* (Protein Purification Techniques), Moscow, Mir, 1985.
13. Osterman, L.A., *Khromatografiya belkov i nukleinovykh kislot* (Chromatography of Proteins and Nucleic Acids) (Nauka, Moscow, 1985).
14. Bystrykh, L.V., Dvorokova, J., and Volfova, O. Alcohol oxidase of methylotrophic thermo- and acidotolerant yeast Hansenula sp., *Folia Microbol*., 1989. V. 34. № 1. P. 233.
15. Plakunov, V.K. *Osnovy enzimologii* (Fundamentals of Enzymology) (Logos, Moscow, 2001).
16. Mizgunova, U.M., Shekhovtsova, T.N., and Dolmanova, I.F., Fermentnye metody opredeleniya alifaticheskikh spirtov (Enzymatic methods for the determination of aliphatic alcohols), *J. Anal. Chem. (Moscow)*, 1998. V. 53. № 10. P. 1014.
17. Azevedo, A.M., Prazeres, D.M., Cabral, J.M., and Fonseca, L.P. Ethanol biosensors based on alcohol oxidase, *Biosens Bioelectron.*, 2006. V. 21. № 12. P. 2306.

THEORETICAL AND PRACTICAL ASPECTS OF THE DEVELOPMENT OF A BALANCED LIPID COMPLEX OF FAT COMPOSITIONS

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Abstract: In the paper, the effect of the lipid complex in fats and oils on human organism and their role in physiology of nutrition are reviewed. Aspects of impairment of the nutritional status upon fat consumption, in particular, the excess consumption of saturated fats, *trans*-isomers of fatty acids, and cholesterol and the deficiency of polyunsaturated fatty acids and phospholipids, are discussed. Data on oil and fat in modern structure of nutrition are described. Aspects of the development of balanced fat compositions, accounting for normal physiological needs of modern people for lipids and their structural components, are reviewed. Data on the construction of fatty bases from milk fat, natural, and modified vegetable oils and fats providing for predetermined consumer properties of functional fatty milk products are presented.

Keywords: fat consumption; nutritional status; saturated, monounsaturated, and polyunsaturated fatty acids; balanced fat compositions; development of fatty bases

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INTRODUCTION

Development of new technologies in food industry and creation of a wide range of qualitatively new products with controlled modification of the chemical composition and properties is an important direction of modern nutritiology aiding the improvement of nutrition and preservation of health of the population.

Purposeful modification of fatty acid composition of lipid fraction is one of the main conditions in design of balanced food product compositions to approach the optimal ratio between the saturated, monounsaturated, and polyunsaturated fatty acids.

The concept of the development of an assortment of dairy products with increased content of milk fat identifies a number of directions, among which preferable are the directed regulation of fatty acid composition of the product by means of introduction of vegetable oils or compositions of non-milky fats, decrease of the calorific index due to the change of the ratio between fat and non-fat components for the benefit of the latter ones and allowance and feasibility of application of fatty bases improving agents, including the structure stabilizers, antioxidants, etc., for which the main principles of selection are oriented at the group of compounds of natural origin possessing maximal functionality.

The area of combining milk and plant raw materials seems promising for the development of qualitatively new dairy products of modified composition and properties. This methodology provides for a potential possibility of mutual enrichment of the products' ingredients with one or several essential factors and allows for the development of balanced-composition products, including products developed for specific needs.

Therefore, optimization of the composition and properties for the development of products that meet the formula of balanced nutrition the most predetermines the directions of new technology advancement. Design of the composition of products and diets in adherence with the requirements on balance of fatty acid, amino acid, mineral, and vitamin composition is the subject of priority in scientific and applied research [3].

THEORETICAL ASPECTS OF THE DEVELOPMENT OF A BALANCED LIPID COMPLEX OF FAT COMPOSITIONS

When characterizing the lipid component of milk fat, it should be noted that the question of modification of its fatty acid composition is a timely one. Special attention is paid to the development of new types of fat products using fatty compositions of milk fat with vegetable oils and fats.

Earlier studies on the increase of food and biological value of milk fat provided ground for the development of a new group of products, that is, spreads with modified fat phase, including milk fat and compositions of non-milk fats. Selection of the components of fat phase should be performed according to scientific principles, which are based on the requirement of preservation of the nutritive value of milk products and their organoleptic parameters with possible correction of the negative properties of milk fat. Rational combining of several sources of lipids is important from the economical point of view since it allows the producers to minimize the costs spent on raw materials and decrease the dependence of the production on seasonal fluctuations of milk input.

The need for a comprehensive evaluation of nutritive value of fats is connected with the fact that they hold an important place in human diet.

Considerable amount of research is dedicated to the investigation of the multifaceted role of lipids in vital processes [3].

According to the modern concepts, food fats are not only a concentrated form of energy, exceeding the energy content of all other food substances (oxidation of 1 g of fat in the organism yields 37.66 kJ or 9 kcal), but also a carrier of essential nutrition factors. Lipids are cell components playing an important role in membrane organization; they are solvents for vitamins A and D providing for their consumption; finally, they influence the intensity of protein and carbohydrate metabolism. A number of biologically active compounds enter the organism together with the fats; these include polyunsaturated fatty acids, phospholipids, sterols, fat-soluble vitamins, and other compounds possessing targeted functional effects. The role of lipids as a material for structural organization, first of all, the obligatory elements of biological membranes and the associated biosynthesis of physiologically active peroxidized lipids, by its importance has been compared by the scientists with the plastic function of proteins. This justified the establishment of the plastic function of lipids in the organism. While all fatty acids fit for utilization as an energy source, a specialized set of fatty acids is required for the plastic processes. The fraction of polyunsaturated acids with 20–22 carbon atoms, the most important representative of which is the arachidonic acid $C_{20:4}$, is well represented in the structure of cell membrane. These acids arrive in small amounts with food and are products of biosynthesis from their elementary precursors, linoleic $C_{18:2}$ and linolenic $C_{18:3}$ acids, in the organism. A smaller fraction of membrane lipids is represented by the products of metabolism of oleic acid $C_{18:1}$ [4].

Fat improves the taste properties of food and increases its nutritive value. Insufficient fat supply may lead to a number of disorders of the central nervous system, weakening of the immunobiological mechanisms, and other negative consequences. Excess fat consumption, especially, the animal fat, is unadvisable and leads to the risk of obesity, cardiovascular, and other diseases. A correlation between excess consumption of animal fat components and atherosclerosis progression has been established [2, 4].

Biological efficiency of lipids is determined on one hand by the structural characteristics of fatty acids, and on the other, by the ratio and content of components of various nature and functionality in fat.

A special role in fat composition belongs to essential polyunsaturated fatty acids, linoleic $C_{18:2}$, linolenic $C_{18:3}$, and arachidonic $C_{20:4}$. These fatty acids, as well as some amino acids of proteins, are referred to as essential and are not synthesized in the organism; the requirement for them may be satisfied only with food. Arachidonic acid is synthesized from linoleic acid with the involvement of pyridoxine (vitamin B6) and tocopherols. Tocopherol not only helps the transformation of linoleic acid to arachidonic, but also activates it. By their biological properties, these polyunsaturated fatty acids are referred to the vitally important nutrients, and thus are being proposed as a vitamin F complex [4].

The most important biological property of polyunsaturated fatty acids (PUFA) is their participation as structural elements in highly active biological complexes, such as phospholipids, lipoproteins, etc. PUFAs are an essential element of the myelin sheath of the connective tissue. In the organisms, hormone-like organic compounds prostaglandins, which are involved in regulation of intracellular metabolism, blood pressure, and platelet aggregation, are synthesized from arachidonic acid and influence smooth musculature and vitally important secretory functions [8]. Connection between PUFAs and cholesterol exchange manifested in the ability to increase cholesterol clearance from the organism by transformation into labile, easily soluble forms thus preventing and alleviating atherosclerosis has been established. In the absence or lack of PUFAs, cholesterol is considered to form esters with saturated acids, which are hardly oxidized in the metabolism and, due to their chemical stability, accumulate in blood and are deposited on artery walls. Essential acids, when in sufficient amounts, form esters with cholesterol that are oxidized to low molecular weight compounds in the course of the metabolism and are easily eliminated from the organism [2, 10].

In this connection, there is a need for increased PUFAs in the diet to prevent cardiovascular and other diseases. Besides, PUFAs increase the organism resistance to infections. The effect consists in suppression of pathogenic microorganisms due to replacement of bacterial lipids with PUFAs.

PUFAs produce normalizing effect on blood vessel walls, increase their elasticity, decrease permeability, participate in the exchange of vitamins of group B (pyridoxine, thiamine) and choline, which, under conditions of lack of PUFAs, decreases or looses completely its lipotropic properties. Data on stimulatory role of PUFAs in protective functions of the organism, in particular, increase in the stability of infectious diseases and γ-radiation effects, were obtained. All these functions are performed only by *cis*-isomers of PUFAs.

Vegetable oils are among the most PUFA-rich food products with linoleic acid contents reaching 50–60%; spreads contain up to 20% PUFAs, and animal fats, 3–5%.

In terms of biological activity, linolenic acid is 9-fold less active than linoleic acid. According to some reports, linolenic acid is transformed to arachidonic acid only partially in the organism. It occurs in vegetable oils in comparatively small amount, except for linseed (60%), hempseed (18%), soybean (10%), and rapeseed (9%) oils [6].

Oleic acid does not possess the activities of the essential acids, but enhances the effects of linoleic acid acting as its synergist.

The greatest biological effect is produced by arachidonic acid. It is not contained in plant products and in animal fats its content is insignificant: 0.25% in dairy butter, 0.6% in beef fat, and 2.1% in pork fat. However, as indicated above, arachidonic acid may be

formed from linoleic acid thus satisfying the organism's need for the essential fatty acids.

For quantitative evaluation of how well fatty acid composition of food fats matches the organism's requirements, a parameter termed coefficient of metabolization efficiency (CME) of essential fatty acids was introduced. Violation of the optimal proportions of fatty acids in the diet or a specific metabolic situation in the organism may cause replacement of arachidonic acid by other PUFAs with 20–22 carbon atoms, which are products of metabolism of oleic and linolenic acids. These modifications cause changes of the CME value, which is the ratio of arachidonic acid fraction in cell membrane lipids composition to the sum of fractions of other 20–22 carbon atom PUFAs. CME is in a tight correlative bond with an integral parameter of the efficiency of plastic processes—gain of body wait of rapidly growing organisms,—as well as multiple parameters of membrane functional state. Unsaturated fatty acids with the double bond position between the third and the fourth carbon atoms, ω-3 fatty acids (linolenic, eicosapentaenoic, and docosahexaenoic acids), have been established to be the most effective ingredients of the group. Fatty acids with double bond position between the sixth and the seventh carbon atoms (numeration starts from carbon atom of the methyl group) belong to the ω-6 family and include linoleic, γ-linolenic, and arachidonic acids.

The recommended ω-6/ω-3 ratio in the diet of a healthy man is 10 : 1, and in a dietotherapy, from 3 : 1 to 5 : 1 [5].

Fatty acids of the ω-6 family—eicosapentaenoic and docosahexaenoic—are present in the lipid complex of fish and marine animals. The exclusion of PUFAs (linoleic, linolenic, and arachidonic) from animal and human diet leads to impairment of vital processes: retardation of growth and development, dermatitis, liver and kidney disorders, changes in cell membranes and their properties, declination of the reproductive function, etc. In the course of detalization of the issue, it was noted that only linoleic and arachidonic acids possess the high level of physiological activity. Linolenic acid, initially considered essential, is only a substituent for linoleic acid in case the latter one is absent. In the case of the presence of both acids, their transformations in the organism compete with each other due to the commonness of the enzymatic systems responsible for their transformations, which ultimately decreases the biological efficiency of fat. Taking into account that arachidonic acid is synthesized from linoleic acid by the organism, linoleic acid is the very essential polyunsaturated fatty acid.

Multiple studies are focused on the establishment of the sufficient level of linoleic acid in the organism. The level has been determined as 5–8% of calorific value of the diet, which makes 8–10 g linoleic acid per day on average (in individual cases linoleic acid content may be increased to 20 g). For children, the values of physiological needs in fatty acids of the ω-6 and ω-3 groups are 4–12 and 1–2% to the calorific value of a daily ration, respectively [5].

Excess consumption of linoleic acid by the organism is not desirable, which is due to its high proneness to oxidation and the ability to form free radicals, hampering normal course of the metabolic processes in the organism.

Previously, the data on linoleic acid content in this or that fatty product were the main characteristics of its biological properties. The results of the studies performed in the recent decades proved that not only the absolute amount of linoleic acid, but also its combination with other acids is considerably important upon determination of the efficiency of food fats. The ratio between linoleic and linolenic acids in an ideal fat should be close to 10 : 1.

The next step on the way to elucidation of the effect of fatty acid composition of food fats on the nature of their biological effects was the establishment of the physiological role of isomers of unsaturated acids. The question on the nature of the biological effect of fatty acid isomers has a practical importance, since *trans*-isomers of monoene and diene acids are present in many natural and modified fats. For example, the presence of up to 40% *trans*-isomers in the total amount of linoleic acid in milk fat makes the product an even more insignificant nutrition factor. Modification of the spatial configuration of fatty acids comprising triglycerides occurs under the effect of a number of factors: high temperature, effect of catalysts, etc. In this connection, modified vegetable oils and fats contain various amounts of *trans*-isomerized fatty acids [11].

Most authors consider hydrogenated fats possess lower nutritive and biological value than the intact vegetable oils. In the course of hydrogenation, *cis/trans*-isomerization of unsaturated acids is possible, which makes linoleic and linolenic acids physiologically inactive. While *trans-cis* or *cis-trans* linoleic acid possesses lower biological activity, *trans-trans* linoleic acid completely loses it and is not converted to arachidonic acid, which may damage biomembrane structure and prostaglandin synthesis.

Studies on the metabolic fate of *trans*-isomers of monoene and polyene fatty acids demonstrated considerable differences in the amount of *cis*- and *trans*-forms incorporated into tissue lipids of the organism. It was found that the more specialized are the membrane structures, the less is the fraction of *trans*-isomers in them. No considerable increase in their incorporation in membrane lipids occurs upon the increase in the content of *trans*-isomers in diet, which evidences the presence of some limiting factors. The effect of spatial configuration of fatty acids on the level of activation of the enzymes involved in cholesterol esterification and in the processes of dehydrogenation and chain elongation of fatty acids was noted. In this connection, the content of *trans*-isomers of fatty acids in food fats and products is regulated [2, 11].

The nature of saturated acids contained in fat products can also considerably affect the nature of biological effect of the fat. In this connection, fatty acids with intermediate carbon chain length—caprylic $C_{8:0}$, capric $C_{10:2}$, lauric $C_{12:0}$, and myristic $C_{14:0}$ acids—should be pointed out.

These fatty acids are present in sufficient amounts only in milk fat and coconut butter. Coconut butter

served source for preparation of new types of fat products, investigation of which showed that they are consumed in the digestive tract without the involvement of fatty acids and pancreatic lipase. Upon entering the internal medium of the organism, they are not deposited but rather subjected to β-oxidation. Transformation of intermediate chain length fatty acids produces a pronounced effect on biosynthesis of exogenous fatty acids and cholesterol. The indicated specific features of metabolization of intermediate chain length fatty acids provided ground for the attempts to use them in dietary correction of a number of lipid exchange disorders. Their content in usual diets also cannot be ignored upon evaluation of biological properties of fat components of food.

Animal fats may contain saturated fatty acids with chain length of twenty and over carbon atoms, they are characterized by solid texture and high melting temperature. The fats include lamb, beef, pork fats, etc. High consumption of saturated fatty acids is a risk factor in the development of diabetes, obesity, cardiovascular, and other diseases.

Saturated fatty acid consumption for adults and children should make not more than 10% to the calorific value of daily diet.

Taking as an axiom the thesis that lipids are absorbed at the minimal level for each of the fractions (that is, if $C_{i1} < C_{i2} < C_{i3}$, then all fatty acid fractions are absorbed at the level C_{i1}, and the excess of each fraction equal to $(C_{i2} – C_{i1})$ and $(C_{i3} – C_{i1})$ is deposited in the organism or spent on energy needs), lipid utilization coefficient is calculated similar to that of proteins. For a proposed arbitrary etalon, $C_{i1} = C_{i2} = C_{i3} = 1$ and the coefficient of utilization is also equal to 1. Although the fatty acid formula is the important characteristics of fat products, it is not a comprehensive one. Such constituents of lipocomplex as phospholipids, sterols, and fat-soluble vitamins also produce a pronounced effect on the nature of biological effects of food fat [3].

None of the fats individually can completely provide for the organism's need for nutrients. Animal fats, including milk fat, contain vitamins A and D, as well as lecithin, possessing a lipotropic effect. However, they contain little essential PUFAs and cholesterol. Vegetable oils contain enough PUFAs and tocopherols (vitamin E). The presence of β-sitosterol, promoting normalization of cholesterol exchange in the organisms, and insignificant amounts of vitamins A and D have been noted in them.

According to the modern perception, the most appropriate is the consumption of a balanced composition of fats with each meal rather than fat products of different composition throughout the day.

An important group of lipids are the phospholipids. Being part of cell membranes, they play a considerable role in membrane permeability and exchange of compounds between the cells and intercellular space, promoting better absorption of fats and preventing liver fattening, exhibiting pronounced lipotropic effect, and preventing cholesterol accumulation in the organism enhancing its elimination [2, 10].

The highest content of phospholipids is noted in unrefined vegetable oil, 1–2% (up to 6% in soybean oil). The content of phospholipids of 5–7 g/day is considered optimal in the diet of an adult.

The physiological role of cholesterol contained in the products of animal origin should also be noted. Cholesterol is a precursor in biosynthesis of vitamin D and a number of hormones and is involved in bile acids exchange. At the same time, it is known that high blood level of cholesterol is known to be a factor of atherosclerosis development. Approximately 80% of cholesterol is formed in the organism, and we receive 20% with food. The amount of cholesterol in a daily ration of adults and children should not exceed 300 mg. The same amount of vegetable sterols (phytosterols) is required [7].

Together with the large number of works confirming the positive effects of PUFAs on human body and firstly their anti-atherosclerotic effects, there are studies proving negative effect of diets in which fat composition is based exclusively on vegetable oil. Vegetable oil contains little acids with short or intermediate chains and many polyunsaturated acids that may cause negative effects due to formation of excess peroxides. None of the vegetable oils contains cholesterol, which is required for a growing organism for the development of immune system to protect the organism from a number of diseases in the following life.

The presented characteristics of the main components of fats evidence that man equally needs animal and vegetable fats. In this connection, the design of biologically comprehensive combined fat products is timely and practically important.

PRACTICAL ASPECTS OF THE DEVELOPMENT OF A BALANCED LIPID COMPLEX OF FAT COMPOSITIONS

The following ratio of balanced fatty acids may be considered a biologically optimal formula: 10–20% polyunsaturated fatty acids (PUFA), 30–40% saturated fatty acids (SFA), and 50–60% monounsaturated fatty acids (MUFA). On average, this is provided by the ratio of 50% animal and 50% vegetable fat in the diet.

The presented ratio between the lipid fractions depends considerably on the target designation of the developed fatty composition and may change in a defined range. For dietary nutrition of people with impaired fat exchange and atherosclerosis patients, fats with increased content of linoleic acid (at least 40%) and the ratio between saturated and unsaturated fatty acids of 1 : 2 are needed.

Among the diversity of food fats, milk fat is the most valuable by its biological and dietary properties. It is absorbed by human organism better than any other fat. This is promoted by a rather low fat melting temperature (28–33°C), as well as its being presented in a finely dispersed phase in milk. Biological value of milk fat is increased by the presence of considerable amount of phosphatides (up to 400 mg %) and tocopherols (2–5 mg %), arachidonic acids, and vitamins A and D in it. Due to its good organoleptic properties and favorable compatibility with other food products, milk fat has a rather wide range of applications.

In recent years, a tendency of decreased milk fat

consumption has been observed, which is in connection with its critics as of a factor increasing blood levels of cholesterol. Therefore, increasing attention is paid to the problems of milk fat composition correction to get the required ratio between the fatty acids.

Saturated acids dominate the fatty acids involved in formation of glycerides of milk fat, with saturated acid content in summer period being 62.9–67.3% and in winter period, 65.9–75.9%, and low molecular weight saturated acid content being 5.6–7.6% and 7.61–10.8%, respectively.

The content of unsaturated fatty acids in milk fat of the summer period is within the range from 33.1 to 36% and in the winter period, from 18.6 to 27.9%. The amount of polyunsaturated essential fatty acids in the milk fat in summer is 3.9–6.5%, and in winter, 2.9–3.8% [1]. Contents of these fatty acids do not meet modern medical and biological requirements of balanced nutrition.

Daily need for such vitally important compounds as phospholipids and PUFA exceeds considerably their content in consumed amounts of milk and dairy products. In these connection, to increase the nutritive value of these products, mass fraction of phospholipids and PUFAs in them should be increased.

Regulation of the fatty acid composition of milk products may be performed in three ways:

1) introduction of special feed supplements with high content of PUFAs to cow nutrition;
2) chemical modification of milk fat, with fractionation and re-esterification being the most common methods; and
3) partial substitution of milk fat with fat of a different origin.

The first two directions did not find wide application due to their low efficiency. In the first case, there is a need to correct the amount of feed supplements introduced in accordance with seasonal and regional factors, as well as the breed, age, and lactation stage of the animals. Also, it has been found that unsaturated fatty acids may pass to milk in an unchanged form, therefore, fat of such milk differs from the normal milk fat. Products produced from the modified milk have a more pronounced tendency to separation of a liquid phase at room temperature than the products prepared from normal milk [1].

In the case of correction of fatty acid composition of milk fat by its fractionation, there is a need to find rational use for solid fractions, which leads to increased product cost.

The method of milk fat composition and properties modification by mixing with fats of non-animal origin found the widest appreciation. Many years of studies on the chemical composition and physicochemical properties of food fat and lipocomplex of dairy products allowed to prove theoretically the possibility to use fat of non-animal origin in production of fat-containing dairy products with partial substitution of milk fat with vegetable oils, modified fats, or mixtures thereof taking into account the formula of balanced fatty acid composition. This method is available and convenient for use under industrial settings.

To regulate the fatty acid composition of dairy products, various vegetable oils and fats are used, including sunflower-seed, soybean, rapeseed, maize, palm, and other oils and mixtures thereof. A specially selected mixture of modified fats and oils may be used.

By the content of PUFAs, vegetable oils are divided into three main groups:

– with very high PUFA content (80% and more to the total content of fatty acids), linseed and hempseed oils;
– with high PUFA content (40–60%), sunflower-seed, soybean, cotton, and maize oils;
– with low PUFA content, but high content of oleic acid (80% and more), olive, earth-nut, and almond oils.

Fatty acid composition is an important characteristics of fat products, and such lipid fractions as phospholipids, sterols, and fat-soluble vitamins determine the biological effect of food fat.

Phospholipids are an important part of vegetable oils. The highest content of phospholipids is present in soybean (6%), cotton (up to 2.5%), sunflower-seed (up to 1.5%), and maize (up to 1.5 mg %) oils. High content of phospholipids is noted in unrefined oils. In the process of refining, vegetable oils get completely free of phospholipids [6].

The third biologically active component of vegetable oil is sterols, the best known of which is β-sitosterol. It normalizes cholesterol exchange forming insoluble complexes with cholesterol that prevent cholesterol absorption. By the content of sterols, vegetable oils are divided into the following groups:

– with very high content of sterols (1000 mg/100 g and more), wheat germ and maize oils;
– with high content of sterols (300 mg/100 g and more), sunflower-seed, soybean, rapeseed, linseed, and olive oils;
– with intermediate content of sterols (200 mg/100 g and more), earth-nut and cacao oils;
– with low content of sterols (60 mg/100 g), palm and coconut oils.

Considerable part of sterols is lost upon refining, which is why refined deodorized oils contain less sterols. Vegetable oils are completely free from cholesterol.

The fourth group of biologically active compounds present in vegetable oils is fat-soluble vitamins: tocopherols and carotenoids. High content (100 mg/100 g and more) of tocopherols is typical of wheat germ, soybean, and maize oil; sunflower-seed, cotton, and rapeseed oils are characterized by somewhat lower content of tocopherols (60 mg/100 g and more); insignificant amount of tocopherols is contained in olive and coconut oil.

Due to the absence of water and mineral substances, vegetable oils are not affected by microorganisms. During storage, only chemical changes occur with oxidation being the most important. Self-oxidation happens upon product contact with oxygen contained in the air, and the effect of oxygen targets the unsaturated double bond of fatty acids. In the process of the reaction, lipids, fatty acids, fat-like compounds, and vitamins are destroyed. Decay products with specific taste and smell that may be toxic are formed. The oxidation processes are catalyzed by

enzymes, heavy metal ions, light, and heat [3]. In this connection, to prevent oxidative destruction of fat products it is reasonable to add antioxidants–or substances that enter the self-oxidation process with the formation of stable intermediates, thus blocking the chain reaction of oxidation. Intact vegetable oils contain certain amount of natural antioxidants, among which tocopherols (vitamin E) and carotenoids play the most important role. However, upon refining and deodoration, vegetable oils are freed from their natural protective properties.

Analysis of composition and properties of fat raw materials (Table 1) allows for a conclusion that in nature there are no fats and oils that would completely meet the requirements of a hypothetically ideal fat. Liquid vegetable oils are rich in polyunsaturated fatty acids, but they lack saturated acids, the content of which is rather high in milk fat. Therefore, combining milk fat with vegetable oils and fats of this or that group provides for a possibility to bring the fatty acid composition of a product closer to the hypothetically ideal fat. Upon correction of the optimal ratio of milk fat–vegetable oil, it is important to take into account not only the structural and rheological characteristics of the product, but the medical and biological requirements on consumption of this or that essential acid as well.

Table 1. Fatty acid composition of fats and oils

Product	Fatty acids		
	saturated	monounsaturated	polyunsaturated
Milk fat	67.7	28.6	3.7
Sunflower-seed oil	10.1	26.8	63.1
Soybean oil	14.7	20.9	61.2
Rapeseed oil low erucidic acid high erucidic acid	 7.0 3.1	 59.1 73.4	 33.9 23.5
Olive oil	12.5	70.9	16.6
Palm oil	50.0	39.0	11.0
Hydrogenated fat	13.0	82.0	5.0
Re-esterified fat	27.0	50.0	23.0
Palm olein	35	53.5	11.5

Based on the facts presented above, we attempted to model the lipid constituent of fatty bases which would maximally resemble the hypothetic ideal fat with the following fatty acid composition: 30% saturated fatty acids, 60% monounsaturated acids, and 10% polyunsaturated fatty acids. Not only the amount, but also qualitative composition should be taken into account. An obligatory controlled parameter for a fatty base under development is the content of *trans*-isomerized acids.

As found in practice, in order to regulate their fatty acid composition it is reasonable to formulate binary and multicomponent compositions in two stages: determination of optimal content of the ingredients and evaluation of the efficiency of lipid constituent of the designed composition.

At the first stage of practical studies, binary mixtures of milk fat and liquid vegetable oil of various fatty acid groups are discussed as raw components to design fatty bases for optimization of their fatty acid composition.

Table 2 presents the physicochemical parameters of fatty bases prepared from milk fat and liquid vegetable oils [4].

In the composition, oils of various fatty acid groups were used, including the linoleic–linolenic (soybean oil), linoleic–oleic (sunflower-seed oil), oleic–palmic (olive oil), and erucidic (rapeseed oil) groups. The fraction of vegetable oil in the binary composition varied from 5 to 25%. Alteration of the quantitative ratio of milk fat–vegetable oil changes the fatty base characteristics, which ultimately determines the designation and area of application of the products.

Introduction of 20–25% vegetable oil into a composition allows for production of a fatty base characterized by a rather soft texture with firmness of 25–42 g/cm in function of the oil used. The base comprising partially hydrogenated oils possessed the highest firmness value. Decrease in the fraction of vegetable oil to 5–10% allows to produce a product of dense texture with firmness of 80–98 g/cm. The best qualitative characteristics are typical of compositions of milk fat with sunflower-seed, rapeseed, and partially hydrogenated vegetable oils [4].

Analysis of physicochemical parameters of a fatty base comprising milk fat and a liquid vegetable oil demonstrated the impossibility of preparation of a product with balanced composition and the required melting temperature and firmness indicated in Table 2.

Introduction of a vegetable oil in the amount of 10–15% allows for bringing the product fatty acid composition closer to a hypothetically ideal by only one set of acids, that is, polyunsaturated acids, while the content of saturated and monounsaturated acids is insignificantly modified.

Therefore, binary fatty compositions do not ensure the desirable ratio of the controlled output parameters and do not allow for the design of a product with the necessary range of parameters and of predetermined composition and quality.

This fact implies the need for the development of a fatty composition that would take into account two issues (raw material- and technology-related) and ensure a reasonable compromise in the design of a combined fatty composition.

Table 2. Physicochemical parameters of fatty bases from milk fat and liquid vegetable oils

Milk fat fraction, %	Vegetable oil fraction,%	Parameters of the fatty base of a combined oil				
		Melting temperature, °C	Firmness, g/cm	Fatty acid composition		
				SFA	MUFA	PUFA
With sunflower seed oil						
95	5	30.9	93	64.9	28.5	6.6
90	10	29.8	77	62.1	28.3	9.6
85	15	28.7	61	59.3	28.1	12.6
80	20	27.6	45	56.5	27.9	15.6
75	25	26.5	30	53.7	27.8	18.5
With rapeseed oil						
95	5	31.2	96	64.6	30.2	5.2
90	10	30.2	81	61.6	31.7	6.7
85	15	29.4	67	58.6	33.2	8.2
80	20	28.6	52	55.5	34.8	9.7
75	25	27.8	37	52.5	36.3	11.2
With soybean oil						
95	5	30.8	90	65.1	28.2	6.7
90	10	29.6	74	62.4	27.8	9.8
85	15	28.4	57	59.7	27.5	12.8
80	20	27.2	41	57.1	27.1	15.8
75	25	26.0	25	54.4	26.9	18.7
With olive oil						
95	5	31.2	96	64.5	31.2	4.3
90	10	30.4	82	62.2	32.8	5.0
85	15	29.6	68	59.4	35.0	5.6
80	20	28.7	53	56.6	37.1	6.3
75	25	28.1	39	53.9	39.2	6.9

At the next stage, study on the design of fatty bases comprising milk fat and natural and modified solid vegetable fats and oils was performed.

The main constituents of combined fatty phases are the milk fat, natural and modified vegetable oils, and fats, physicochemical and rheological properties of which determine directly the properties of the final product. Varying the ratio of the components one may obtain a wide range of combined fat phases with required properties.

In the world practice, a wide array of dairy products has been developed and studied using solidified modified fats obtained in the process of hydrogenation, re-esterification, and fractionation. The development and assimilation of production of products with combined fatty phase, on one hand, promotes the realization of the requirements of balanced nutrition with respect to fatty acid composition, and on the other, is promising from the positions of decrease of the production raw material capacity. Important are the studies on composition and properties of natural and modified fats and oils.

Taking into account the requirements on limitation of the content of *trans*-isomers in fatty products, quantitative determination of fatty acids with *trans*-configuration is needed in frames of the studies of fatty acid composition of modified fats. This is essential for the establishment of the regulated quantity of hydrogenated fatty source used in the receipt composition of combined products.

Therefore, the mass fraction of *trans*-isomerized fatty acids in the starting raw materials (hydrogenated, hydro-re-esterified, and re-esterified fats) determines the specific features of the fatty base design, taking into account the medical and biological requirements for the content of lipocomplex constituents in the final product.

In selection of fat constituents for a fatty phase, one should perform a complex evaluation of composition and properties of each of the raw materials determining the quality of produced combined products.

Two aspects of the design of fatty base should be noted. The first one is aimed at the solution of the problem of development of balanced product in terms of its food and biological value, including products for prophylaxis and dietary nutrition. The second one is technological, allowing the production of a product with the required structural and rheological parameters, of defined composition and properties, and taking into account its designation and specificity of use even upon changes in quantitative ratio of the fatty acid set.

To reach the high consumer appeal of combined fatty phases, multicomponent fatty bases incorporating raw milk (milk fat, dairy butter, cream), vegetable fats, and oils, both natural and modified, should be used.

Important parameters of a fatty base are the melting temperature, firmness, and the solid phase content in a certain range of temperatures.

Fatty phase melting temperature determines the fusibility of a product, which is characterized by completeness of fat melting at physiological temperature. This parameter should be in the interval of temperatures below 36°C. Use of high-melting fats not melting at the temperature of 35–36°C in the fatty composition worsens the parameters of the final product imparting it with tallow flavor [4].

Firmness of the fatty base, determined at 15°C, may be corrected by the content of the solid phase; it characterizes one of the important properties of solid fats and oils— its ability to attain required structure at given temperature. The higher is the content of the solid phase in a fat, the higher is its firmness. At solid phase content of 30, 40, and 50% firmness of fatty base makes 75, 200, and 300 g/cm, respectively.

The content of a solid phase in the range of temperatures form 5 to 35°C determines the plasticity of fatty product, which characterizes the ability of fat, under mechanical effect, change shape without fracturing, that is to maintain shape upon the stress removal. Fat with good plasticity does not change the ratio between solid and liquid glyceride content in a wide temperature range. The good elastoplastic properties of butter are determined by the composition of its solid fraction, which is heterogeneous and is transformed to liquid phase in a wide temperature range. In this connection, butter is easily deformed upon mechanical action.

For a dietary nutrition of individuals with impaired lipid exchange, fatty bases typically contain increased amount of natural vegetable oil, with the linoleic acid content reaching 20% to the total fatty acid content. Fatty bases with increased content of glycerides of linoleic acid have decreased firmness (30–50 g/cm).

At the same time, introduction of much liquid vegetable oils decreases the fat resistance to oxidation. In this connection, special attention should be paid to selection of efficient compositions of antioxidants, determining the stability of the fatty phase of the product during storage.

Performing a complex evaluation of each of the raw material factors, it should be noted that upon the fatty base construction the quality, composition, and properties of each of the ingredient should be taken into account.

Considerable amount of monounsaturated fatty acids (up to 75%) in hydrogenated fats increases the fatty phase stability to oxidation. The content of hydrogenated fat in the compositions is regulated and determined based on the mass fraction of *trans*-isomerized fatty acids in them; the amount of the latter in final product should not exceed 8%.

A distinctive feature of re-esterified fats is their high plasticity and the ability to crystallize into a stable finely crystalline polymorphous form. Introduction of such fats into a fatty base considerably improves the structural and mechanical properties of the final creamy vegetable spreads and allows producing various products from a limited assortment of raw fat.

Variation of the fraction of liquid vegetable oil produces considerable effect on texture of the fatty phase, changing it from dense to plastic and soft. The quantitative ratio of solid fats and liquid vegetable oils not only influences the structure and texture of final product, but also determines the essential factor and how well the fatty composition is balanced.

Therefore, the most important quality parameters— melting temperature, solid fat content, and firmness— are determined by the crystalline structure of the base, formation of which causes a number of interrelated factors, the determining of which is the chemical composition of the formulation and, particularly, the content of saturated and unsaturated acids.

Table 3 presents the composition and properties of fatty bases utilizing various compositions of milk fat with natural and modified oils and fats [4].

Table 3. Fatty acid compositions based on milk fat, vegetable oil, and modified fats

Fatty phase components	Number of components	Melting temperature, °C	Firmness, g/cm	Fatty acid composition, %			*Trans*-isomer content, %
				SFA	MUFA	PUFA	
Milk fat Palm oil Sunflower-seed oil	50 30 20	30.0	59	51.2	36.7	72.1	3.6
Milk fat Palm oil Rapeseed oil	30 50 20	30.7	61	47.0	39.7	13.3	1.2
Milk fat Palm olein Re-esterified fat	50 20 30	28.9	96	48.8	39.7	11.5	6.5
Milk fat Palm olein Sunflower-seed oil	50 40 10	28.6	65	48.9	37.4	13.7	2.0
Milk fat Hydrogenated fat Palm olein	20 23 57	29.2	86	36.4	53.6	10.0	7.7
Milk fat Re-esterified fat Palm olein	20 30 50	28.5	70	38.6	46.8	14.6	5.3
Milk fat Re-esterified fat	85 15	31	115	59.1	28.4	12.5	3.2
Milk fat Re-esterified fat Sunflower-seed oil	50 45 5	30.9	97	46.5	38.3	15.2	2.1

Analysis of the compositions demonstrates that the modification of structural and rheological properties in fatty models milk fat–re-esterified fat–liquid vegetable oil and milk fat–palm oil–liquid vegetable oil is affected by the mass fraction of vegetable oil since the values of firmness, melting temperature, and solid glyceride content for milk and re-esterified fats and palm oil are in the same range and practically match each other.

In this connection, in this block of work, the following fatty system was studied: milk fat–re-esterified fat–palm olein.

Analysis of the results of performed studies allowed for a design of multicomponent fatty bases with composition corresponding to the medical and biological requirements. Realization of the principle of balanced ratio between the saturated, monounsaturated, and polyunsaturated fatty acids allowed to design the formulations of fatty phases with the required structural, rheological, and physicochemical parameters.

The data of the literature review and a complex of studies on the topic discussed allows for a conclusion that the production of products with combined fatty phase may be a subject of further scientific research and technological studies aimed at providing for healthy nutrition.

REFERENCES

1. Vyshemirskii, F.A., *Maslo iz korovégo moloka i kombinirovannoe* (Cow milk and combined butter) (GIORD, Saint-Petersburg, 2004).
2. Ipatova, L.G., Kochetkova, A.A., Nechaev, A.P., and Tutel'yan, V.A., *Zhirovye produkty dlya zdorovogo pitaniya. Sovremennyi vzglyad* (Fat products for healthy nutrition. Modern view) (DeLi Print, Moscow, 2009).
3. Kornena, E.P., Kalmanovich, S.A., Martovshchuk, E.V., et al., *Ekspertiza masel, zhirov i produktov ikh pererabotki* (Regulatory analysis of oil, fat, and processing products), ed. by Poznyakovskii, V.M. (Sib. Univ. Izd., Novosibirsk, 2007).
4. Tereshchuk, L.V., *Teoreticheskie i eksperimental'nye issledovaniya po sozdaniyu kombinirovannykh masel iz molochno-rastitel'nogo syr'ya* (Theoretical and experimental studies on development of combined butters from milk and vegetable raw materials), doctor of technical sciences thesis, Kemerovo, 2002.
5. Tutel'yan, V.A., *Gosudarstvennaya politika zdorovogo pitaniya naseleniya: zadachi i puti realizatsii na regional'nom urovne: Rukovodstvo dlya vrachei* (State policy on the healthy nutrition of the population: Problems and possible solutions at regional level: Guidance for doctors), ed. by Tutel'yan, V.A. and Onishchenko, G.G. (GEOTAR-Media, Moscow, 2009).
6. *Khimicheskii sostav i energeticheskaya tsennost'pishchevykh produktov: Spravochnik Makkansa I Uiddounsa* (McCance and Widdowson's Chemical Composition of Foods, 6th edition), translated from English, ed. by Baturin, A.K. (Professiya, Saint-Petersburg, 2006).
7. *Khimiya pishchevykh produktov* (Fennema's Food Chemistry), translated from English, ed. by Damodaran, S., Parkin, K.L., and Fennema, O.R. (Professiya, Saint-Petersburg, 2012).
8. Chavarro J.E., Rich-Edwards, J.W., Rosner, B.A., and Willet, W.C., Dietary fatty acid intakes and the risk of ovulatory infertility, *Am. J. Clin. Nutr.,* 2007. V. 85, no. 1, P. 231–237.
9. Kiihn, M.C., European Functional Foods: Challenges Beyond the Regulatory Milestone, *Food Highlights*, 2007, NM5.
10. Handbook of Nutriceuticals and Functional Foods, ed. by Wildman, R.E.S. (CRC Press, New York, 2007).
11. Chajès, V., Thiébaut, A.C., Rotival. M., Gauthier, E., Maillard, V., Boutron-Ruault, M.C., Joulin, V., Lenoir, G.M., Clavel-Chapelon, F., Association between serum trans-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC Study, *Am. J. Epidemiol.*, 2008. V. 167, P. 1312–1320.

DEVELOPMENT AND INTRODUCTION OF NEW DAIRY TECHNOLOGIES

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Abstract: Recently the Russian dairy industry has undergone large changes, predetermined by the increasing use of nondairy raw materials (vegetable fats and proteins, natural fruit and vegetable fillers, etc.). This is associated with the increased demand for new products that not only have traditional nutritive properties but also make up for the deficit of certain nutrients in the ration. Practically all types of dairy products can be combined with various vegetable components. This article attempts to solve the problem of creating healthy foods by considering natural ingredients and technological specifics of functional food production.

Keywords: scientific developments, functional food products, biologically active substances, milk, cheese products, nutritive and biological value, essential nutrients, dietary fibers, galacto-oligosaccharides, probiotics, dairy products

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INTRODUCTION

At the Kemerovo Institute of Food Science and Technology (Russia), a scientific school has formed that solves important scientific and technical problems of the development of the dairy industry. The founder of this scientific school is Lev Aleksandrovich Ostroumov, who has dedicated more than 50 years of his life to the development of the dairy industry. He is known in Russia and abroad as a scientist who deeply understands trends in the global practices of dairy engineering and technology.

Ostroumov's scientific school is universally recognized, and his scientific works in the sphere of milk processing have a great impact on the formation and development of the industry's scientific potential. Ostroumov was awarded the Honored Worker of Science and Technology for excellence in scientific research.

Many doctors of sciences and professors at the Kemerovo Institute of Food Science and Technology have passed through the school of Prof. Ostroumov. Among his disciples are Anatolii M. Popov, Irina A. Smirnova, Lyubov' V. Tereshchuk, Aleksandr Yu. Prosekov, Aleksei M. Osintsev, Lyudmila M. Zakharova, Irina V. Buyanova, and Svetlana M. Lupinskaya.

Investigations of Ostroumov's scientific school are aimed at the implementation of topical problems of the dairy industry and are undertaken in the following areas.

– Improving the technology of large rennet cheeses and developing the concept of the biotechnology of these cheeses, thus facilitating the production of cheeses of accelerated ageing and guaranteed quality. The research findings have been introduced into practice to clarify production processes, as well as the storage modes for hard rennet cheeses, to improve consistency, reduce cheese-ripening times, and improve salting modes. Research has helped to intensify high-temperature cheesemaking processes of curd scalding and to design new technologies.

– The development of theoretical and practical basics of soft cheese production, which is promising for the industry.

AN OVERVIEW OF NEW DAIRY TECHNOLOGIES

Cheese with its high nutritive and biological value, balanced composition of the main components, and wide spectrum of organoleptic indicators refers to widespread foods. Cheeses should comprise the daily ration of various categories and age groups of people. The problems of increasing production and expanding and adjusting the assortment of cheeses produced in Russia have been topical for many years. It is unlikely that cheese production will be increased in the near future due to limited primary resources, milk suitable for cheesemaking.

In recent years the production of soft cheeses has gained wide acceptance. The technological specifics of these cheeses are the processing of milks of a certain degree of maturity, the use of high temperatures of milk pasteurization, acid–rennet clotting of milk, soft modes of granular curd production and processing, lengthy cheese mass self-pressing, granular curd salting, and the absence of the ripening stage. Soft cheeses are characterized by a short production cycle, reduced raw-material consumption per unit of output, and a more rational use of milk components.

A promising way of soft cheese production is the use of raw materials nontraditional for cheesemaking alongside milk raw materials, in particular, vegetable additives. Soft cheeses are a good basis for the creation of new-generation foods. First, they refer to protein products; therefore, the adjustment of their composition improves the biological and nutritive value of a product. Second, the production process of soft cheeses

makes possible a wide use of nondairy raw materials, introducing them at the stages of milk preparation for coagulation and for granular curds before molding, at the pressing stage, and at the stage of the finished product. The third condition to ensure the creation of soft cheeses is a good compatibility of the fermented-milk taste of cheese mass with the taste of introduced components. Fruit, vegetable, and wild-growing raw materials, as well as marine products and vegetable oils are used to enrich cheeses. The use of nondairy components is of a polyfunctional nature due to the presence in them of many biologically active ingredients (vitamins, minerals, amino acids, polyunsaturated fatty acids, dietary fibers, and other essential nutrients).

The production of cheeses with vegetable components is quite widespread in Southeast Asian and Western European countries, as well as on the American continent. Japan, China, Korea, and other countries make cheeses with added soybean products, isolates, concentrates, or curds (tofu). Germany manufactures potato cheese, and France, cheeses with onions, pepper, and fruit. Cheeses with nuts, fruit, vegetables, and spice plants are popular in Greece, Italy, Spain, and Portugal.

In Russia the assortment of cheeses in which the milk base is combined with plant additives is limited. The analysis of economic and technological features of making various cheeses shows that the production of soft cheeses is promising. Their production is more economical because 1.5-times less milk is consumed to produce them. Moreover, no specific requirements are imposed on milk fitness for cheesemaking and on expensive cheesemaking equipment. The production of such cheeses ensures a quicker turnover of committed finances and smoothes the seasonality of cheesemaking. The cheeses of this group have good consumer properties and a high nutritive value. Production may be organized practically at any dairy factory. A social advantage is that the cheeses of this group are cheaper and accessible to low-income groups. In addition, the use of nondairy raw materials, nontraditional for cheesemaking, makes it possible to enrich the nutritive balance with large amounts of vegetable components that are currently not used or used inefficiently.

Taking into account the topicality of this problem, the Kemerovo Institute of Food Science and Technology (Russia) has conducted a number of integrated studies on the development of various soft cheese technologies with complex feed compositions, widely using local vegetable raw materials (berries, vegetables, wild-growing plants, oil-bearing crops and products processed from them, legumes and products processed from them, and grains and products processed from them). It has been established that vegetable additives blend well with dairy raw materials and are characterized by a high content of biologically valuable substances, such as indispensable amino acids, micro- and macroelements, vitamins, dietary fibers, and polyunsaturated fatty acids. A computer database has been created, Classification and Composition of Nontraditional Raw Materials Used in Dairy Production. Requirements to nontraditional raw materials used in soft cheese production and their preparation methods that ensure the preservation of their biochemical composition, sanitary reliability, and extended storage life, have been established.

Computer software has been developed for designing products of complex primary compositions, which uses preset specifications for essential substances, can determine the type and relative content of the feed components, calculate their energy values, and recalculate their formulas accordingly. Mathematical models of cheeses with chemical compositions adequate to biomedical requirements that take into account the necessary balance of indispensable amino acids, vitamins, as well as dietary fibers, and the preset ratio of saturated, monounsaturated, and polyunsaturated fatty acids, as well as individual minerals, have been developed. A good sensory compatibility of vegetable additives with the dairy feed stock in the ratio obtained by mathematical design has been confirmed experimentally.

Scientific approaches to the production of new cheeses of complex composition have been developed. The physicochemical and technological regularities of cheesemaking have been studie. The mechanisms and regularities of interaction between dairy and nondairy feed components during acid–rennet coagulation have been considered and established. The influence of the main technological factors (mix-curdling temperature; curd-processing temperature; self-pressing duration; and salting, ripening, and storage conditions) on the efficiency of the primary components used and on the formation of quality indicators for soft cheeses of complex compositions has been revealed. Rational production parameters have been determined for guaranteed-quality products. Their nutritive, energy, and biological values, as well as physicochemical, microbiological, and organoleptic indicators during storage, have been analyzed.

Research undertaken in this area has allowed us to create a galaxy of cheese products, over 20 names of new soft cheeses with complex feed compositions.

Technologies have been created that make it possible to enrich cheeses with vitamins and minerals. As is known, cheeses contain practically all vitamins but in low amounts. Thus, 100 g of cheese meet less than 5% of the average daily demand for vitamins B_1, B_6, PP, C, E; about 10% of vitamins B_{12} and D; 16% of vitamin A; and 17.5% of vitamin B_2. Good results have been obtained using vegetable raw materials (buckthorn, chokeberry, viburnum, currant, garlic, celery, etc.) as the sources of these vitamins. The inclusion of representatives of this promising feed group in the cheese composition can adjust the contents of individual amino acids, carbohydrates, vitamins, macroelements, essential oils, and other compounds in cheeses.

Methods of enriching cheese mass with these components have been developed. The components can be introduced in their natural or conserved forms, as well as in the form of various vitamin preparations made from these raw materials. A practical result of the

research is the creation of the following cheeses: *Lesnoi* with fern; *Ryabinka* with chokeberry; *Chesnochnyi* with garlic; *Vesennii* and *Sibirskii* with parsley, dill, and celery; etc. These cheeses best combine traditional consumer properties with the requirements of positive nutrition by containing physiologically valuable natural ingredients.

Integrated studies have been conducted on the use of grain-processed products in the production of soft cheeses in order to enrich them with essential nutrients. Grain and cereal processors that deal with wheat, rye, barley, oats, corn, rice, etc., have large amounts of secondary products whose biological value is unquestionable, and at the same time not all of them are used fully in food production.

The bran share from processing wheat grain into flour reaches 26% of the total amount of the raw material, and the amount of protein in bran is 25–30% of its total content in the raw material; during grain milling, wheat bran retains 65% of minerals, 40% of fat, and 100% of dietary fibers. Wheat bran usually consists of an aleurone layer, characterized by a specific chemical composition. Bran contains more vitamins (PP, 9.47–14.01; B_1, 0.97–1.14; B_2, 0.22–0.28; B_6, 0.83–1.05; E, 31.7–35.78 mg/100 g) and minerals (potassium, 1121–1498; magnesium, 371–447; phosphorus, 900–950; calcium, 97–123; sodium, 46–55; and iron, 1.2–1.3 mg/100 g) than various grades of wheat flour. The proteins of wheat bran are richer in their amino acid content. A higher amount of deficit amino acids (lysine, threonine, and methionine) in the bran proteins is predetermined by their specific fractional makeup and a relatively high content of albumins and globulins. In addition, bran contains more than 50% of dietary fibers. The nutrient materials of fiber texture comprise second-order polysaccharides (cellulose, cellular tissue, hemicellulose, pentosan, lignin, pectic substances, etc.).

Thus, relatively high amounts of protein (14–16%), vitamins, minerals, and ballast substances that go into wheat bran during grain milling make it possible to use this raw material not only for feed but also for food purposes. The use of wheat bran in food production makes it possible:

– to increase the food protein resources;
– to improve the efficiency of grain processors;
– to create functional additives that contain valuable components for improving the quality of traditional foods and for creating new foods; and
– to develop foods with a balanced composition of their main components.

A soft cheese technology has been proposed using wheat and rye bran. The *Zernovoi* acid–rennet soft cheese is made using wheat bran, and the *Tryufel'nyi* cheese, using rye bran.

The *Zernovoi* and *Tryufel'nyi* cheeses are biologically wholesome products, containing indispensible amino acids, vitamins, macro- and microelements. Thanks to bran addition, cheeses are enriched with dietary fibers, whose content is 5–6 g in 100 g of cheese. As a result of mixing vegetable and animal proteins, which complement one another by their amino-acid composition, cheeses are enriched with sulfur-containing amino acids (methionine + cystine) and lysine, which are most valuable for humans. The score of these amino acids increases by 2.1 and 1.8%. Thanks to bran addition, the vitamin contents increase in the raw product: thiamin by 4.7, pyridoxine by 2.8, niacin by 4.1, and tocopherol by 7 times. Moreover, the content of dietary fibers in cheese increases substantially its value as a dietary and therapeutic product. Cheese products have a good sour-milk flavor and odor with a taste of bran, as well as a flexible consistency.

A reduced consumption of milk raw materials in production and an increased product output due to bran addition are the main economic advantages of the soft acid–rennet cheese products, like *Zernovoi* and *Tryufel'nyi*.

Of certain interest were studies on cheese products using wheaten germinal flakes, which are a promising vegetable raw material with a wide range of therapeutic (antioxidant, antitoxic) properties, a unique biochemical composition, and a set of biologically active substances. The use of wheat germs is especially topical in the current environmental conditions, in which the food ration should necessarily contain biologically active substances that improve human resistance to unfavorable environmental impacts. Thanks to their unique composition, wheat germs, if taken regularly, have bracing, restorative, and antiradiation effects; increase working capacity and resistance to various infectious diseases; and normalize the activity of the gastrointestinal tract. In addition, a number of latest studies show that wheat germs also have a hypoallergic effect.

In this respect, we have studied the physicochemical indicators of wheat germs that were produced at OAO Mel'korm, Kemerovo, from high-grade wheat, which complies with the GOST 9353-85 requirements, using the Buhler (Switzerland) processing equipment, which complies with the TU 9295-001-00932169-96 specifications.

The studies of the chemical composition of wheat germs have revealed that they contain 21.5–28.3% of proteins, 8.5–11.2% of lipids, 8.6–10.4% of mono- and disaccharides, 18.6–24.2% of starch, and 4.6–5.1% of ashes.

Note that the analysis of the lipid fraction of the germs under study has revealed that its composition is characterized by high contents of polyunsaturated fatty acids (linoleic acid, 49.2% and linolenic acid, 15.2%) and tocopherols (265 mg/100 g), among which both general-vitamin (α-tocopherols, 75–80 mg/100 g) and antioxidant (γ- and δ- tocopherols, 185–190 mg/100 g) forms have been identified.

The study of the vitamin complex of wheat germs has revealed contents of thiamin, 1.6–4.1; riboflavin, 0.9–1.9; niacin, 3.3–4.8; and pyridoxine, 0.8–1.0 mg/100 g.

The mineral composition is represented by phosphorus, 210–230; potassium, 320–400; calcium, 245–265; magnesium, 248–254, sodium, 6–13; iron, 12–15; manganese, 1.0–1.2 mg/100 g; and other elements, the contents of heavy metals not exceeding the permissible rates.

Along with the chemical composition of wheat germs, we have studied their functional properties. A water-absorbing ability (200%) of the product has been identified, its maximum value being obtained at (85 ± 2)°C.

These studies have laid the foundation for the development and approval of technical documentation for the *Pshenichnyi* acid–rennet soft cheese.

Assessing the quality of the amino acid composition of the *Pshenichnyi* cheese, note that its proteins are well balanced in the content of indispensible amino acids and have a high biological value.

The use of wheat germs in the production of the *Pshenichnyi* cheese changes significantly its fatty–acid composition. Our estimations show that the amount of linolenic acid in the *Pshenichnyi* cheese increases by 5–8 times compared to regular soft cheeses and the amount of linolenic acid, by 3–4 times.

Of special notice is the enrichment of the *Pshenichnyi* cheese with vitamin E. In 100 g of this cheese, the average content of tocopherols is from 5.0 to 7.0 mg, which meets 50% of the daily demand of the human organism for this vitamin. We have proved experimentally the stimulating effect of wheat germs on the growth of bifidobacteria.

Soybean and its processed products have been considered as a potential protein source in the production of soft cheese products without ageing. As is known, partial or full replacement of animal proteins with soybean proteins in the human ration leads to largely reduced cholesterol contents in the blood, which, in turn, reduces the risk of cardiovascular diseases. Acid–rennet and thermal acid cheeses with soybean have been developed, *Soft Combined Cheese*, *Novinka*, *Ideal,* etc.

In order to create new cheeses balanced by their fatty–acid composition, we have studied the composition and properties of the most widespread vegetable oils, sunflower, corn, cameline, olive, soybean, etc. The addition of vegetable oils increases the content of polyunsaturated fatty acids in cheese, and their necessary presence in the human ration is well known.

The comparative analysis of the new products shows their higher nutritive value compared to traditional cheeses, which, in turn, depends on vegetable additives in them. Thus, we have modeled the compositions and developed the technologies of new cheese products, which allow us to economize on milk raw materials, use valuable vegetable inputs, and simultaneously extend the assortment of competitive products with improved organoleptic, nutritive, and functional properties, so attractive for the Russian consumer. The newly developed soft cheese products meet the state-of-the-art food hygiene requirements for various strata and neutralize the negative environmental effects on the human organism.

A currently large research area is the integrated processing of milk raw materials and the development of principally new technologies and milk-based functional foods. Research in this area is carried out in the Department of Milk and Dairy Products Technology under the leadership of Prof. Ostroumov, Dr. Sci. (Eng.). Postgraduate students, masters of sciences, students, and specialists of the dairy industry are actively engaged in research in the following fields:

– the improvement of traditional milk technologies by more efficient use of raw materials, longer storage life, higher nutritive and biological values;

– the adjustment of the fatty–acid and amino–acid composition of dairy products by partially replacing milk raw materials with vegetable and animal raw materials; and

– the creation of scientific and practical basics for the development of principally new functional foods, enriched with biologically active components, capable to reduce the negative effects of hazardous food factors on human health and to improve the general condition of the human organism.

The past decade is characterized by an increase in diseases related to nutritive disorders. Sound nutrition ensures the normal growth and development of the human organism and contributes to higher working capacity and immunity, disease prevention, and human adaptation to the worsening environmental conditions.

Domestic producers are constantly expanding the assortment of products, improving their production technology, packaging and wrapping materials, and trying to meet consumer needs more fully. However, unfortunately, functional foods with high nutritive and biological values and enriched with vitamins, minerals, and ballast substances are few in the Russian food market, and these products are mainly of foreign makes.

Sound nutrition is perceived as a major element of a healthy lifestyle. For individual consumers, healthy nutrition is a rule that they try to stick to every day. Such people use mainly natural products, include the maximum of fruit and vegetables into their ration, and watch their daily norms of food consumption. For other consumers, this is rather a declaration of a healthy way of life.

In order to reveal the general perception of the category of milk-based functional products in Kemerovo, a market research of the functional food market was conducted using depth interviews.

Women aged from 25 to 45 years who are regular consumers of dairy products were chosen as a target audience. The majority of the participants in the survey characterized their lifestyle as active, i.e., a full working day and family chores. The tense daily routine of the respondents may tell negatively on their nutrition. For many participants in the survey, the use of cultured milk foods during the day is a way to neutralize nutrition faults (diet violations, heavy foods) and the effects of negative external factors (lack of vitamins, bad environment, stresses, etc.). Among the most important life priorities of the Kemerovo consumers are the health of close relatives (especially, children), a good psychological atmosphere in the family, good living conditions (material security, permanent employment, etc.), the balance of mind, the preservation of attractiveness (especially topical for more aged women), and self-development. Many participants in the survey noted their own or their children's digestion problems at present or in the past.

Proceeding from the above, we may distinguish the following motives for using functional dairy products at the rational level:
– digestion normalization (from the preventive or curative point of view);
– the diet and desire to normalize weight;
– the purification of the body and waste removal;
– the supply of additional vitamins; and
– the appeasement of hunger (it largely refers to drinking yogurts with various flavor additives).

At the emotional level, the use of functional products contributes to the meeting of the following needs:
– tonicity arousal, good spirits (as a result of well-being), stress management; and
– the opportunity to look attractive.

The survey has revealed the following main factors that affect the choice of a new brand of dairy products: brand distinction; colorful and attractive packaging; the prefix BIO in the product name; the statement of the nutritive effect of the product on its packaging; the presence of special ingredients (bifidobacteria, a vitamin complex, etc.); a short shelf life, which indicates the absence of preservatives and the naturalness of the product; and a low fat content. An additional incentive for sales is an affordable price. Such factors as the volume of packaging and the manufacturer are less important for the consumers of functional products. The most optimal in terms of convenience is packaging functional products in volumes of 0.3–0.4 L. Packages of 1 L are bought exclusively for household use.

Overall, the survey has shown that the category of functional products is well formed in the consumer consciousness. The term *functional* is associated with the presence of "useful additives." Unlike traditional cultured milk foods, they are ascribed the following properties: a large amount of useful "living" bacteria and the ability to normalize digestion. In addition, it has been revealed that elderly women are more perspicacious in the category of functional products compared to young consumers.

In the present living conditions with unfavorable factors, large attention is paid to the creation of directional products, which can stimulate the human immune system and which can be used to prevent a number of diseases.

Among nutritive factors that are of special importance for health support, a major role belongs to the full-fledged and regular supply of the human organism with all the necessary vitamins and minerals. Especially great is the role of vitamins and minerals in children's and adolescence ages, which is associated with the intensity of growth processes, the formation of the child's organism, and the strain of exchange processes during this period.

The creation of new foodstuffs that can reduce the negative effects of the environment on the human organism and correct various physiological disorders, that are designed for the mass prevention of many diseases, and that meet the current requirements of nutrition hygiene of various strata is a major problem for food research institutes, food higher education establishments, and food processing technologists.

An area of the design of such products is the use of natural components in their production.

Secondary products of wheat processing have a great future in the production of baked goods, and now many goods have been developed and produced with bran for preventive and dietary purposes. Wheat bran as food products and biologically active additives is used increasingly widely in the perfume and medical industries. However, it is still not widespread in the dairy industry.

The Department of Milk and Dairy Products Technology has conducted research into the composition and technological properties of wheat bran, found rational methods of technological processing, studied the synergic effect in the use of food ingredients that has made it possible to reduce the component doses and the production cost of products, developed recipes and technologies for products with higher biological and food values and enriched with ballast substances, and studied the quality indicators of new products.

Protein products have been created on the basis of combination of milk and the main milk ingredients with wheat dietary bran.

A new protein product, Laktoaleiron, has been developed for mass consumption, as well as for dietary and preventive nutrition. The product is made from normalized milk mixture with the addition of a vegetable component, viz., wheat dietary bran. The use of wheat bran in the production of milk-protein products saves milk raw materials: the introduction of wheat bran before milk pasteurization increases the curd yield almost by 18%. In addition, the bran intensifies the souring process, because the acidity of the milk–vegetable mixture increases much faster. The raw-material components are selected by method of mathematical modeling with preset limitations to the function magnitude and adjustable indicators: the energy value of the designed product is minimal; the optimal ratio of the three most deficient amino acids, tryptophan : methionine : lysine is 1 : (2–4) : (3–5); the optimal consumption of indispensible amino acids is (25–26) g/day; the optimal ratio of minerals, Ca : P : Mg is (0.8–1) : (1.0–1,5) : (0.3–0.5); the optimal supply of ballast substances is 25 g/day; the balance of the main components, protein : fat : carbohydrates is 1 : 1.2 : 4. The finished product has a clear sour-milk taste and odor with a pleasant flavor and bran aroma, a uniform and tender consistency, and a cream-white color; the mass fraction of fat is no more than 5%; that of moisture, 75%; and that of saccharose, 3%. It should be noted that the ratio of leucine to isoleucine is 1.81, which indicates a high biological value of the proteins of the curd product enriched with wheat bran. We should specially consider the enrichment of the curd product with dietary fibers (7%). They are the basic category of functional nutrition; they affect positively the digestion processes and, consequently, reduce the risk of diseases associated with these processes. As is known, the dietary fibers of wheat bran bind most actively cholic acid and other products of cholesterol exchange; they

are capable of excluding the conjugates of foreign substances from the hepatoenteric circulation, reducing the frequency of large intestine tumors, and of lowering diabetic sugar. Due to wheat bran, the average vitamin E content in 100 g of the curd product is 4.0–5.0 mg.

Studies on the functional–technological (water-binding) properties of wheat germs show their wide use not only as an additive but also as stabilizers in ice-cream production. A technology has been designed for new ice-cream types, *Zernyshko-1* and *Zernyshko-2,* using processed-wheat products based on a cream mix.

The first option is an ice-cream with roasted wheat germ flakes added directly into the product after freezing, which makes it possible to reduce the product cost by increasing the output, increase the nutritive and biological values of the ice-cream, and create a greater variety of products.

The second option is an ice-cream with roasted ground wheat germ flakes as an additive. This makes it possible to reduce the content of an expensive stabilizer and, at the same time, to obtain a well-structured product.

The *Zernyshko-1* technology includes the following stages: the formulating of the ice-cream mix, the preparation of the formulation components, mix filtering, heating, pasteurization, homogenization, cooling, mix aging, mix freezing, the introduction of roasted wheat germ flakes, filling and hardening, and ice-cream storage.

The *Zernyshko-2* process uses roasted ground wheat germ flakes that are introduced in the amount of 2% of the mix mass before pasteurization, which significantly increases the mix viscosity and positively affects the quality of the finished product. The rest of technological stages are similar to those of the *Zernyshko-1* process.

Organoleptically and physicochemically, this is a cream-colored product with a sweetish nutty taste and a light nutty aroma; the content of fat is no more than 10%; that of dry substances, 34%; that of saccharose, 14%; and the acidity is no more than 22°T.

A functional ingredient for the production of functional foods is probiotics, which include microorganisms, in particular, milk microflora, for example, acidophilic lactate bacilli, bifidobacreria, etc. Experts of the International Dairy Federation call them *health products*. At present, special attention is paid to the production of cultured milk foods with probiotics. Such products have increased contents of vitamin C and fat-soluble vitamins A, E, and D. These cultured milk foods contain a large number of bifidobacteria and lactobacilli, capable of developing in the human organism and suppressing the development of pathogens. Regular consumption of such products within the food ration agrees with the principles of healthy nutrition, improving the state of human health and significantly reducing the risk of diseases.

Currently, the development of new synbiotic cultured milk foods for functional purposes is topical, since the creation of such products helps meet the physiological needs of the human organism for nutrients and energy, adapt to unfavorable environmental conditions, and ensure the prevention of many diseases. In this context, the enrichment of food products with probiotics and prebiotics, which are well balanced against each other, looks promising.

Taking the above into account, the Kemerovo Institute of Food Science and Technology has developed technologies of functional products enriched with probiotics and prebiotics.

A major problem faced during the creation of new products for functional nutrition is the choice of the optimal dose of an additive that confers the necessary properties and does not affect negatively the quality of finished products.

Within the development of the technology of functional lactoprotein products, research has been conducted to find out the effect of doses of introduced prebiotics (dietary fibers) on the acid formation process. The properties of dietary fibers in the human organism are diverse: they are not hydrolyzed and are not adsorbed in the upper gastrointestinal tract; they are a bifidogenic factor; they control the cholesterol level, bind and remove part of toxic substances from the human organism, and improve the assimilability of calcium and magnesium.

The dose of dietary fibers introduced varied from 1.2 to 8.7% in increments of 2.5%. For fermentation, we used a culture consisting of bifidobacteria (Bifidobacterium bifidum and Bifidobacterium longum) and a lactic streptococcus at a ratio of 1 : 3. Souring lasted for 4 h until pH 4.6–4.7 was reached.

These studies have shown that acidity increases with the increase of the dietary fiber dose. This is explained by the fact that they are supplemental feeding for souring microorganisms, and the larger the dose introduced, the faster the lactic fermentation occurs. The highest acidity was in a sample in which the dose of dietary fibers was 8.7%.

Dietary fibers, being a prebiotic, selectively stimulate growth and improve the biological activity of the useful intestinal microflora. The combination of a probiotic (bifidobacretia) and a prebiotic (dietary fibers), which mutually amplify the effect on the physiological functions of the human organism, allows us to view the resultant lactoprotein product as a synbiotic product. The developed product, owing to its introduction into the composition of dietary fibers and probiotic microorganisms, has a functional effect and may be recommended for use by all population groups.

Cottage cheese is becoming increasingly popular among the consumers due to its good organoleptic indicators. However, the technology of this product is associated with certain difficulties. A major factor that affects the quality of the finished product is the quality of the raw material used.

Since the content of protein and nonfat milk solids in raw milk is insufficient, it was proposed to adjust its composition by introducing milk protein, obtained by microfiltration and dried by the spray drying method. The protein additive is introduced into fat-free milk before pasteurization and before the nonfat milk solids reach a mass fraction of 9.0–9.5%.

In order to make cottage cheese acquire functional properties, the product is enriched with probiotics and prebiotics. Bifidobacteria, which represent the normal

intestinal microflora and perform numerous and important protective functions in the human organism, are used as a probiotic. Dietary fibers are used as a prebiotic. The distinctive feature of the developed technology is that dietary fibers and bifidobacteria are introduced into cream. Therefore, the optimal dose of dietary fibers was established to stimulate the development of probiotic microflora. Such a dose of dietary fibers (3.0%) ensures the necessary growth of bifidobacteria during cream souring and does not affect negatively the organoleptic properties of the finished product. The amount of viable cells at the chosen dose is $4 \cdot 10^9$ CFC/g.

The dietary fibers contribute to obtaining a thicker cream consistency and allow producing a less caloric product by simulating a higher fat content in cream, as well as by its water-binding ability, and extend the shelf life and increase the output of the finished product.

Organoleptically, the developed product has a clear, lactic taste and odor; a nonuniform consistency with clearly distinct granules of various sizes, covered with cream; and a white color with a cream tint.

Taking into account the importance of the creation of products with probiotic properties, the possibility of enriching sour-milk drinks with whey protein, viable bifidoflora has been studied. The high antagonistic activity of bifidobacteria, their ability to destroy toxic metabolites, accumulate amino acids, produce volatile fatty acids, and synthesize vitamins indicate the practicability of these microorganisms in the production of dairy products for infusing them with functional properties. A high viability of the vegetative cells of bifidobacteria during the storage of a product enriched with whey protein has been established, no less than $2.0 \cdot 10^6$ CFC/g. Considering the above, we may state that the addition of whey protein during the production of sour-milk drinks influences favorably the development of bifidoflora.

Technical documents for the above products have been developed, approved, and duly registered. The developed assortment is produced at the enterprises of the Russian agroindustrial complex.

The advantages of the developed technologies are the production of dairy products of high biological and nutritive values, enriched with essential nutrients and possessing functional properties, the increased output of products owing to the use of rational raw materials and commercially available equipment. The compatibility of these developments is ensured by their topicality, scientific validity, and technological and economic practicability. Their broad promotion will contribute to the introduction of the ideas of positive nutrition into the minds of consumers.

The introduction of functional products into nutrition practices is regarded from the medical point of view as an important link in the program of government measures aimed at the formation of a healthy lifestyle. These products are to improve the nutrition structure of various population groups with their specific needs for foodstuffs. The provision of the country's population with high-quality products is inseparable from the development of progressive technologies that take into account the latest achievements in basic and applied research in the science of food. Note that recently the problem of foodstuffs of predicted and guaranteed quality has increasingly been coming to the fore compared to the problem of the amount of food produced. In addition, the most urgent problem is the creation of products with preset chemical compositions and with account for biomedical recommendations (functional products).

Overall, there are sufficiently ample grounds to assume that the field under consideration in the production of functional products will develop very dynamically in the years to come.

REFERENCES

1. Zakharova, L.M., *Nauchno-prakticheskie aspekty proizvodstva funktsional'nykh produktov iz moloka i zlakov* (Scientific and Practical Aspects of Making Functional Products from Milk and Cereals / Laboratoriya Mnozhitel'noi Tekhniki KemTIPP, Kemerovo, 2005).
2. Ostroumov, L.A. and Zakharova, L.M., Soft cheese production: A promising direction, *Syrodelie maslodelie (*Cheesemaking Buttermaking), 2006. № 1. P. 29–30.
3. Zakharova, L.M., Ovchinnikova, T.A., and Shaposhnikova, E.Yu., Consumer demand for functional dairy products, *Molochnaya prom–st'* (Dairy Industry), 2006. № 8. P. 8–9.
4. Zakharova, L.M., The scientific school of L.A. Ostroumov, *Obrazovanie. Kar'era. Obshchestvo* (Education. Career. Society), 2009. № 1 (23). P. 108–110.
5. Zakharova, L.M. and Zakharchenko, S.G., Cottage cheese for functional purposes, *Molochnaya prom–st'* (Dairy Industry), 2009. № 7. P. 62.
6. Zakharova, L.M. and Mazeeva, I.A., The technology of new functional milk-based foodstuffs, *Khranenie pererabotka sel'khozsyr'ya (Storage Processing Agric. Raw Mater.)*, 2010. № 11. P. 63–67.
7. Zakharova, L.M., Orekhova, S.V., Zakharchenko, M.A., and Lozmanova, S.S., The study of technological parameters of making a functional fermented milk product, in *Tekhnika i tekhnologiya pishchevykh proizvodstv* (Food Production Techniques and Technology) (Kemerovo, 2012), pp. 37–42.
8. Zakharova, L.M., Lozmanova, S.S., and Krokhaleva, L.V., The study of the technological characteristics of a functional fermented milk product and its nutritive value, *Aktual'naya biotekhnol.* (Topical Biotechnol.), 2014. № 1 (8). P. 12–15.

BIOTECHNOLOGY

SCREENING AND IDENTIFICATION OF PIGMENTAL YEAST PRODUCING L-PHENYLALANINE AMMONIA-LYASE AND THEIR PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS

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Abstract: The results of the analysis of DNA sequences encoding L-phenylalanine ammonia-lyase (PAL) synthesis, performed to obtain universal primers complementary to conserved regions of the *pal* gene, are presented in the article. The fragment of *pal* gene was amplified in organisms under study. Nucleotide sequence of the *pal* gene in microorganisms exhibiting L-phenylalanine ammonia-lyase activity was determined by DNA sequencing. The results of its comparison with the corresponding sequences of known species are presented. Phenotypic characteristics and biochemical properties of selected cultures were studied. An investigation aimed to choose a superproducer strain of L-phenylalanine ammonia-lyase was conducted. It was found that L-phenylalanine ammonia-lyase synthesis was the most active in the following strains: *Aureobasidium pullulans* Y863, *Rhodosporidium infirmominiatum* Y1569, *Candida glabrata* Y2813, *Candida maltose* Y242, *Debaryomyces robertsiae* Y3392, *Rhodosporidium diobovatum* Y1565, *Rhodotorula lactose* Y2770, *Saccharomyces cerevisiae* Y1127, *Tilletiopsis washingtonensis* Y1650, *Torulopsis apicola* Y566, *Tremella foliacea* Y1624, *Rhodotorula rubra* Y1193, and *Debaryomyces castellii* Y968. This allows recommending them for further research aimed to obtain the enzyme preparation of L-phenylalanine ammonia-lyase.

Keywords: L-phenylalanine ammonia-lyase, enzyme, *pal* gene, pigmental yeast, nucleotide sequence, amino acid sequence, phylogenetic tree

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INTRODUCTION

L-phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyzes the reaction of reverse deamination of L-phenylalanine to *trans*-cinnamic acid and ammonia [1]. It is the key enzyme of phenylpropanoid metabolism in plant and fungi, where it is involved in biosynthesis of secondary metabolites (flavonoids, furanocoumarines, and cell wall components), existing in multiple isoforms [2, 3].

PAL plays an important role in catabolic processes of microorganisms, providing for utilization of L-phenylalanine as a sole source of carbon and nitrogen [4]. Among the microorganisms, the highest PAL activity is exhibited by yeasts, especially the red basidiomycetes of the Rhodotorula family [5]. *Sporobolomyces roseus* and *Sporidiobolus pararoseus* are also PAL-producing yeasts [6].

Therapeutic potential of PAL with respect to neoplasms was evaluated due to its selective activity to phenylalanine and amino acids that are consumed by mammalian cells from external sources. PAL was shown to inhibit neoplasm growth in vitro [7].

The enzyme is also of interest as a therapeutic agent for phenylketonuria treatment and may be used for both direct therapy of phenylketonuria and production of food products free of phenylalanine [8]. Besides the medical applications, the enzyme may be used in biotechnology for L-phenylalanine production from *trans*-cinnamic acid [9].

Considerable contribution to the development and assimilation of the technology of specialized food products was made by G.B. Gavrilov, N.B. Gavrilova, V.I. Ganina, N.I. Dunchenko, I.A. Evdokimov, V.I. Kruglik, K.S. Ladodo, L.A. Ostroumov, A.N. Petrov, V.O. Popov, G.Yu. Sazhinov, V.A. Tutel'yan, V.D. Kharitonov, I.S. Khamagaeva, and A.G. Khramtsov, and to the technology of the enzyme preparation of PAL, by V.I. Mushtaev, M. Jason Mac Donald, H. Orum, and O.F. Rasmussen.

The development of new and improvement of existing technologies of the PAL preparation production requires new, more intensive sources of its superexpression, which is impossible without studies on the specific features of its genetics in known producers. Only 26 sequences of genomes of microorganisms exhibiting PAL activity were found in the databases of genetic sequences (EMBL and GenBank). Therefore, the search for microorganisms exhibiting L-phenylalanine ammonia-lyase activity based on sequence analysis of their genomes is urgent.

The aim of the work was to screen and identify pigmented yeasts producing L-phenylalanine ammonia-lyase and describe their physiological and biochemical characteristics.

OBJECTS AND METHODS OF RESEARCH

Agarose (Chemapol, Czech Republic); low gelling-temperature agarose (Ultra Pure, BRL, United States); β-mercaptoethanol, PEG-1500, PEG-6000 (Loba Feichemie, Austria); imidazole (Diaem, Russia); dATP, dCTP, dGTP, dTTP (Bioline, Germany); bactoagar, yeast extract (Difco, United States); bactotriptone (Ferak, Germany); D-glucose, urea, bromine (99.8%, for synthesis) (Merck, Germany); bromphenol blue, ammonium persulfate, *N,N'*-methylene-bis-acrylamide (Reanal, Hungary); acetyl phosphate, ethidium bromide, DTT, IPTG, Triton X-100, EDTA, $MgCl_2$, BSA, DMSO, Tris, $(NH_4)_2SO_4$, $CaCl_2$ (Sigma, United States); L-phenylalanine ammonia-lyase, acrylamide, *N,N'*-methylene-bis-acrylamide, ammonium persulfate (Sigma, Germany); RNase A, CH_3COOK, C_6H_5OH, $CHCl_3$, NaCl (Reakhim, Russia); MilliQ deionized water (Millipore, France); orthophosphoric acid (85.1%, imported) (Lenreaktiv, Russia); tris(hydroxymethyl)aminomethane, PIPES, SDS, X-Gal, tetracycline hydrochloride, chloramphenicol (Serva, Germany); L-phenylalanine (Acros Organics, Belgium); *trans*-cinnamic acid (Brituer Co. Ltd., China); boric acid (99.8%, extra pure), sodium tungstate dihydrate (99.1%, extra pure) (AppliChem, United States); (3-aminopropyl)triethoxysilane (98.0%, for synthesis), acrylic acid (99.8%, for synthesis), sodium caseinate (92.0%, extra pure), potassium monophosphate dihydrate (98.5%, extra pure), sodium carbonate (99.9%, for synthesis), sodium hydroxide (9.1%, pure for analysis), sodium chloride (99.8%, extra pure), hydrochloric acid (36.0%, extra pure) (Khimlaborpribor, Russia); sodium phosphate dibasic dodecahydrate (98.2%, extra pure), L-tyrosine (99.9%, extra pure), trichloroacetic acid (99.0%, for synthesis), acetic acid (98.5%, extra pure), phenolphthalein (98.5%, extra pure).

Sequences of the *pal* gene of ascomycetes and basidiomycetes presented in the NCBI international database are reported in Table 1.

Table 1. Sequences of microorganism strains possessing L-phenylalanine ammonia-lyase activity

Strain	Strain number	Species name
1	83976309	*Rhodotorula_graminis*
2	3293	*Rhodosporidium_toruloides*
3	3284	*Rhodotorula_mucilaginosa*
4	317034460	*Aspergillus_niger*
5	115437191	*Aspergillus_terreus*
6	497418	*Arabidopsis_thaliana*
7	242780352	*Talaromyces_stipitatus*
8	212533750	*Penicillium_marneffei*
9	169610841	*Phaeosphaeria_nodorum*
10	317157281	*Aspergillus_oryzae*
11	238493630	*Aspergillus_flavus*
12	121698870	*Aspergillus_clavatus*
13	119480760	*Neosartorya_fischeri*
14	71001127	*Aspergillus_fumigatus*
15	389639669	*Magnaporthe_oryzae*
16	116206211	*Chaetomium_globosum*
17	164422921	*Neurospora_crassa*
18	15824530	*Ustilago_maydis*

Table 1. Ending. Sequences of microorganism strains possessing L-phenylalanine ammonia-lyase activity

Strain	Strain number	Species name
19	331236172	*Puccinia_graminis*
20	507833891	*Letharia_vulpina*
21	4127288	*Amanita_muscaria*
22	299751359	*Coprinopsis_cinerea_okayama*
23	170097945	*Laccaria_bicolor*
24	409924409	*Tricholoma_matsutake*
25	482667462	*Pleurotus_eryngii*
26	1666264	*Agaricus_bisporus*

Strains of the microorganisms for study of their L-phenylalanine ammonia-lyase activity obtained form the All-Russian Collection of Industrial Microorganisms, GosNIIGenetika, are presented in Table 2.

Table 2. Strains of the microorganisms for investigation of the PAL activity

No.	Name	Microorganism number in the Collection of Microorganisms of GosNIIGenetika
1	*Aureobasidium pullulans*	Y863
2	*Bullera alba*	Y1581
3	*Bullera piricola*	Y1577
4	*Candida glabrata*	Y2813
5	*Candida maltosa*	Y242
6	*Cryptococcus laurentii*	Y227
7	*Cryptococcus macerans*	Y2763
8	*Cystofilobasidium capitatum*	Y1573
9	*Cystofilobasidium capitatum*	Y1852
10	*Debaryomyces castellii*	Y968
11	*Debaryomyces robertsiae*	Y3392
12	*Dioszegia hungarica*	Y3208
13	*Dioszegia sp.*	Y3320
14	*Geotrichum klebahnii*	Y3053
15	*Phaffia rhodozyma*	Y1666
16	*Phaffia rhodozyma*	Y1668
17	*Rhodosporidium capitatum*	Y1567
18	*Rhodosporidium diobovatum*	Y1565
19	*Rhodosporidium infirmo-miniatum*	Y1569
20	*Rhodotorula aurantiaca*	Y985
21	*Rhodotorula glutinis*	Y77
22	*Rhodotorula lactosa*	Y2770
23	*Rhodotorula minuta*	Y2777
24	*Rhodotorula rubra*	Y1193
25	*Saccharomyces cerevisiae*	Y1127
26	*Saccharomyces kluyveri*	Y2559
27	*Sporobolomyces holsaticus*	Y991
28	*Sporobolomyces roseus*	Y987
29	*Tilletiopsis washingtonensis*	Y1650
30	*Torulopsis apicola*	Y566
31	*Tremella foliacea*	Y1624
32	*Tremella mesenterica*	Y1625

BLAST2 software was used to search for homologous sequences; Generunner and Chromas, for analysis of nucleotide and amino acid sequences; Clustal Omega, for multiple nucleotide sequence alignment; and BioEdit 7.0.0, for editing and alignment, as well as translation into amino acid sequence. Analysis of an optimal model for amino acid substitutions was performed on a ProtTest 3 on-line server according to the Akaike information criterion (AIC) (the sequence of the *pal* gene of *Arabidopsis thaliana* was used as an out-group). The phylogenetic tree was built using a distance method in a Phylip 3.69 software package, and using the Bayesian method, in a MrBayes 3.2 software; the trees were visualized in a TreeGraph 2, and the logo diagram was built in a WebLogo software.

Sanger sequencing was performed on an ABI3730xl (Applied Biosystems, United States) automated sequencer according to the manufacturer's protocol using the BigDye® Terminator v3.1 Cycle Sequencing kit.

Oligonucleotides were obtained on an ABI3900 (Applied Biosystems) synthesizer. The results of the experiments were processed using the methods of mathematical statistics.

The primers RalF and RalR were synthesized by JSC Sintol. Primer operational parameters are presented in Table 3.

Table 3. Operational parameters of RalF–RalR primers

Primers	
Forward primer RalF	CTCACCAACTTCCTCAA CCACGGCA
Parameters	
Length	25 bp
Molecular weight	7475.9
CG content	56%
Melting temperature	69°C
Annealing temperature	64°C
Reverse primer RalR	ATGCCCTCGTCGTCCTT GACCTTGA
Parameters	
Length	25 bp
Molecular weight	7559.9
CG content	56%
Melting temperature	69°C
Annealing temperature	64°C

PCR amplification was performed on a SMART CYCLER (Cepheid, United States) thermocycler in 20–50 μL solution prepared on the basis of a 10-fold buffer for Taq polymerase, which contained 200 μmol of each of deoxyribonucleotides, 0.5 μmol primers, 2 μmol $MgSO_4$, 10 ng template, 2 units of Taq DNA polymerase, and 0.1 unit of Pfu DNA polymerase. Oligonucleotide annealing temperature was calculated using an empirical formula: $T_m = 67.5 + 34[\%GC] – 395/n$, where %GC = (G + C)/n, and n is the number of nucleotides. PCR products were analyzed by electrophoresis in 1-% agarose gel.

Table 4 presents the amplification parameters for RalF–RalR primers.

Table 4. Amplification parameters for RalF–RalR primers

Step	Temperature, °C	Time	
Initial heating	90–95	1 min	
Denaturation	95	30 s	30 cycles
Primer annealing	64	30 s	
Elongation	72	1 min	
Elongation	72	1 min	

DNA electrophoresis in agarose gel. Samples of DNA were separated by electrophoresis in Tris–acetate buffer (0.04 M Tris–acetate; 0.002 M EDTA) in a 0.7–0.8% agarose gel (Bio-Rad, United States) containing 0.5 μg/mL ethidium bromide under the voltage of 2–5 V/cm. GeneRuler™ 1 kb DNA Ladder (Fermentas, Lithuania) were used as standard molecular weight markers. DNA bands were detected upon gel irradiation with UV light using the Gel Doc XR Plus (Bio-Rad) system.

Isolation of DNA fragments from agarose gel. Samples of DNA were separated by electrophoresis in Tris–acetate buffer in a 0.7–0.8% agarose gel (Bio-Rad) containing 0.3 μg/mL ethidium bromide and analyzed by fluorescence under ultraviolet light at 254 nm. Gel pieces containing fragments of interest were cut out and transferred into microcentrifuge tubes, then DNA fragments were eluted from the gel using the "Isolation of DNA from agarose gels" kit (Boeringer Mannheim, Germany). Sodium perchlorate was added to the tubes in the amount of 400 μL per 100 mg weight of the cut out gel. The mixture was heated to 65°C, then agarose was dissolved in salt buffer. Glass milk microbeads were introduced into the suspension at the amount of 20 μL per 100 mg of gel weight. In the salt solution, DNA contained in the gel adsorbed on the surface of the microbeads. They were washed (consecutive precipitation–resuspension) with the same salt solution once and with 70% ethanol, two times. DNA was desorbed from the beads by resuspension in TE buffer (10 mM Tris-HCl buffer, pH 7.4, 1 mM EDTA) in the amount of 50 μL per 100 mg gel weight.

L-phenylalanine ammonia-lyase activity was determined according to a protocol by Sigma with little modifications in the preparation of the reaction mixture. All solutions were prepared in deionized MilliQ water.

Composition of standard incubation mixture (1 cm^3): 0.2 mol Tris-HCl, pH 8.5, 0.5 cm^3; 0.05 mol L-phenyl-alanine, 0.04 cm^3; deionized water, 0.42 cm^3.

After mixing and pre-incubation (at least 5 min at 30 ± 0.1°C), the reaction was initiated by the addition of 0.04 cm^3 diluted enzyme (0.025–0.125 U/cm^3 PAL).

In the control sample, 0.04 cm^3 water was added instead of the enzyme.

The reaction course was registered continuously at 270 nm in a Shimadzu UV-1800 (Shimadzu, Japan) spectrophotometer equipped with a thermocontrolled chamber in quartz cuvettes with a 1-cm optical path. Data collection and analysis was performed by a UV-probe v (Shimadzu) software. PAL activity was calculated according to the formula using the value of millimolar extinction coefficient of *trans*-cinnamic acid (Sigma's protocol):

$$Activity\ \left(U/_{cm^3}\right) = \frac{\left(\Delta OD_{270}/min_{exp} - \Delta OD_{270}/min_{control}\right) \times V_{reac.mix} \times f}{19.73 \times V_{sample}},$$

where $V_{reac.mix.}$ is the reaction mixture volume, mL; f, coefficient of dilution of the initial PAL preparation; 19.73, millimolar coefficient of extinction of *trans*-cinnamic acid at 270 nm; V_{sample}, sample volume, cm^3; min_{exp}, $min_{control}$, duration of enzymatic activity measurement in experiment and control samples, respectively.

The amount of PAL that catalyzed the transformation of 1 μmol L-phenylalanine into *trans*-cinnamic acid and NH_3 within 1 min at pH 8.5 at 30 ± 1°C was considered an activity unit.

Study of specific features of phenotype and biochemical properties of the selected pigmented yeast strains. Shape and size of cells of the microorganisms were described and determined in cultures of different age on dense and liquid nutritive media. The first imaging and cell size measurement was performed in two–three-day cultures grown at 25–28°C. Then, the cultures were left at room temperature (17–18°C) and described again in four weeks.

To determine cell size, length and width were measured with a micrometer in at least 20 cells, and the extreme values were indicated.

RESULTS AND DISCUSSION

Today, to determine the species reference of a microorganism and the presence of a certain gene in it, the method of 16S rRNA gene sequence comparison may be used, since the gene carries both the conserved and variable regions of the nucleotide sequence. The data on nucleotide sequences of various microorganisms are contained in the international databases GenBank and EMBL-EBI.

Therefore, comparative analysis of twenty six nucleotide sequences of the *pal* gene contained in the GenBank database of genetic sequences were compared, including *Rhodotorula graminis* 83976309, *Rhodosporidium toruloides* 3293, *Rhodotorula mucilaginosa* 3284, CBS 513.88 *Aspergillus niger* 317034460, NIH2624 *Aspergillus terreus* 115437191, *Arabidopsis thaliana* 497418, ATCC_10500 *Talaromyces stipitatus* 242780352, ATCC 18224 *Penicillium marneffei* 212533750, SN15 *Phaeosphaeria nodorum* 169610841, RIB40 *Aspergillus oryzae* 317157281, NRRL3357 *Aspergillus flavus* 238493630, NRRL 1 *Aspergillus clavatus* 121698870, NRRL 181 *Neosartorya fischeri* 119480760, Af293 *Aspergillus fumigatus* 71001127, 70-15 *Magnaporthe oryzae* 389639669, CBS_148.51 *Chaetomium globosum* 116206211, OR74A *Neurospora crassa* 164422921, *Ustilago maydis* 15824530, f. sp. tritici CRL 75-36-700-3 *Puccinia graminis* 331236172, *Letharia vulpina* *507833891,* *Amanita muscaria* 4127288, 7.130 *Coprinopsis cinerea okayama* 299751359, S238N-H82 *Laccaria bicolor* 170097945, NBRC 30605 *Tricholoma matsutake* 409924409, P810 *Pleurotus eryngii* 48266746, and *Agaricus bisporus* 1666264, deposited in the GenBank of the National Center for Biotechnology Information (NCBI).

Phylogenetic relationships between the microorganisms established on the basis of comparative analysis of the nucleotide sequences may be presented as a dendrogram (phylogenetic tree), an arbitrary graphical representation reflecting the affinity between the genetic macromolecules, biological species, or higher rank taxa (Fig. 1).

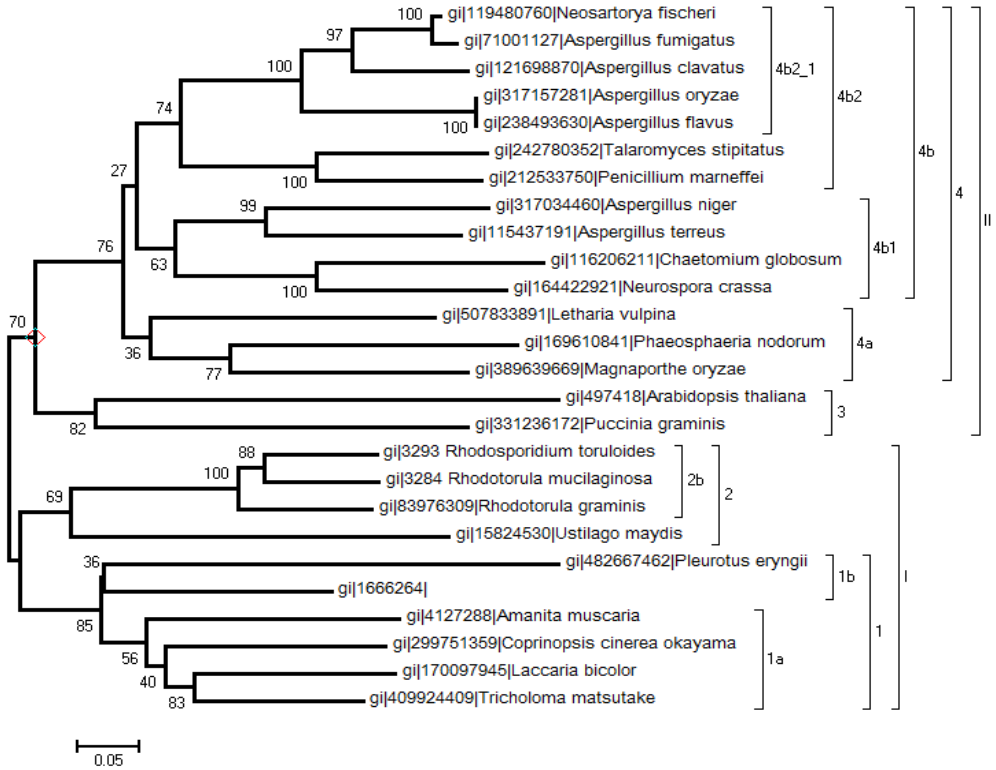

Fig. 1. Dendrogram of nucleotide sequences of the pal gene built with the NJ method using the MEGA 4.0.2 software. Figures indicate the statistical reliability of the branching order determined with bootstrap analysis.

Figure 1 presents the molecular phylogenetic tree obtained using the neighbor-joining method. The genetic distances have been calculated according to Kimura-2 method.

The performed phylogenetic analysis demonstrates rather high similarity between the *pal* gene sequences. In the phylogenetic tree, all *pal* gene sequences under study may be grouped into two big clusters, I and II.

Cluster I comprises the smaller clusters 1, with bootstrap support of 85%, and 2, with bootstrap support of 69%; *pal* gene sequences of *Agaricus bisporus* and *Pleurotus eryngii* correspond to clade 1b with bootstrap support of 36%. In clade 1a, *Amanita muscaria* forms an individual branch, and *Coprinopsis cinerea okayama, Laccaria bicolor*, and *Tricholoma matsutake* form a small clade with support of 40%, and *Laccaria bicolor* forms a separate consortium with *Tricholoma matsutake* with the support of 83%.

Sequences of *pal* gene in the representatives of the *Rhodotorula* and *Rhodosporidium* genera, *Rhodotorula graminis, Rhodosporidium toruloides,* and *Rhodotorula mucilaginosa*, form a separate clade 1b in cluster 1 with the highest bootstrap support of 100%. They are neighbored by *Ustilago maydis,* which forms an individual branch.

Cluster II may be divided into a small cluster 3 and a large cluster 4.

Cluster 3 comprises the sequences of the representtatives of the *Arabidopsis thaliana* and *Puccinia graminis* species with bootstrap support of 82%.

Representatives of the *Aspergillus* genus—*A. oryzae, A. flavus, A. clavatus, Neosartorya fischeri* (synonym with *A. fischeri),* and *A. fumigatus*—form a monophyletic clade 4b2 1 with a 100-% bootstrap support. They are neighbored by another clade with a 100-% support, which includes *pal* sequences of *Talaromyces stipitatus* and *Penicillium marneffei.*

Clade 4 b1 also comprises the representatives of *A. niger* and *A. terreus,* which form a monophyletic consortium with support of 99%, and *Chaetomium globosum* and *Neurospora crassa,* a consortium with bootstrap support of 100%.

Sequences of the *pal* gene from *Magnaporthe oryzae* and *Phaeosphaeria nodorum* form a consortium with 77-% support. The neighboring *Letharia vulpine, Letharia vulpine, Magnaporthe oryzae,* and *Phaeosphaeria* form a separate branch comprising the 4a clade with support of 33%. Clades 4a and 4b together make up cluster 4 with bootstrap support of 76%.

Multiple alignment of selected nucleotide sequences for each of the gene clusters encoding L-phenylalanine ammonia-lyase deposited in GenBank and EMBL-EBI were performed with the Clustal X V 1.75 software.

The results of the study demonstrated that the *pal* sequences in the analyzed microorganisms are poorly conserved; therefore, the area of search for universal primers was narrowed. For this purpose, *pal* gene sequences in basidiomycete yeasts *Rhodosporidium toruloides* and *Rhodotorula glutinis* were analyzed, because these very organisms possessed the highest homology with the pigmented yeast strains.

Based on the obtained conserved fragments, as well as the theoretical rules of selection, the following universal primers were selected:

– forward primer PAL1F (5'- CGC GGY CAY TCK GCK GT -3')

– and reverse primer PAL1R (5'-CAT YTC TGC CGG YTG AAC RTG -3').

Melting temperature and amplification parameters for the designed primers were calculated using the Olig 4.0 software. The results of the studies are presented in Table 3.

Analysis of the literature data demonstrated that L-phenylalanine ammonia-lyase was detected in a number of microorganisms, including the pigmented yeasts. Colorless yeasts do not contain the enzyme. In this connection, the search for culture with PAL activity was performed among the pigmented yeast from the microorganism collection (GosNIIGenetika): *Aureobasidium pullulans* Y863, *Bullera* Y1581, Y1577, *Candida* Y2813, Y242, *Cystofilobasidium* Y1573, *Debaryomyces* Y968, Y3392, *Phaffia* 1666, 1668, *Rhodosporidium* Y1567, Y1565, Y1569, *Rhodotorula* Y985, Y2770, Y2777, Y1193, *Saccharomyces* Y1127, Y2559, *Sporobolomyces* Y987, *Tilletiopsis* Y1650, *Torulopsis* Y566, and *Tremella* Y1624, Y1625.

As a result of amplification reaction, a PCR product of expected length was obtained for each of the samples. However, we failed to sequence it, since the reaction yielded additional non-specific products of various length, including the one very close to the target fragment. We decided to substitute one of the primers with another one and decrease the length of expected fragment. At the second stage the PAL2F primer (CAT YTC TGC CGG YTG AAC RTG) was used. Therefore, the pair of primers RAL2F–RAL1R flanks the *pal* gene fragment located between nucleotides 292 and 1319 of the *pal* gene in NCBI database; the size of the amplified fragment was 1027 bp.

The results of amplification were controlled with electrophoresis in 1.5% agarose gel under voltage of 5 V/cm in an SE-1 horizontal electrophoresis chamber equipped with an Elf-4 power supply (Khelikon, Russia). Artificially synthesized *pal* gene of *Rhodosporidium toruloides* was used as a positive control.

After enzymatic purification with a mixture of exonuclease I and alkaline phosphatase, the fragments were sequenced with the PAL2R primer on an ABI 3130xl (Applied Biosystems) analyzer according to standard techniques.

To prove that the obtained amplificates are indeed the *pal* gene fragments, direct sequencing of PCR fragments was performed using an ABI3730xl (Applied Biosystems) automated sequencer and BigDye® Terminator v3.1 Cycle Sequencing Kit for the following strains: *Aureobasidium pullulans* Y863, *Bullera alba* Y1581, *Bullera piricola* Y1577, *Candida glabrata* Y2813, *Candida maltosa* Y242, *Cryptococcus laurentii* Y227, *Cryptococcus macerans* Y2763, *Cystofilobasidium capitatum* Y1573, *Cystofilobasidium capitatum* Y1852, *Debaryomyces castellii* Y968, *Debaryomyces robertsiae* Y3392, *Dioszegia hungarica* Y3208, *Dioszegia sp.* Y3320, *Geotrichum klebahnii* Y3053, *Phaffia rhodozyma* Y1666, *Phaffia rhodozyma* Y1668, *Rhodosporidium capitatum* Y1567, *Rhodosporidium diobovatum* Y1565,

Rhodosporidium infirmo-miniatum Y1569, *Rhodotorula aurantiaca* Y985, *Rhodotorula glutinis* Y77, *Rhodotorula lactosa* Y2770, *Rhodotorula minuta* Y2777, *Rhodotorula rubra* Y1193, *Saccharomyces cerevisiae* Y1127, *Saccharomyces kluyveri* Y2559, *Sporobolomyces holsaticus* Y991, *Sporobolomyces roseus* Y987, *Tilletiopsis washingtonensis* Y1650, *Torulopsis apicola* Y566, *Tremella foliacea* Y1624, and *Tremella mesenterica* Y1625.

The results evidence that strains *Bullera alba* Y1581, *Cryptococcus laurentii* Y227, *Cryptococcus macerans* Y2763, *Cystofilobasidium capitatum* Y1852, *Dioszegia hungarica* Y3208, *Dioszegia sp*. Y3320, *Geotrichum klebahnii* Y3053, *Phaffia rhodozyma* Y1666, *Rhodosporidium capitatum* Y1567, *Rhodotorula aurantiaca* Y985, *Rhodotorula minuta* Y2777, *Sporobolomyces holsaticus* Y991, and *Sporobolomyces roseus* Y987 contain no *pal* gene and thus were excluded from further study.

Phylogenetic analysis of *pal* gene sequences in strains under study and reference strains from the GenBank database of genetic sequences (Table 1) was performed.

Upon multiple alignment of the sequences (CLUSTAL Omega) and their editing (intron excision), they were translated using the BioEdit 7.0.0 software. For further analysis, testing of various models of amino acid substitutions was performed with a ProtTest 3 service.

Structure of the PAL protein of *Rhodosporidium toruloides* was obtained from the PDB bank (1T6P, DOI:10.2210/pdb1t6p/pdb) in the PyMOL Molecular Graphics System, version 1.5.0.4 (Schrodinger, LLC). As follows from Fig. 2, the fragment lies in the internal part of the molecule and includes the region of a beta-sheet, two turns, and several alpha-helix regions.

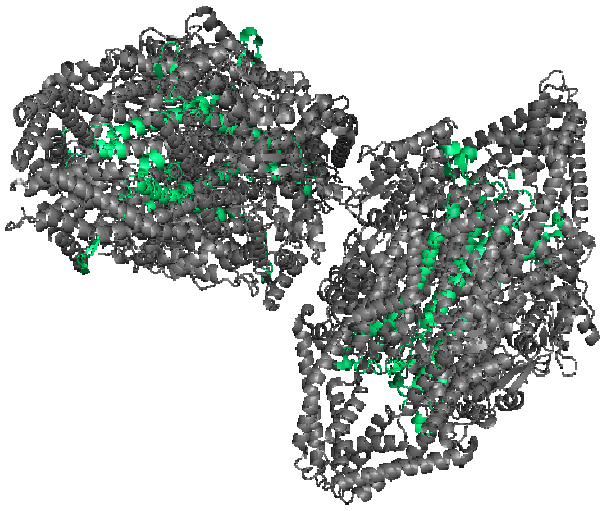

Fig. 2. Structure of PAL protein from *Rhodosporidium toruloides* (DOI:10.2210/pdb1t6p/pdb*)*. Green color indicates amino acids 398–472 (numbers are given according to the GI:3294 sequence) based on the results of alignment with the fragments under study in Clustal Omega software.

For a phylogenetic analysis in the PHYLIP 3.69 software package, 1000 bootstrap replicas of amino acid alignment were created, then the distances between the sequences (JTT substitution model, gamma = 1.78, based on a previously chosen optimal model) were calculated and the trees were built by a neighbor-joining method (NJ). The PAL sequence of *Arabidopsis thaliana* was used as an out-group. Figure 3 presents the phylogenetic consensus tree; joining was performed according to the 50% majority rule.

Also, MrBayes 3.2 software was used to built Bayes trees (based on a previously chosen model, Jones+G template of amino acid substitutions). The number of mcmc iterations was 600000. Figure 4 presents the phylogenetic tree with the possibility of node formation noted in the nodes. Again, the PAL sequence of *Arabidopsis thaliana* was used as an out-group.

Based on the analysis of phylogenetic trees one may conclude that the studied sequences are divided into two clades. Subgroup Ia includes the following families: *Aureobasidium, Candida, Cystofilobasidium, Debaryomyces, Phaffia, Puccinia, Rhodosporidium, Rhodotorula, Saccharomyces, Tilletiopsis,* and *Tremella.* Clade II includes the following families: *Aspergillus, Chaetomium, Letharia, Magnaporthe, Neosartorya, Neurospora, Penicillium, Phaeosphaeria, Talaromyces,* and *Uncinocarpus.*

Upon the analysis of the tree obtained with Bayesian statistics one may note that the sequences also rather reliably divided into two clades, and the first one (lower clade) corresponds to subgroup Ia, while the second (upper) one corresponds to subgoup Ib and clade II (Figs. 3 and 4).

The template of identity of nucleotide and amino acid sequences (Tables 6 and 7) was created for some of the investigated strains under study (all sequences obtained in the current study, as well as *Arabidopsis thaliana, Aspergillus niger, Neurospora crassa, Puccinia graminis, Rhodosporidium toruloides, Rhodotorula graminis, and Rhodotorula mucilaginosa* sequences) using the CLUSTALW software.

Analysis of the data presented in Tables 5 and 6 evidences that the rate of identity of both nucleotide and amino acid sequences inside a clade is very high and makes up to 93–97% for some species.

A logo-diagram (Fig. 5) was designed for the Ia subgroup demonstrating the fragment is rather conserved.

Therefore, using the phylogenetic analysis the investigated gene has been shown to belong to the *pal* family, and the affinity of the sequences was evaluated.

The performed comparative analysis of sequences from genomes of pigmented yeasts with completely characterized genes encoding L-phenylalanine ammonia-lyase amplified and sequenced using the developed pair of primers demonstrated undoubtedly the sequences belong to the *pal* genes.

Therefore, we developed a universal primer system revealing genes encoding L-phenylalanine ammonia-lyase.

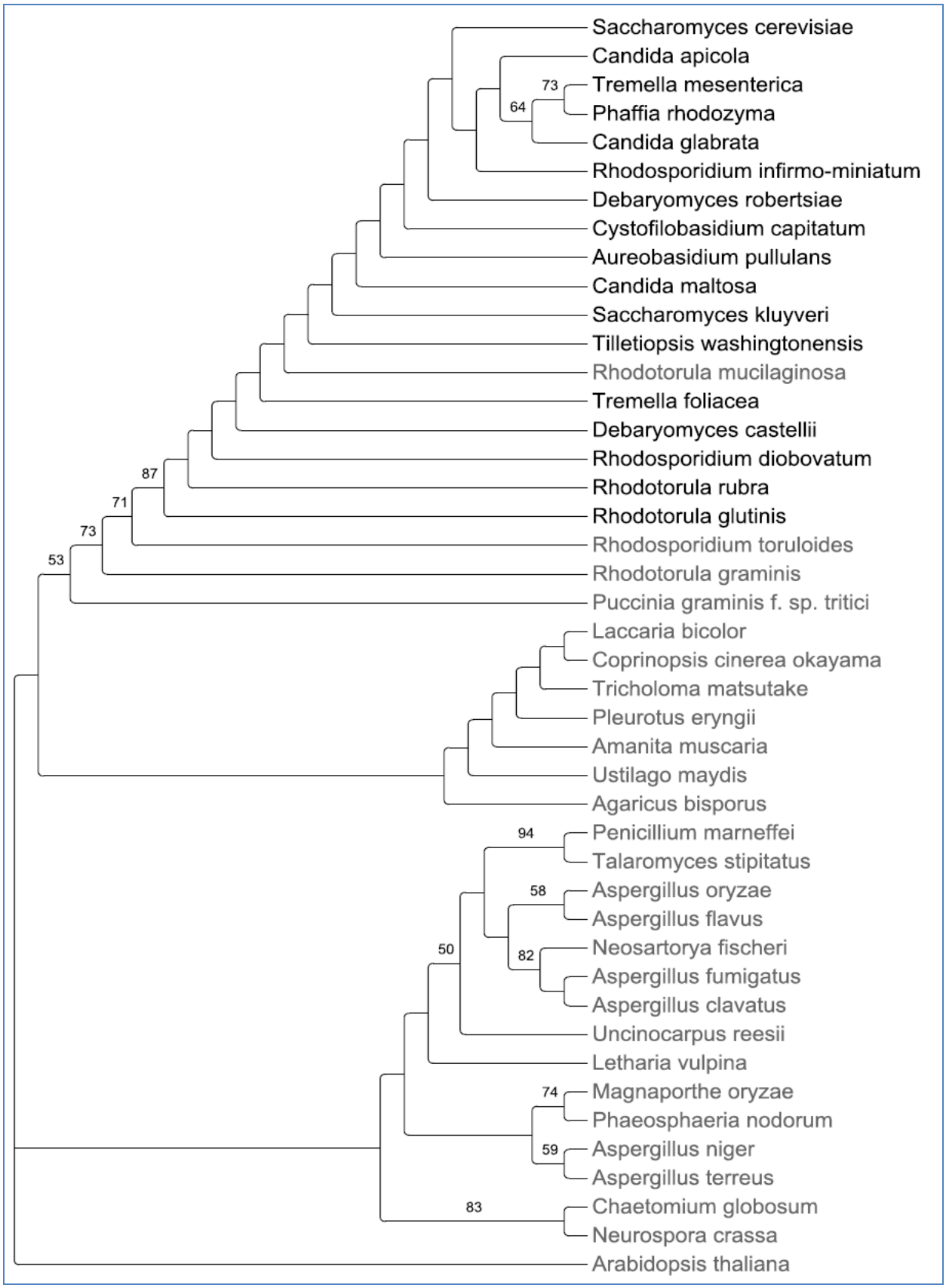

Fig. 3. Consensus tree designed with the PHYLIP 3.69 software using the data on PAL amino acid sequences obtained (trees were built using the neighbor-joining method, JTT substitution model, gamma = 1.78). In the nodes, the percent of bootstrap support is indicated for 1000 replicas. Bold font indicates the PAL sequences obtained in the work, and light font, sequences from NCBI databases.

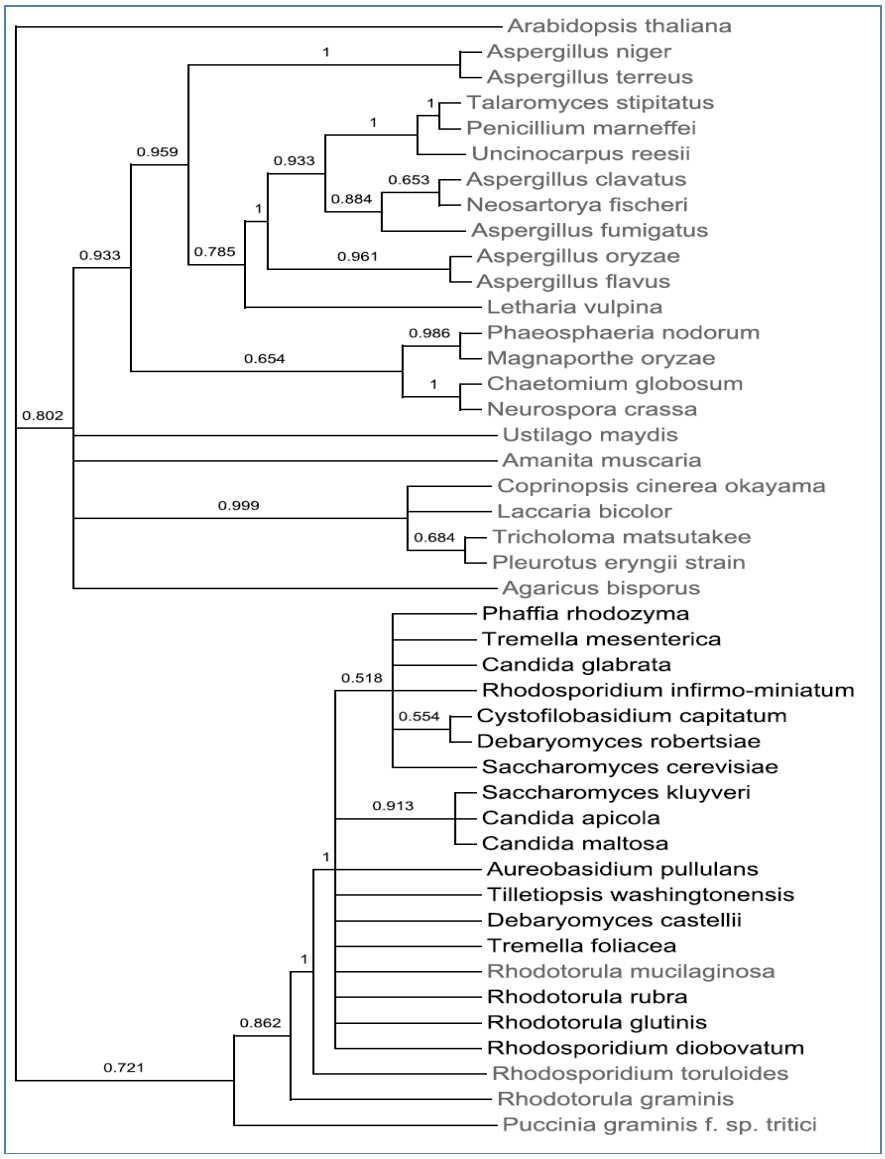

Fig. 4. Bayesian tree built using the MrBayes 3.2 software and the data on the obtained amino acid sequences of PAL (Jones+G substitution model). Node formation possibilities are indicated with the figures. Black letters indicate the sequences obtained in the work, and gray color, sequences from NCBI databases.

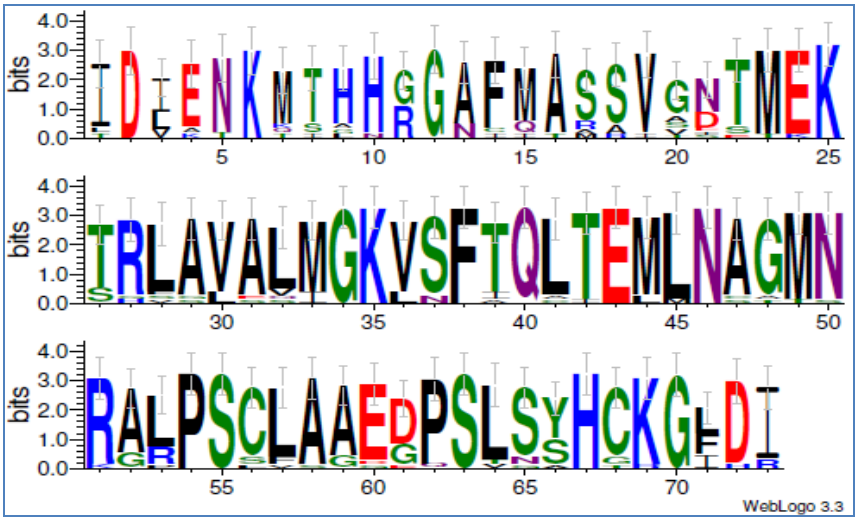

Fig. 5. Logo diagram of the PAL fragment, clade I.

Table 5. Identity percent of the *pal* gene fragment nucleotide sequences in some species

	Arabidopsis thaliana	*Aspergillus niger*	*Aureobasidium pullulans*	*Candida apicola*	*Candida glabrata*	*Candida maltosa*	*Cystofilobasidium capitatum*	*Debaryomyces castellii*	*Debaryomyces robertsiae*	*Neurospora crassa*	*Phaffia rhodozyma*	*Puccinia graminis f. sp. tritici*	*Rhodosporidium diobovatum*	*Rhodosporidium infirmo-miniatum*	*Rhodosporidium toruloides*	*Rhodotorula glutinis*	*Rhodotorula graminis*	*Rhodotorula mucilaginosa*	*Rhodotorula rubra*	*Saccharomyces cerevisiae*	*Saccharomyces kluyveri*	*Tilletiopsis washingtonensis*	*Tremella foliacea*
Aspergillus niger	60																						
Aureobasidium pullulans	23	24																					
Candida apicola	25	22	96																				
Candida glabrata	15	22	93	93																			
Candida maltosa	23	22	96	97	93																		
Cystofilobasidium capitatum	20	22	95	96	93	94																	
Debaryomyces castellii	26	25	90	86	92	93	95																
Debaryomyces robertsiae	18	22	95	96	92	94	96	95															
Neurospora crassa	52	62	21	21	16	21	18	22	18														
Phaffia rhodozyma	9	9	76	77	80	78	77	65	77	8													
Puccinia graminis f. sp. tritici	52	59	20	19	19	23	22	30	19	59	16												
Rhodosporidium diobovatum	37	40	87	84	91	92	95	97	94	33	59	43											
Rhodosporidium infirmo-miniatum	18	22	95	96	94	95	96	96	96	19	78	18	94										
Rhodosporidium toruloides	12	26	71	69	71	73	72	71	72	20	55	27	72	73									
Rhodotorula glutinis	32	35	86	83	89	90	92	94	91	31	58	38	99	92	62								
Rhodotorula graminis	43	47	35	34	32	34	34	41	34	49	21	57	58	33	43	50							
Rhodotorula mucilaginosa	23	22	88	86	83	88	86	87	86	20	65	27	89	86	75	86	38						
Rhodotorula rubra	29	34	80	77	82	84	85	88	85	27	51	35	89	85	72	87	48	97					
Saccharomyces cerevisiae	16	23	91	90	94	91	95	95	96	21	74	27	95	95	70	91	39	85	86				
Saccharomyces kluyveri	20	22	90	90	87	91	89	92	88	18	73	22	93	89	72	91	33	83	84	88			
Tilletiopsis washingtonensis	20	24	95	94	93	94	95	94	96	20	76	24	91	96	71	89	36	86	84	93	90		
Tremella foliacea	23	23	95	94	92	94	95	97	95	18	76	24	97	95	73	93	38	87	87	95	90	96	
Tremella mesenterica	17	22	84	82	82	83	84	78	82	16	67	19	79	84	62	77	32	76	71	82	80	84	83

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Table 6. Identity percent of the *pal* gene fragment amino acid sequences in some species

	Arabidopsis thaliana	*Aspergillus niger*	*Aureobasidium pullulans*	*Candida apicola*	*Candida glabrata*	*Candida maltosa*	*Cystofilobasidium capitatum*	*Debaryomyces castellii*	*Debaryomyces robertsiae*	*Neurospora crassa*	*Phaffia rhodozyma*	*Puccinia graminis f. sp. tritici*	*Rhodosporidium diobovatum*	*Rhodosporidium infirmo-miniatum*	*Rhodosporidium toruloides*	*Rhodotorula glutinis*	*Rhodotorula graminis*	*Rhodotorula mucilaginosa*	*Rhodotorula rubra*	*Saccharomyces cerevisiae*	*Saccharomyces kluyveri*	*Tilletiopsis washingtonensis*	*Tremella foliacea*
Aspergillus niger	41																						
Aureobasidium pullulans	40	41																					
Candida apicola	38	35	92																				
Candida glabrata	30	36	86	85																			
Candida maltosa	39	39	91	94	87																		
Cystofilobasidium capitatum	41	39	88	92	82	89																	
Debaryomyces castellii	43	43	92	84	84	90	87																
Debaryomyces robertsiae	38	38	90	91	86	87	93	90															
Neurospora crassa	43	53	41	40	35	41	43	44	41														
Phaffia rhodozyma	26	26	63	63	63	60	65	60	65	31													
Puccinia graminis f. sp. tritici	46	46	45	44	45	50	49	50	45	49	29												
Rhodosporidium diobovatum	45	41	91	86	86	91	93	95	93	43	53	50											
Rhodosporidium infirmo-miniatum	38	38	87	91	86	90	90	92	91	41	58	47	86										
Rhodosporidium toruloides	47	47	73	71	67	73	72	80	69	50	51	57	84	71									
Rhodotorula glutinis	47	39	91	87	85	91	93	95	91	43	53	52	100	87	85								
Rhodotorula graminis	50	47	68	65	60	67	65	70	64	46	48	58	78	63	79	79							
Rhodotorula mucilaginosa	42	42	94	92	84	94	90	96	89	43	63	52	100	89	79	100	72						
Rhodotorula rubra	44	40	87	83	81	87	88	90	87	44	60	51	100	83	81	100	77	94					
Saccharomyces cerevisiae	38	39	94	92	89	87	93	92	94	42	63	46	93	91	72	93	67	91	88				
Saccharomyces kluyveri	40	37	83	85	83	92	87	85	85	40	63	46	89	87	77	89	66	88	85	83			
Tilletiopsis washingtonensis	41	42	93	88	86	90	90	95	91	43	63	49	93	91	75	93	68	94	88	94	88		
Tremella foliacea	40	39	90	86	86	91	91	93	95	42	65	49	97	90	77	95	70	96	90	93	87	95	
Tremella mesenterica	27	28	72	69	71	66	72	69	71	31	58	33	82	69	59	81	50	71	74	75	68	72	68

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Study of the Specific Features of Phenotype and Biochemical Properties of the Selected Cultures

Phenotypic characteristics of the selected pigmented yeasts were judged by the combination of micro- and macromorphological traits (the former are studied with a microscope and the latter, visually). Micromorphology includes the features characterizing individual vegetative cells (shape, size), as well as the types of vegetative or asexual reproduction and the structures formed in the process. Macromorphology joins the culture features characterizing culture growth on dense (following the streak or in the form of a giant colony) or in liquid medium.

On a complete yeast medium (g/cm^3 distilled water: peptone, 10; yeast extract, 5; glucose, 20; agar, 20), yeasts form colonies of intermediate size and white or creamy color, colony surface is smooth, dim, elevated by a cone, colony edges are even or slightly wavy.

Description of the isolated yeast strains was performed according to a standard scheme. Shape and size of cells were described and determined in cultures of different ages on dense and liquid media. The first examination fnd measurement of cell size was performed in 2–3-day cultures grown at 25–28°C. Then, the cultures were left at room temperature (17–18°C) and described again after 4 weeks.

To determine cell size, length and width were measured with a micrometer in at least 20 cells, and the extreme values were indicated. Cells of isolated yeasts were found to be of round, round-like, oval, and cylindrical shape.

Size of mature yeast cells varied in different species from 1.0 to 8.0 μm in width and reached 17 μm and more in length in case of elongated cells. The results are presented in Table 7.

Further studies were aimed at determination of L-phenylalanine ammonia-lyase activity in the selected pigmented yeasts. The results of the investigation are presented in Table 8.

Table 7. Phenotypic traits of pigmented yeasts

Strain	Size, μm	Shape	Edge outline	Relief	Surface	Color
Candida glabrata Y2813	2.5 × 6.0	oval	uneven edges	smooth	dim	creamy
Aureobasidium pullulans Y863	8.0 × 6.0	ellipse-like	uneven edges	wrinkled	dim	creamy
Tremella foliacea Y1624	2.0 × 6.5	round	uneven edges	smooth	shiny	creamy
Phaffia rhodozyma Y1668	3.8 × 10.0	round-to-oval	uneven edges	smooth	dim	red orange
Rhodotorula lactose Y2770	2.5 × 8.0	oval-to-elongated	uneven edges	smooth	shiny	red pink
Rhodosporidium diobovatum Y1565	1.0 × 9.0	round-to-oval	uneven edges	wrinkled	dim	red pink
Rhodotorula rubra Y1193	2.3 × 6.5	oval-to-elongated	even edges	smooth	shiny	red pink
Tremella mesenterica Y1625	2.0 × 8.5	round-to-oval	uneven edges	smooth	shiny	creamy
Debaromyces castellii Y 968	3.8 × 8.5	round-to-oval	uneven edges	wrinkled	dim	white
Candida maltose Y242	2.0 × 7.0	round-to-cylindrical	even edges	smooth	shiny	creamy
Rhodosporidium capitatum Y1567	1.0 × 14.0	round-to-cylindrical	even edges	smooth	dim	pink orange
Saccharomyces kluyveri Y2559	5.0 × 12.0	round-to-cylindrical	even edges	smooth	shiny	light creamy
Bullera alba Y 1581	2.0 × 6.5	oval	uneven edges	smooth	dim	creamy
Rhodotorula glutinis Y77	2.3 × 10.0	round-to-oval	even edges	smooth	dim	red pink
Rhodosporidium infirmominiatum Y1569	1.0 × 10.0	round-to-oval	even edges	smooth	dim	red pink
Debaryomyces robertsiae Y3392	2.8 × 8.0	round-to-oval	uneven edges	wrinkled	dim	white
Saccharomyces cerevisiae Y1127	5.0 × 12	round-to-cylindrical	even edges	smooth	shiny	light creamy
Tilletiopsis washingtonensis Y1650	1.0 × 17.0	round-to-cylindrical	uneven edges	wrinkled	dim	creamy
Torulopsis apicola Y566	2.0 × 6.0	round-to-oval	even edges	smooth	shiny	yellowish-creamy

Note:. Width × length.

Table 8. L-phenylalanine ammonia-lyase activity in selected pigmented yeasts

No.	Yeast	L-phenylalanine ammonia-lyase activity, U/mg protein
1	*Aureobasidium pullulans* Y863	0.038
2	*Bullera alba* Y1581	0.010
3	*Bullera piricola* Y1577	0.008
4	*Candida glabrata* Y2813	0.007
5	*Candida maltosa* Y242	0.012
6	*Cryptococcus laurentii* Y227	0.010
7	*Cryptococcus macerans* Y2763	0.008
8	*Cystofilobasidium capitatum* Y1852	0.010
9	*Debaryomyces castellii* Y968	0.007
10	*Debaryomyces robertsiae* Y3392	0.012
11	*Dioszegia hungarica* Y3208	0.010
12	*Dioszegia sp.* Y3320	0.008
13	*Geotrichum klebahnii* Y3053	0.009
14	*Phaffia rhodozyma* Y1668	0.009
15	*Rhodosporidium diobovatum* Y1565	0.022
16	*Rhodosporidium infirmominiatum* Y1569	0.019
17	*Rhodotorula glutinis* Y77	0.008
18	*Rhodotorula lactosa* Y2770	0.049
19	*Rhodotorula rubra* Y1193	0.015
20	*Saccharomyces cerevisiae* Y1127	0.016
21	*Saccharomyces kluyveri* Y2559	0.010
22	*Sporobolomyces holsaticus* Y991	0.009
23	*Tilletiopsis washingtonensis* Y1650	0.017
24	*Torulopsis apicola* Y566	0.011
25	*Tremella foliacea* Y1624	0.019
26	*Tremella mesenterica* Y1625	0.010

The data presented in Table 8 evidence that the highest activity was exhibited by the strains *Aureobasidium pullulans* Y863, *Rhodosporidium infirmominiatum* Y1569, *Candida glabrata* Y2813, *Candida maltose* Y242, *Debaryomyces robertsiae* Y3392, *Rhodosporidium diobovatum* Y1565, *Rhodotorula lactose* Y2770, *Saccharomyces cerevisiae* Y1127, *Tilletiopsis washingtonensis* Y1650, *Torulopsis apicola* Y566, *Tremella foliacea* Y1624, *Rhodotorula rubra* Y1193, and *Debaryomyces castellii* Y968, which allows to recommend them for further studies aimed at the development of an enzyme preparation of L-phenylalanine ammonia-lyase.

Therefore, we analyzed the DNA sequence encoding the synthesis of L-phenylalanine ammonia-lyase (PAL) to create universal primers complementary to conserved regions of the *pal* gene. The pal gene fragment was amplified in the organisms under study. Nucleotide sequence of the *pal* gene in microorganisms possessing L-phenylalanine ammonia-lyase activity was determined by DNA sequencing. The sequence was compared with the relevant sequences of known species. Phenotypic features and biochemical properties of the selected cultures were investigated.

Acknowledgments

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REFERENCES

1. Sarkissian, C.N., and Gamez, A., Phenylalanine ammonia lyase, enzyme substitution therapy for phenylketonuria, where are we now? *Mol. Genet. Metab.*, 2005. V. 86, P. 22–26.
2. Rother, D., Poppe, L., Morlock, G., Viergutz, S., and Re´tey, J., An active site homology model of phenylalanine ammonia-lyase from *Petroselinum crispum*, *Eur. J. Biochem.*, 2002. V. 269, P. 3065–3075.
3. Dixon, R.A., Stress-induced phenylpropanoid metabolism, *Plant Cell*, 1995. V. 7, P. 1085–1097.
4. Bezanson, G.S., Desaty, D., Emes, A.V., and Vining, L.C., Biosynthesis of cinnamamide and detection of phenylalanine ammonia-lyase in *Streptomyces verticillatus*, *Can. J. Microbiol.*, 1970. V. 16, P. 147–151.
5. Gilbert, H.J., The effect of proteinases on phenylalanine ammonia-lyase from the yeast *Rhodotorula glutinis*, *Biochem.*, 1981. V. 199, P. 715–723.
6. Evans, C.T., Hanna, K., Payne, C., Conrad, D., and Misawa, M., Biotransformation of trans-cinnamic acid to L-phenylalanine: Optimization of reaction conditions using whole yeast cells, *Enzyme Microb. Technol.*, 1987. V. 9, P. 417–421.

7. Hodgins, C.W., and Stith, D.S., An in vivo evaluation of the chemotherapeutic potency of phenylilalanine ammonia-lyase, *Cancer Research*, 1973. V. 33, P. 2529–2532.
8. Sarkissian, C.N., Shao, Z., Blain, F., Peevers, R., Su, H., Heft, R., Chang, T.M.S., and Scriver, C.R., A different approach to treatment of phenylketonuria: Phenylalanine degradation with recombinant phenylalanine ammonia lyase, *Proc. Natl. Acad. Sci. USA*, 1999. V. 96, P. 2339–2344.
9. Leyva, A., Liang, X., Pintor-Toro, J.A., and Dixon, R.A., Ciselement combinations determine phenylalanine ammonia-lyase gene tissue-specific expression patterns, *Plant Cell*, 1992. V. 4, P. 263–271.
10. Church, G.M., and Gilbert, W., Genomic Sequencing, *Proc. Natl. Acad. Sci. USA*, 1991. V. 22, P. 1991–1995.
11. Cochrane, F.C., Davin, L.B., and Lewis, N.G., The Arabidopsis phenylalanine ammonia lyase gene family: kinetic characterization of the four PAL isoforms, *Phytochemistry*, 2004. V. 65, P. 1557–1564.
12. Dixon, R.A., and Brown, M., Ward Modulation of L-phenylalanine ammonia-lyase by pathway intermediates in cell suspension cultures of dwarf French bean (Prtaseo/us vulgaris L), *Planta*, 1980. V. 150, P. 279–285.
13. *Genosistematika — chto eto takoe?* (Genosystematics: What is it?) http://www.rusbiotech.ru/article/.
14. Babich, O.O., Prosekov, A.Yu., and Soldatova, L.S., Modeling of a continuous biotechnological process of L-phenylalanine ammonia-lyase production, *Izvestiya vysshykh uchebnykh zavedenii. Seriya: Khimiya i khimicheskie tekhnologii* (Higher Education Institution Herald. Chemistry and chemical technology series), 2013. V. 56 (4), P. 106–109.

ANALYSIS OF THE STRUCTURAL AND MECHANICAL PROPERTIES AND MICROMORPHOLOGICAL FEATURES OF POLYMERIC FILMS BASED ON HYDROCOLLOIDS OF VEGETABLE ORIGIN USED FOR THE PRODUCTION OF BIODEGRADABLE POLYMERS

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Abstract: Modern research and technology approaches to the production of biodegradable polymeric materials based on renewable resources have been reviewed. It has been found that films prepared of cellulose, chitosan, gelatin, polypeptides, casein, soy, wheat, corn, rice, and maize are being commonly used at present. The structural and mechanical properties and micromorphological features of hydrocolloids of vegetable origin promising for the production of biodegradable polymers—starches, pectins, carrageenans, and agar—have been studied. It has been determined that, with respect to strength and suitability for use in films of individual components, all the studied hydrocolloids can be arranged in ascending order as follows: starches, carrageenans, pectins, agar. According to analysis of the structural and mechanical properties of the films, it has been shown that the best parameters are found for the samples based on pectin P1 and agar A2. The breaking stress for these materials is 52 and 77 MPa, respectively. The breaking strain is 11.5 and 8.0%, respectively. Analysis of the micromorphology has revealed the formation of surface microdiscontinuities in the films based on high methoxyl pectins P1 and P4 and unmodified corn starch S3 and the formation of wavy folds in the case of the films of kappa-carrageenan C1; these folds are formed during drying and decrease the tensile strength of the respective films. The found features will be used in the development of technologies for the production of biodegradable polymeric materials based on hydrocolloids of vegetable origin with enhanced performance and processing characteristics.

Keywords: biodegradable materials, hydrocolloids, starch, pectin, agar, carrageenan, micromorphological properties, mechanical properties

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INTRODUCTION

The important environmental issues that are extensively discussed at the present day include the salvation of nature from debris, the direction of development of the industry of biodegradable polymeric materials, and the rates of annual output [1–4].

It is known that most of the plastics used in the global production are based on products of processing of hydrocarbon-containing raw materials (propylene, ethylene, and other organic compounds). However, the further focusing on these technologies is attributed to the rise in prices for hydrocarbon-containing raw materials and negative impacts on the environment. In addition, the amount of plastic waste also increases; the waste disposal requires studies on the development of technologies for the production of stable biodegradable plastics for various purposes. This direction is consistent with the modern concept of improvement and development of the production of useful goods from waste. These raw materials include biological waste; prior to use, it must be processed and modified by physicochemical methods [5].

The previous studies were primarily focused on the design of polymeric materials that are resistant to environmental factors. At present, there is a new approach to the development of polymeric materials; it is based on maintaining their performance characteristics only in the period of their use. Despite all the difficulties faced by scientists, this direction of polymer materials science is part of their research interests, as evidenced by recent publications [6].

Modern biopolymers can be produced from both renewable natural resources and conventional raw materials, i.e., petrochemicals [7]. At present, films based on natural biodegradable polymers, such as cellulose, chitosan, gelatin, polypeptides, casein, and soybean, are commonly used in the food industry [8–11]. Of particular interest is starch because it is the cheapest raw material and the main sources of its industrial production are potatoes, wheat, corn, rice, maize, and some other plants [12–14].

Russian scientists have developed approaches to the preparation of biodegradable polymers based on enzymatic hydrolysate of keratin waste [15].

A research trend of high priority is the development of new approaches to the production of biodegradable polymers exhibiting a high rate of biodegradation, enhanced performance characteristics, and a low cost.

The aim of this study is to analyze the structural and mechanical properties and micromorphological features of polymer films based on hydrocolloids of

vegetable origin that are used for the production of biodegradable polymers.

OBJECTS AND METHODS OF RESEARCH

The objects of research are listed in Table 1.

The films of the studied components were prepared by drying solutions of the test substances. The solutions at a temperature of 60°C were poured onto preformed Teflon substrates (Fig. 1). Drying was conducted under gradual cooling for a few hours. To this end, the samples were placed in a TS-1/20-SPU thermostat (SKTB, Russia) heated to 60°C; after that, the heat supply was turned off and the samples were left in the thermostat overnight. In 10 h, the door of the thermostat was slightly open and the samples were dried for 2 days until the cessation of weight loss. The resulting films were cut in strips measuring 6 cm in length and 1 cm in width. The film thickness was measured using an MSC 25 electronic micrometer (Matrix, China). The thickness of the different films varied from 0.15 to 0.5 mm; this fact was taken into account in the calculations.

Table 1. Objects of research

Designation	Denomination
	Starches
S1	Acetylated distarch adipate C'Tex 06201 food supplement, Cargill B.V.
S2	Modified corn starch C'Tex 06205 food supplement, Cargill B.V.
S3	Corn starch, MP Biomedicals, CAS 9005-25-8
S4	Modified corn starch, Thermtex
	Pectins
P1	Pectin ARA 103, Yantai Andre Pectin
P2	Pectin ARA 104, Yantai Andre Pectin
P3	Pectin ARS 105, Yantai Andre Pectin
P4	Pumpkin pectin, ZAO NPO Evropa-Biofarm
	Carrageenans
C1	Refined kappa-carrageenan E-407, CAS 9000-07-1
C2	Semirefined iota-carrageenan, OOO Nord Plus
	Agar
A1	QP agar-agar, Panreac
A2	Agar, Helicon

To determine the structural and mechanical properties, the prepared films of hydrocolloids with given sizes were fixed using clamps with rubber heads and placed in an LR50KPlus universal tensile testing machine (Lloyd, United Kingdom). The measurement was conducted by uniaxial tension with breaking of the sample. Stress–strain diagrams were recorded in the experiment. The parameters of the diagrams were calculated using the software of the instrument.

The micromorphology of the films based on components for the production of biodegradable polymers was examined by atomic force microscopy in the tapping mode. The scanning of the samples was conducted on a SmartSPM atomic force microscope (AIST-NT, Russia) using fpN01S cantilevers (Nanotyuning, Russia); the radius of curvature was ≤10 nm. The resulting images were analyzed using the Gwiddion software (Czech Metrology Institute, Czech Republic).

For each scan of the film surface, two images are shown below: the surface topography and the image recorded in the amplitude registration mode. Since the film surface is extremely heterogeneous and characterized by a large height difference, the image based on the amplitude registration is more informative because it reveals more details.

RESULTS AND DISCUSSION

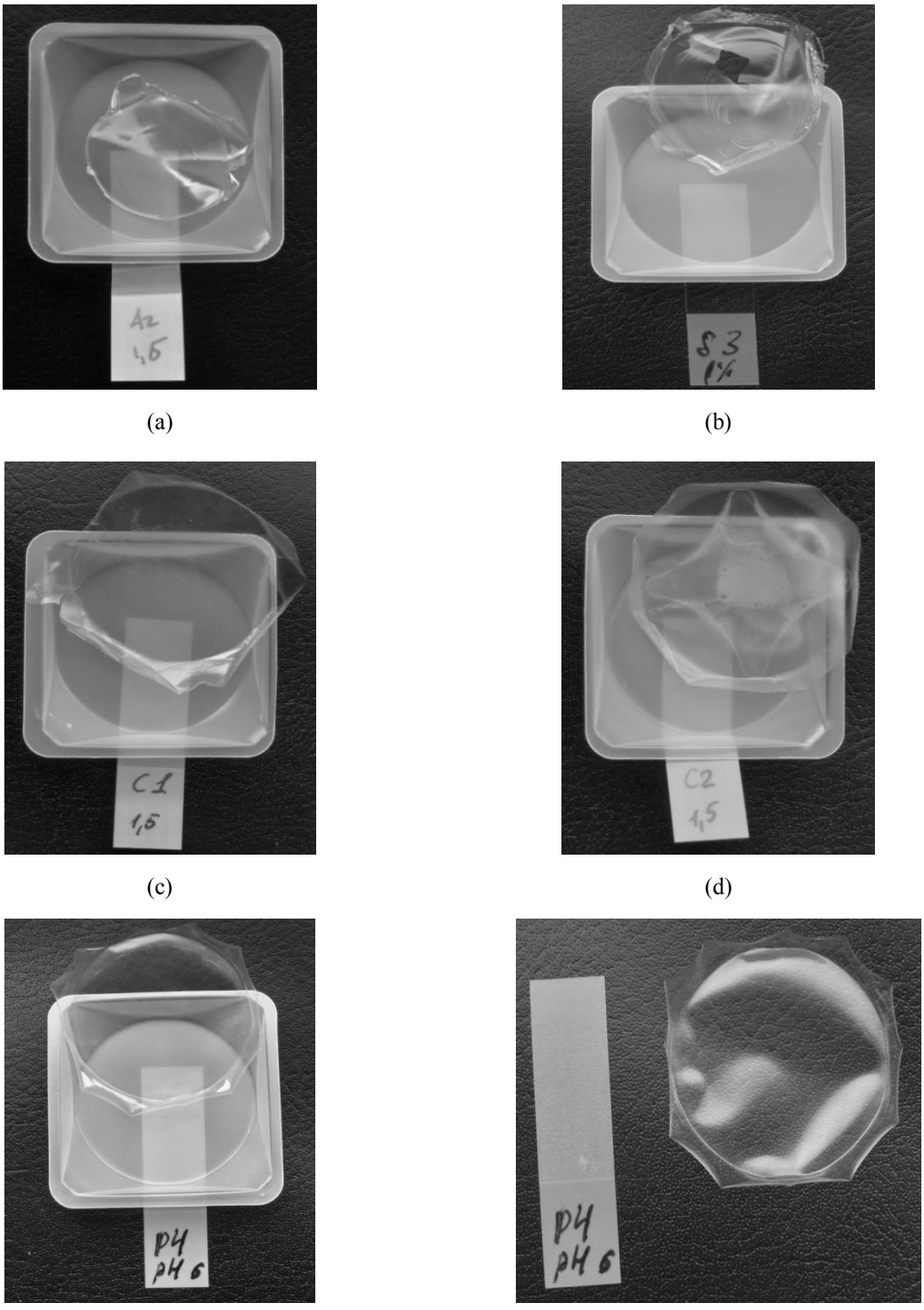
The mechanical properties of the films are among the most important characteristics of gelling agents used in mixtures for producing biodegradable materials. The substances used in the study are capable of forming their own films during drying of the solution. The parameters of the films determined in this study are Young's modulus, breaking stress, and breaking strain. These parameters can be used in predicting the properties of the final biodegradable polymers comprising the test substances.

The studies have revealed that the starch films are the most brittle; they are partially transparent, solidify nonuniformly and form a wavy surface (Fig. 1b). An increase in the starch concentration results in an increase in the brittleness of the material. The films of agar A1 and A2 are thick and dense; they are not brittle and undergo strong deformation during drying (Fig. 1a). The films of carrageenans C1 and C2 differ in properties (Figs. 1c, 1d). Kappa-carrageenan C1 forms very thin cellophane-like films, which are transparent and exhibit a fairly high tensile strength. Iota-carrageenan C2 forms thick brittle varicolored turbid films. Pectins, except for P3 (Fig. 1e), are dried to form smooth thin transparent films. The films of P1 are tinted; however, they preserve transparency. The pectin films, in common with the films of agars, are not brittle.

Despite the mild conditions of drying, some of the film samples were declared unfit for further examination after complete drying. This is primarily attributed to the fact that these compounds cannot retain water as a natural plasticizer. Thus, the films of most starches are extremely brittle; the films of some pectins and agars undergo strong deformation during drying; the carrageenan films do not dry uniformly.

The stress–strain diagrams were recorded for all classes of the test compounds: starches, pectins, agars, and carrageenans (Fig. 2). In general, the stress–strain diagrams derived for agar (A1 and A2) and pectin (P4) were typical for that sort of studies. This is indicative of the correct sample preparation and the possibility in principle to use solutions of these substances for preparing films suitable for research. The diagram for carrageenan C1 is not typical, which can be indicative of the nonuniform drying of the sample.

Some parameters of the resulting films, such as Young's modulus, breaking stress, and breaking strain, can be estimated from the stress–strain diagrams (Table 2).



(a) (b) (c) (d) (e)

Fig. 1. Examples of films of the test substances: (a) 1.5% agar-agar A1, (b) 1% starch S3, (c) 1.5% carrageenan C1, (d) 1.5% carrageenan C2, and (e) 1.5% pectin P4 prepared in a universal buffer solution with a pH of 6.

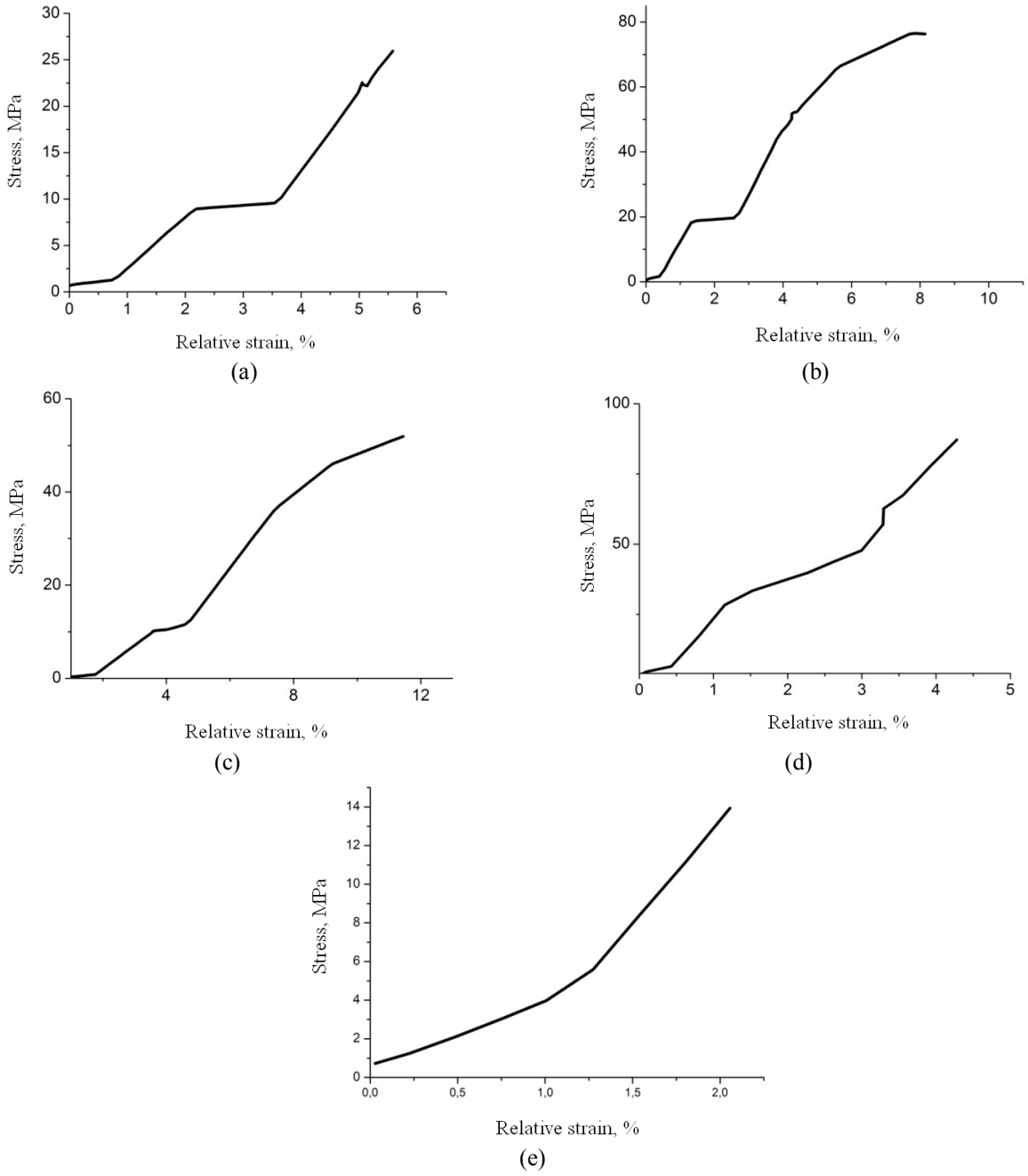


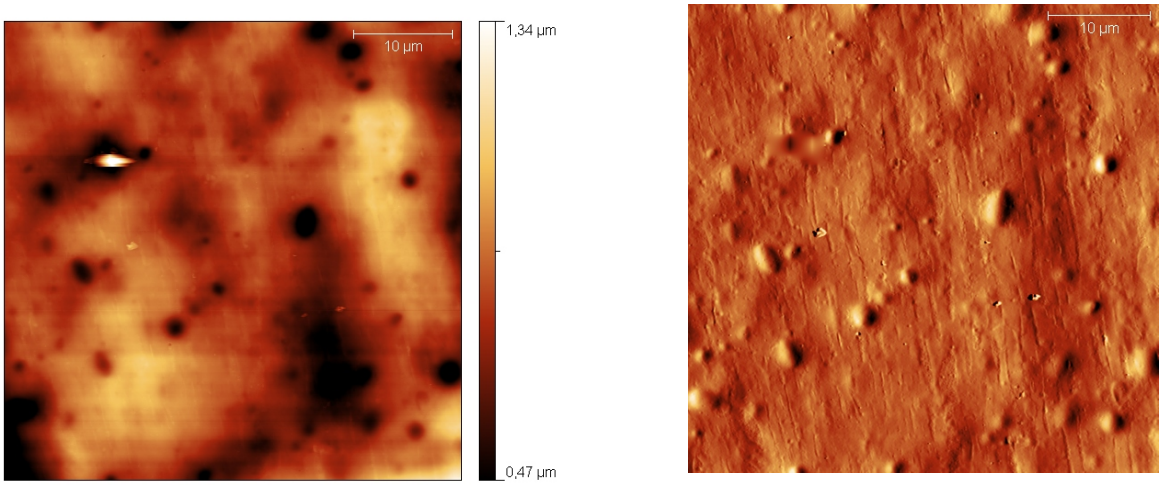
Fig. 2. Examples of stress–strain diagrams for the studied substances: (a) 1.5% agar A1, (b) 1.5% agar A2, (c) 1.5% pectin P1, (d) 1.5% pectin P4, and (e) 1.5% carrageenan C1.

Table 2. Structural and mechanical properties of films based on the components for the production of biodegradable polymers

Sample	Breaking stress σ_p, MPa	Breaking strain ε_p , %	Young's modulus *E*, MPa
C1-1	14.0	2.10	1025
C1-2	8.0	2.10	1250
C2-1	4.2	0.44	1600
C2-2	12.6	2.20	3800
A1-1	26.6	6.00	923
A2-2	77.0	8.00	2203
P1-3	52.0	11.50	904
P4-3	90.0	4.30	2200

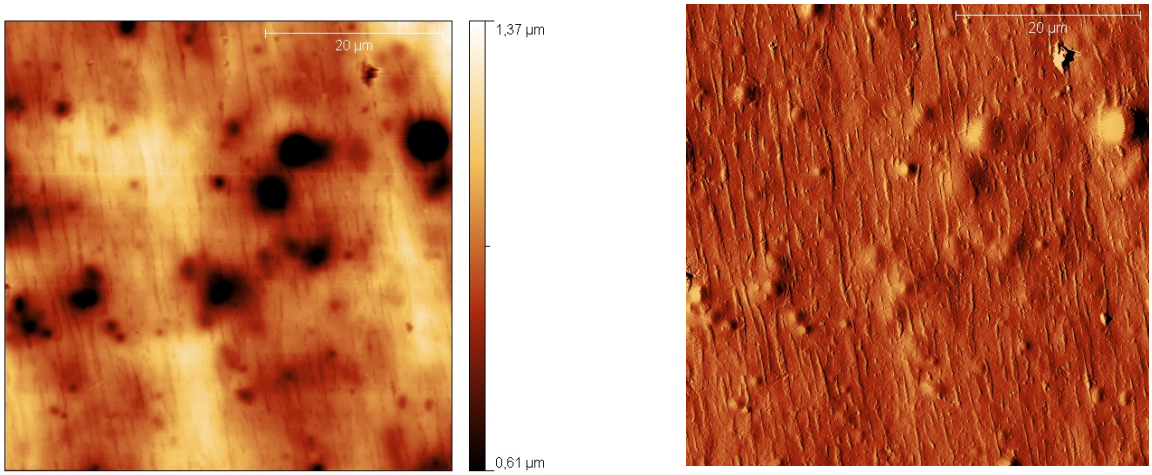
Films with the highest strength are formed from solutions of agar A2 and pectin P4; the breaking stress of these samples is greater than 75 MPa. The films of pectin P1 withstand the highest deformation before breaking; the breaking strain of these films is more than 10%. The lowest strength is exhibited by the films of carrageenans C1 and C2; for these samples, the breaking stress is lower than 15 MPa and the breaking strain is less than 2.5%.

Results of examination of the micromorphology of the films show that the samples of pectins with the highest degrees of esterification form films with transverse striations (Figs. 3, 4), which apparently results from the drying of the film.



(a) (b)

Fig. 3. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of pectin P1 in the amplitude registration mode.



(a) (b)

Fig. 4. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of pectin P4 in the amplitude registration mode.

A reduction in the size of the scanned surface, in the case of the samples of pectins P1 and P4, made it possible to visualize individual chains of polysaccharides (Figs. 5b, 6b). These images recorded in the amplitude registration mode show branched molecules of rhamnogalactouronan, which is one of the components of pectins. The size of the rhamnogalactouronan molecules varies in a range of up to 400 nm, which is consistent with published data [16, 17].

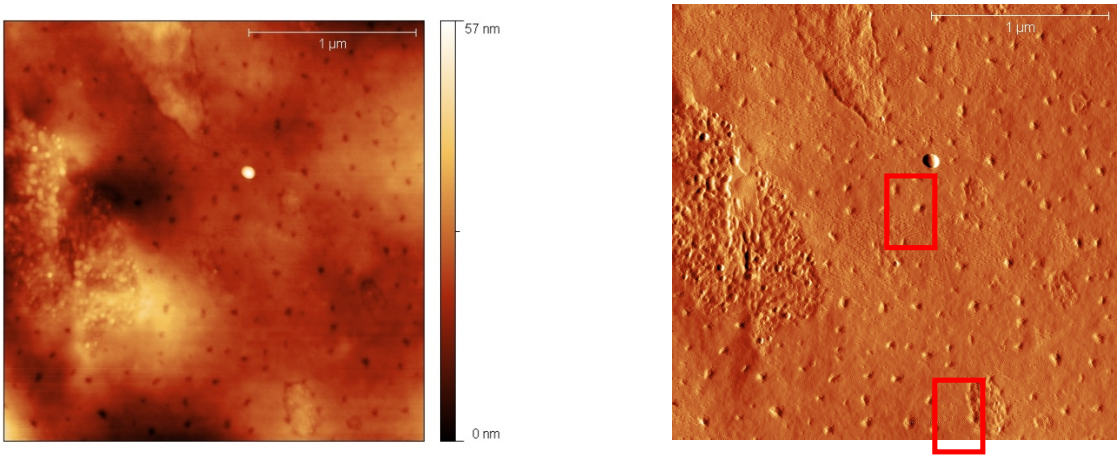
Unlike the samples of pectins P1 and P4, pectin P2 with a lower degree of esterification forms films with a grained surface (Fig. 7) in which individual chains of polysaccharides are not visualized.

The drying of a 1% aqueous solution of unmodified corn starch S3, similarly to pectins P1 and P4, results in the formation of a film with transverse striations caused by the formation of surface discontinuities (Fig. 8). At the same time, all the studied samples of modified corn starches are characterized by the formation of films in which smooth surface regions alternate with rough regions (Figs. 9–11). Apparently, modification of corn starch leads to an increase in the stability of the respective films and to the formation of surface microdiscontinuities during drying.

Analysis of the micromorphology of the films resulting from drying of a 1% aqueous solution of

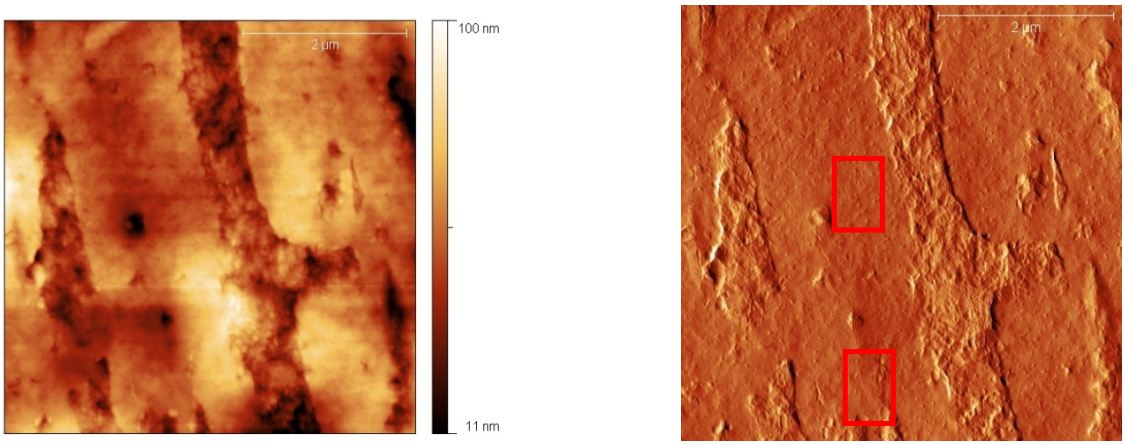
kappa-carrageenan C1 revealed wavy contractions; this fact explains why the films are readily torn off after tearing from the edge (Fig. 12).

According to the micromorphological studies, the surface of the films based on agars A1 and A2 was characterized by almost uniformly distributed roughnesses and the absence of transverse striations (Figs. 13, 14).



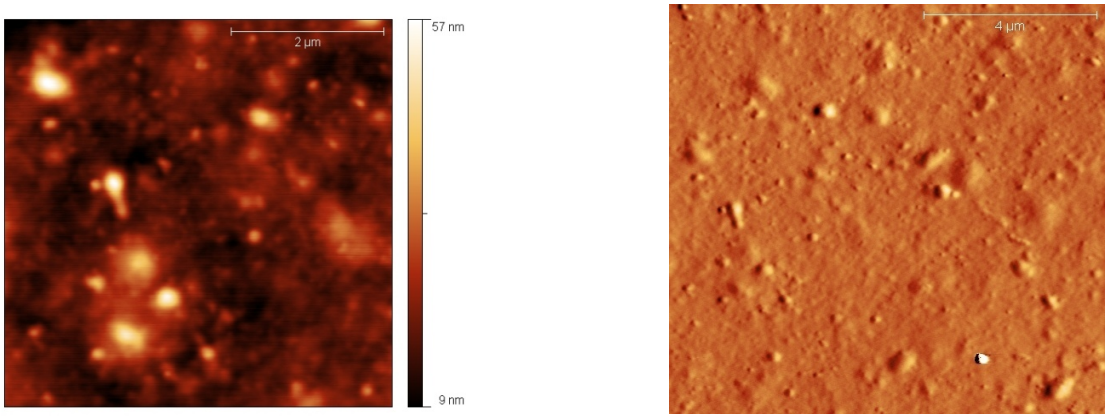
(a) (b)

Fig. 5. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of pectin P1 in the amplitude registration mode.



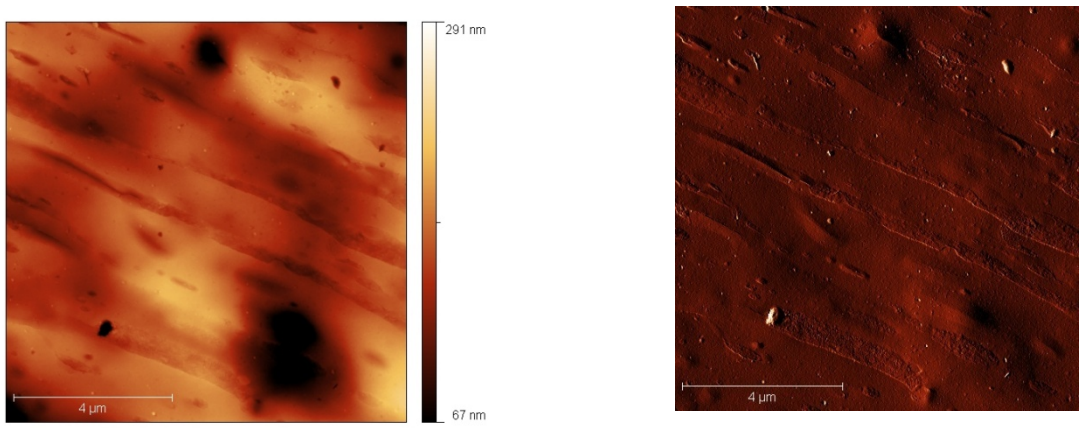
(a) (b)

Fig. 6. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of pectin P4 in the amplitude registration mode.



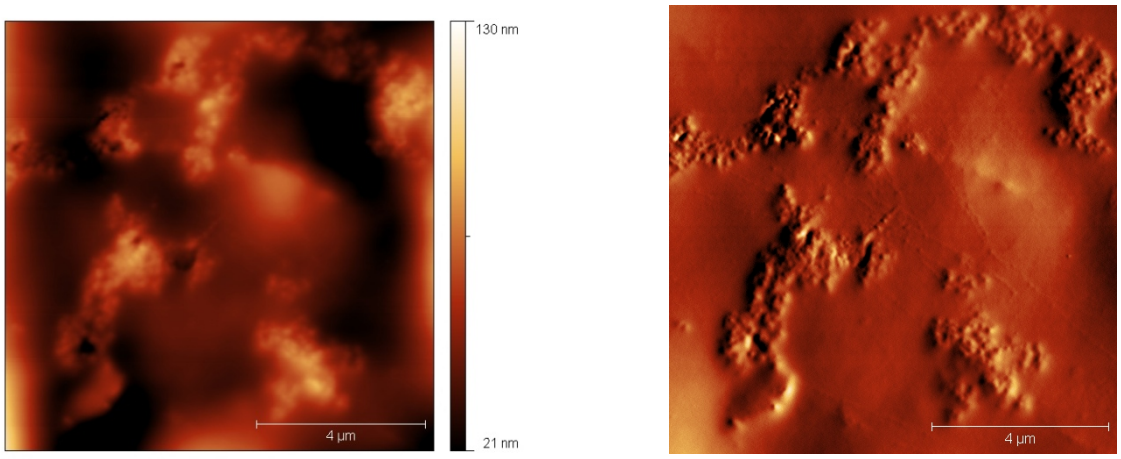
(a) (b)

Fig. 7. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of pectin P2 in the amplitude registration mode.



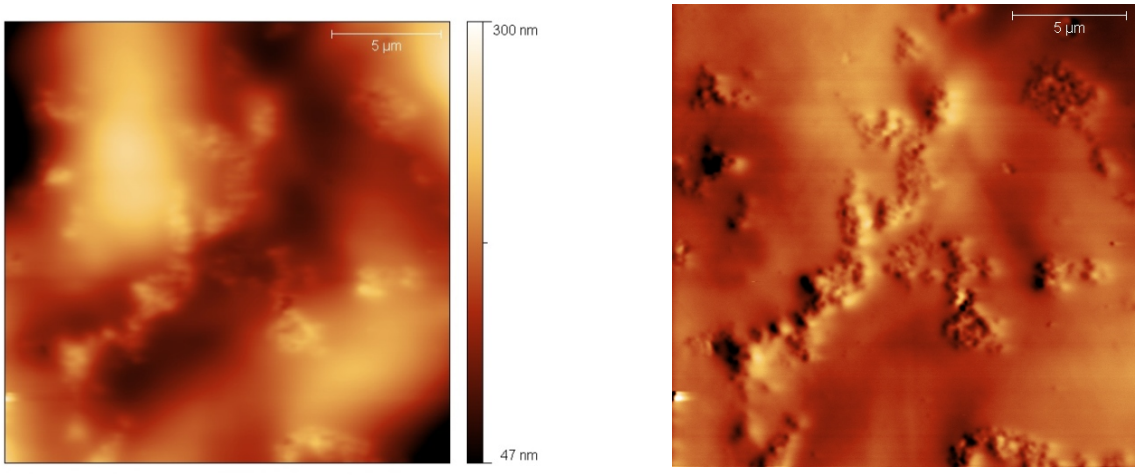
(a) (b)

Fig. 8. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of native corn starch S3 in the amplitude registration mode.



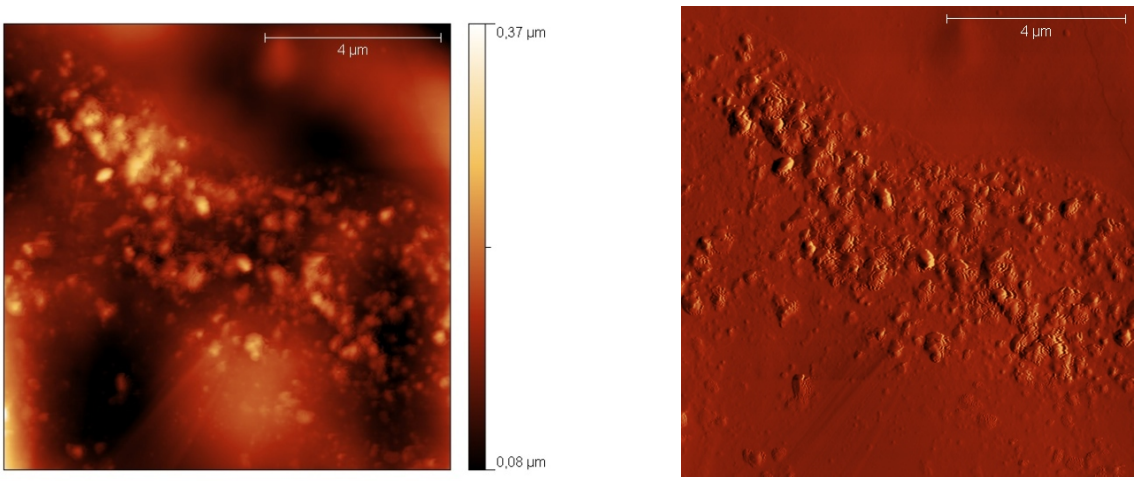
(a) (b)

Fig. 9. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of modified corn starch S1 in the amplitude registration mode.



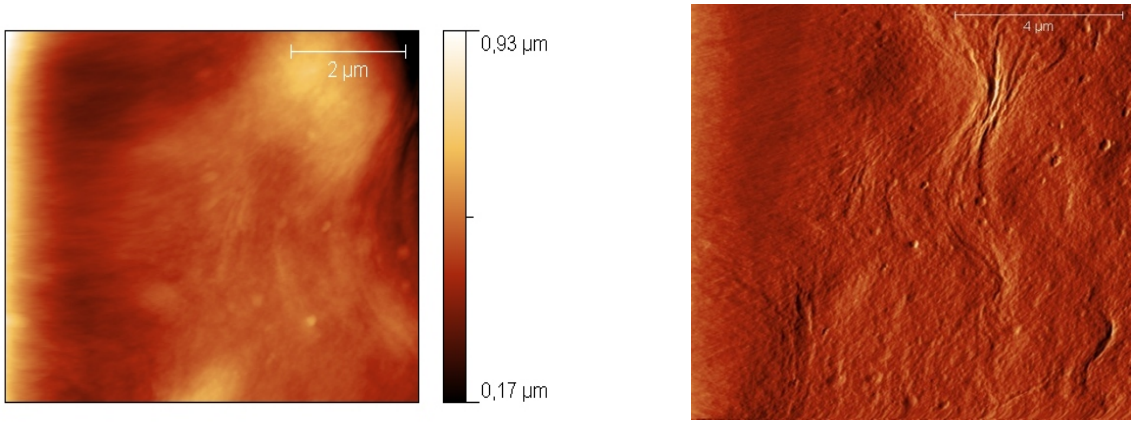
(a) (b)

Fig. 10. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of modified corn starch S2 in the amplitude registration mode.



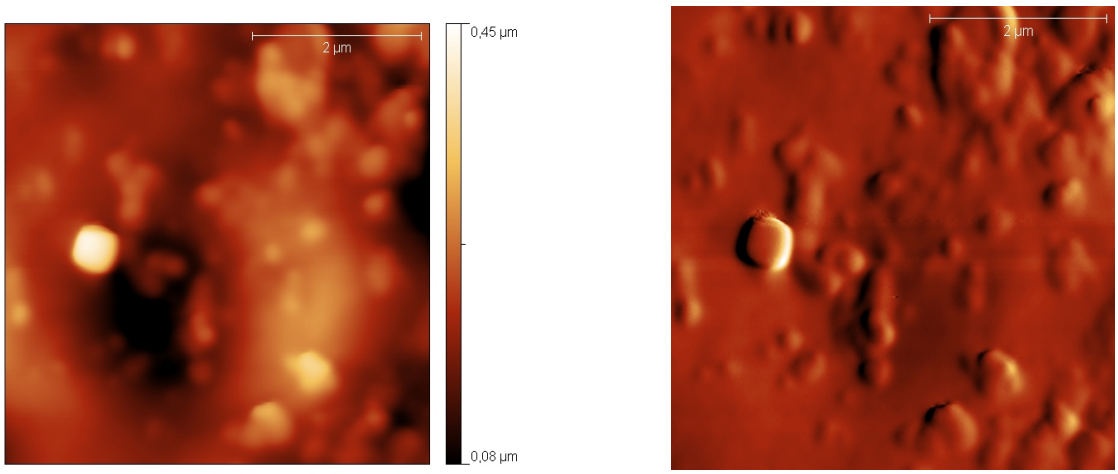
(a) (b)

Fig. 11. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of modified corn starch S4 in the amplitude registration mode.



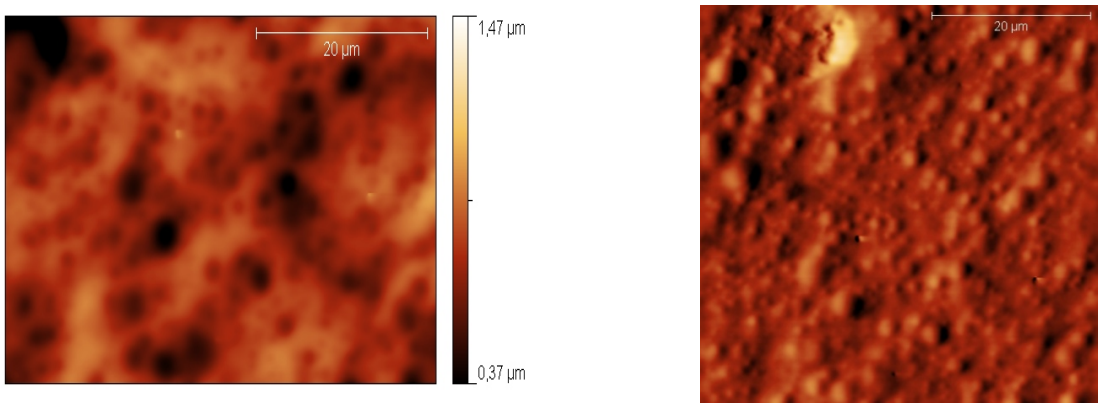
(a) (b)

Fig. 12. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of kappa-carrageenan C1 in the amplitude registration mode.



(a) (b)

Fig. 13. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of agar A1 in the amplitude registration mode.



(a) (b)

Fig. 14. (a) Topography and (b) image of the surface of the film prepared by drying a 1% aqueous solution of agar A2 in the amplitude registration mode.

Results of the structural and mechanical analysis and microscopic studies suggest the following.

1. By drying solutions under mild conditions, films for all the studied components—agar-agar, starches, carrageenans, and pectins—have been prepared. It has been found that some components are incapable of independently forming films suitable for examination of structural and mechanical properties and morphological features. This fact limits their use in the composition of technological mixtures for producing biodegradable polymers.

2. Parameters of the prepared films for all classes of the studied components have been determined. With respect to strength and suitability for use in films of individual components, all the studied components can be arranged in ascending order as follows: starches, carrageenans, pectins, agar.

3. Analysis of the structural and mechanical properties of the films has revealed the best results for the samples based on pectin P1 and agar A2. The breaking stress of these films is 52 and 77 MPa, respectively. The breaking strain is 11.5 and 8.0%, respectively.

4. Analysis of micromorphology has revealed the formation of surface microdiscontinuities in the films based on high methoxyl pectins P1 and P4 and unmodified corn starch S3 and the formation of wavy folds in the case of the films of kappa-carrageenan C1; these folds are formed during drying and decrease the tensile strength of the respective films.

REFERENCES

1. Fomin, V.A. and Guzeev, V.V., Biorazlagaemye polimery, sostoyanie i perspektivy ispol'zovaniya (Biodegradable polymers: State-of-the-art and prospects), *Plast. Massy* (Plastics), 2001. № 2. P. 42.
2. Buryak, V.P., Biopolimery. Est' li al'ternativa? (Biopolymers: Is there an alternative?), *Polim. Mater.* (Polym. Mater.), 2006. № 1. P. 32.
3. Vlasov, S.V. and Ol'khov, V.V., Biorazlagaemye polimernye materialy (Biodegradable polymer materials), *Polim. Mater.* (Polym. Mater.), 2006. № 7. P. 23.
4. Shah, A.A., Hasan, F., Hameed, A., and Ahmed, S., Biological degradation of plastics: A comprehensive review, *Biotechnol. Adv.,* 2008. V. 26. P. 246.
5. Arshakyan, A.D., Rozanova, E.N., Kometiani, I.B., and Grekhneva, E.V., Per'evoy keratin v sinteze biorazlagaemykh polimernykh materialov na osnove akrilamida i metilmetakrilata (Feather keratin in synthesis of biodegradable polymer materials based on acrylamide and methyl methacrylate), *Uchenye Zapiski: Elektron. Nauchn. Zhurn. Kursk. Gos. Univ.* (Sci. Notes: Electron. Sci. Journ. Kursk. State Univ.), 2013. V. 2. № 3 (27) (scientific-notes.ru/pdf/032-020.pdf).
6. Pavelzhak, R., Upakovka iz kukuruzy – fantastika ili real'nost' (A pack of corn: Fiction or reality), *Paket* (Packaging), 2006. № 5. P. 40.
7. Buryak, V.P., Biopolimery – nastoyashchee i budushchee (Biopolymers: Present and future), *Polim. Mater.* (Polym. Mater.), 2005. № 11 (78). P. 8.
8. Rinaudo, M., Chitin and chitosan: Properties and applications, *Prog. Polym. Sci.,* 2006. № 1. P. 603.
9. Zhang, J.W. and Chen, F., Development of novel soy protein-based polymer blends, *Green Polym. Chem.: Biocatal. Biomater.,* 2010. № 1043. P. 45.
10. Ofokansi, K., Winter, G., Fricker, G., and Coester, C., Matrix-loaded biodegradable gelatin nanoparticles as new approach to improve drug loading and delivery, *Eur. Journ. Pharm. Biopharm.,* 2010. № 76 (1). P. 1.
11. Averous, L., Polylactic acid: Synthesis, properties and applications, in *Monomers, Oligomers, Polymers and Composites from Renewable Resources,* ed. by Belgacem, N. and Gandini, A. (Elsevier, Amsterdam, 2008). P. 433.
12. Suvorova, A.I., Tyukova, I.S., and Trufanova, E.I., Biorazlagaemye polimernye materialy na osnove krakhmala (Biodegradable polymeric materials based on starch), *Usp. Khim.* (Russ. Chem. Rev.), 2000. V. 69. № 5. P. 498.

13. Averous, L., Biodegradable multiphase systems based on plasticized starch: A review, *Journ. Macromol. Sci., Part C: Polym. Rev.,* 2004. № 44 (3). P. 231.
14. Martin, O., Averous, L., and Della Valle, G., In-line determination of plasticized wheat starch viscoelastic behavior: Impact of processing, *Carbohydr. Polym.,* 2003. № 53 (2). P. 169.
15. Filimonov, I.S., Loginov, D.S., Trushkin, N.A., Ponomareva, O.A., and Koroleva, O.V., Sozdanie biorazlagaemykh polimerov na osnove fermentativnogo gidrolizata keratinsoderzhashchego syr'ya (Design of biodegradable polymers based on enzymatic hydrolysate of keratin-containing raw materials), *Sovr. Probl. Nauki Obraz.* (Mod. Probl. Sci. Educ.), 2012. № 6. P. 8.
16. Pose, S., Kirby, A.R., Mercado, J.A., Morris, V.J., and Quesada, M.A., Structural characterization of cell wall pectin fractions in ripe strawberry fruits using AFM, *Carbohydr. Polym.,* 2012. № 88 (3). P. 882. doi 10.1016/j.carbpol.2012.01.029.
17. Austarheim, I., Christensen, B.E., Hegna, I.K., Petersen, B.O., Duus, J.Ø., Bye, R., Michaelsen, T.E., Diallo, D., Inngjerdingen, M., and Paulsen, B.S., Chemical and biological characterization of pectin-like polysaccharides from the bark of the Malian medicinal tree Cola cordifolia, *Carbohydr. Polym.,* 2012. V. 89. № 1. P. 259.

THE USE OF MOLECULAR GENETIC MARKERS AND PCR FOR DNA DIAGNOSTICS IN RAW MATERIALS DERIVED FROM FRUIT AND BERRIES

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Abstract: A general description of molecular genetic markers is provided in the present article. The classification of DNA markers used for identification of raw materials of plant origin is presented. The most appropriate method for identifying raw materials derived from fruit and berries is chosen using study reports. The use of PCR for determining the quantitative and qualitative composition of the raw materials is considered. DNA regions used for PCR diagnostics of raw material derived from fruit and berries are characterized. The significance of amplification for a PCR test is outlined. The optimal PCR conditions have been selected and the advantages of this method have been revealed in the present study. Amplification profiles of DNA from the samples have been analyzed using different primers. Experiments with different primers allowing for identification of the raw material were carried out. The possibility of using a complex including a common gene and a variable gene for the identification of raw materials derived from fruit and berries has been considered. The sequence of DNA regions has been analyzed. The possibility of using a ribosomal RNA gene for generic and interspecific differentiation of DNA samples has been demonstrated. The significance of oligonucleotide primers and PCR product length for the reliability of the whole genotyping system has been elucidated. A scheme of PCR-based DNA profiling has been developed. Two types of the procedure were compared and the most appropriate type was chosen according to cost efficiency.

Keywords: DNA, genome, identification, genetics, primers, amplification, PCR amplicons, amplification profile

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INTRODUCTION

Identification of plant species from which raw material was derived is among the main areas of application of molecular genetic markers. DNA fragments associated with a specific nucleotide sequence constitute a new class of molecular markers. The number of such markers is severalfold higher than that of the markers characterized previously (isozymes, storage proteins, and morphological features). Besides, expression of DNA markers is neither dependent on the phenotype nor tissue-specific and can be detected at any stage of plant development. The use of DNA markers led to changes in the methods of evaluation of genetic diversity of plants, certification and classification of plant varieties, and genetic monitoring and selection breeding [4].

All studies related to identification of plant samples are based on the assumption that DNA fragments with equal molecular weights and the same activity represent the same genomic fragments within one family of plants. Specific resolved DNA bands are used as tools for the assessment of the level of similarity between multiband DNA profiles. The possibility of detecting identical multiband DNA profiles for two randomly selected individuals is close to $2 \cdot 10^{-9}$ for avocado, $1.5 \cdot 10^{-9}$ for papaya varieties, $4.2 \cdot 10^{-5}$ for apple varieties, and $2.4 \cdot 10^{-3}$ for varieties of raspberries and blackberries [12, 13].

Identification of plants is carried out stepwise in a certain direction:

1. identification of individuals;
2. detection of hybrid forms;
3. investigation of pedigrees of plant specimens.

The purpose of identification of individuals is assignment of a plant specimen to a species, subspecies, variety, etc. or finding a solution for a taxonomic problem.

The main areas of use of molecular genetic markers are the following:

– identification of species, varieties, and other forms of plants;
– assessment of genealogical relationships between plants;
– search for molecular genetic markers associated with desirable traits.

DNA markers must meet several requirements, such as:

– availability of phenotypic manifestations of allelic variants for the identification of different individuals;
– difference between allele replacement at one locus and those at other loci;
– availability of a substantial part of allelic substitutions in the target locus for identification;
– random character of the sample of genetic loci investigated with regard to physiological effects and the degree of variability;
– uniform distribution in the genome;
– relative neutrality.

There are no primers that would meet all these requirements [4, 9].

Molecular genetic markers that are most frequently used in practice can be divided into the following classes:

– markers expressed as visible morphological characteristics;

– markers constituted by structural portions of genes encoding the amino acid sequences of proteins;

– markers constituted by non-coding regions of structural genes;

– markers constituted by various DNA sequences, for which the relation to the structural genes is usually unknown – in other words, short repeats spread throughout the genome (RAPD – randomly amplifiable polymorphic DNA; ISSR – inverted short sequence repeats; and RFLP – restriction fragment length polymorphism), microsatellite loci (tandem repeats of a unit consisting of 2–6 nucleotides), and others.

The most general classification includes three generations of DNA markers:

1. DNA markers based on hybridization (for example, RFLP markers);
2. DNA tags, that is, markers based on nucleotide sequence (SNP);
3. DNA markers based on PCR (RAPD-, SSR-, AFLP-, ISSR-markers, and others).

RFLP (restriction fragment length polymorphism)-based approach was first proposed for studies of the human genome. This method, which detects changes in the location of restriction sites in the DNA sequence, allows for the identification of mutations, deletions, insertions, inversions, or translocations in the DNA sequence and for the detection of variability in cytosine residue methylation patterns.

Isolated DNA is treated with the appropriate restriction enzymes, which are endonucleases capable of recognizing and cleaving specific DNA sequences. Restriction fragments of different length are separated by agarose or polyacrylamide gel electrophoresis. Since multiple DNA fragments are present in the electrophoregram, the detection of a specific fragment requires hybridization with a radioactive or fluorescent probe (labeled oligonucleotide) on a membrane (Southern blotting). The nucleotide sequence of the DNA probes is complementary to the sequence of the fragment of interest.

SNPs (single nucleotide polymorphisms) are usually represented with 2 allelic variants of a single nucleotide site. Polymorphism identification with these markers usually provides an unambiguous result (+/-). More than 10 different approaches to SNP identification are currently available [11].

Detection methods which do not require electrophoretic analysis are the most convenient. An example of such a method is Taq-Man which is based on hybridization of DNA template to an oligonucleotide which contains a reporter moiety at the 5'end and a quencher moiety at the 3'-end; the quencher moiety suppresses the fluorescent signal if the oligonucleotide does not hybridize to the matrix, thus enabling SNP detection.

PCR requires smaller amounts of DNA from plant tissues than RFLP, but contamination of the samples with fungal and bacterial DNA is unacceptable if the former method is used. The use of PCR assay eliminates the need for maintaining a library of clones for hybridization probes, Southern blotting, and the use of radioactive isotopes.

The amplicons produced by PCR are divided into two groups according to the type of primers used:

1. STS markers obtained using specific primers designed for the amplification of certain sequences.
2. Markers amplified with random primers. Amplification of some markers employs primers that combine the properties of specific and random primers [10].

Amplification with specific primers is a controlled process that requires knowledge of the DNA matrix sequence; the primers target a unique site or multiple sites on both DNA strands.

Amplification which does not require prior knowledge of the DNA sequence is termed random. It involves one or several random primers and results in amplification of a single or multiple genomic sites; most of these sites are polymorphic.

Successful implementation of methods for DNA diagnostics stimulated further development and implementation of highly sensitive techniques based on polymerase chain reaction (PCR). Specific DNA amplification is currently in wide use for the development of reliable procedures for identification of the origins of multicomponent products and raw material derived from various fruit and berries [8, 12]. The advantages of species identification based on PCR over the traditional (physicochemical) methods of identification of genera and species serving as sources of plant-derived raw material are versatility, more precise differentiation of species, high reproducibility, and the possibility of quantitative analysis. Furthermore, plant DNA is more stable during food processing than other chemical compounds or biomolecules present in food.

Chloroplast genome, which is unique to plants, is represented by a large number of copies in plant cells and contains large variable and non-coding fragments (introns and intergenic regions), and therefore it can be used for very sensitive species identification of plant-derived raw materials in foods [5]. The size of the amplified fragment is different for different species. This feature was used for identification of tangerine, orange and grapefruit juice by heteroduplex PCR in a study reported in 2006 [7].

The aim of the present study was to investigate the possibility of using PCR for DNA diagnostics of raw materials derived from fruit and berries and to analyze target DNA regions, profiles generated by amplification, and PCR products.

OBJECTS AND METHODS OF THE STUDY

Raw materials derived from fruit and berries, namely, a mixture of orange and tangerine juice, apple, strawberry, pomegranate, blueberry, blackcurrant, and pineapple were the objects of the present study. DNA regions and their sequences were investigated.

RESULTS AND DISCUSSION

Polymerase chain reaction (PCR) is a method for in vitro amplification (repeated copying) that allows for a more than 10^8-fold expansion of the amount of the target DNA in several hours. The significance of this method for molecular biology and genetics is so great and obvious that the author was awarded the Nobel Prize in Chemistry just seven years after the discovery [2, 9].

The method is based on repeated copying of specific DNA fragments; the specificity is determined by complementarity of DNA sequences and the copying is mediated by the enzyme DNA polymerase.

PCR analysis consists of three main procedures:

Amplification (controlled replication of a DNA fragment) is the main stage of PCR. Each amplification cycle consists of three phases:

1. DNA denaturarion;
2. Primer annealing (binding to target DNA);
3. Primer elongation (synthesis).

Cyclic repetition of these three steps leads to enrichment of the reaction mixture with target DNA molecules, since both the target DNA initially present in the sample and the newly synthesized DNA serve as templates in each subsequent cycle. The course of PCR, i.e. the transition from one stage to another and from cycle to cycle, is regulated by changing the temperature of the reaction mixture [3, 7].

The optimal operating mode is determined by the length and specificity of the fragment amplified. The first PCR cycle is usually preceded by pre-incubation of the reaction mixture at 92–96°C for 0.5–10 min, which leads to inactivation of contaminating proteins (which may be present in the sample) and enhances the initial denaturation of template DNA. Introduction of this step increases the efficiency of PCR. After completion of the last cycle, the sample is typically incubated at 72°C for 5–10 min for completion of synthesis of all the polynucleotide chains in the PCR product. The reason for introducing this delay at the last stage of DNA synthesis is the accumulation of a large amount of PCR product; the presence of truncated chains formed during the last cycle is manifested as considerable molecular heterogeneity of the reaction mixture, while the "incomplete" chains formed in the intermediate cycles are completed in the subsequent cycles and do not affect the overall result of the reaction [1, 2].

The duration of primer elongation. The rate of primer elongation in PCR is typically around 50 n/s at 72°C, and therefore 15 seconds are sufficient for the amplification of a DNA fragment shorter than 400 nt (nucleotides). This time can still be reduced, because the process of primer elongation begins during the annealing phase already. Amplification of very long sequences requires increasing the elongation time during PCR to compensate for the inhibitory effect of increasing viscosity of the reaction mixture.

After completion of the elongation step of the first cycle, the working mixture is heated to enable the transition to the denaturation step of the second cycle, and so forth. The length of amplicons formed during the third and following cycles is standard and equals the number of base pairs between the sense and antisense 3'- ends of the template DNA fragment [2, 3].

The number of cycles is inversely proportional to the number of copies of the template in the reaction mixture. The number of copies is usually chosen to ensure the formation of a detectable amount of product after 25–50 cycles. The number of DNA molecules synthesized during the exponential phase of PCR increases until it is in the range of 10^{12}, and afterwards the rate of product accumulation is drastically reduced. There are several major factors that hinder the theoretically predicted exponential accumulation of the product, such as exhaustion of reaction substrates (deoxyribonucleotides or primers), insufficient stability of the components of the reaction mixture, PCR inhibition by the end product, competition for substrates involving nonspecific PCR products and primer dimers, re-annealing of PCR products preventing primer elongation, incomplete denaturation in the presence of large amounts of PCR product, and some others. These factors determine the "plateau effect" that renders it impossible to improve PCR specificity or enhance the accumulation of the specific product by increasing the number of cycles. PCR specificity increases if the number of cycles and the duration of the individual stages (namely, denaturation, annealing, and elongation of primers) are reduced. However, increasing PCR efficiency for the amplification of large fragments of the template (> 1 kb, thousand of nucleotide pairs) requires increasing the duration of the cycle steps. The duration of individual cycle steps is affected by the shape of the reaction mixture droplet, the thickness of tube walls, and structural features of the heating block of the thermal cycler [2, 4].

Volume of the reaction mixture. Standard sample volume for PCR is usually 20–100 μl; however, modern PCR devices allow for the use of 5 μl samples in analytical PCR. Heating and cooling of large samples is inefficient, while PCR in small reaction volumes yields small amounts of the product.

PCR is performed in automatic mode using special instruments (thermocyclers) which perform the necessary number of cycles and allow for choosing the appropriate time and temperature for each reaction cycle from a large and often continuous scale of options.

The final stage of PCR-based DNA analysis is detection of the amplification products that usually involves electrophoretic separation of PCR mixture on agarose or polyacrylamide gels and staining with ethidium bromide [9].

The specificity of DNA amplification is inferred from the position (size) of the amplicon band relative to the marker DNA fragments and a DNA standard.

The main advantages of PCR are high sensitivity and specificity, simplicity of the procedure, the possibility to use a DNA template without time-consuming isolation or purification, and the possibility of analyzing almost any biological material [2].

A scheme illustrating all stages of polymerase chain reaction is shown in Fig. 1.

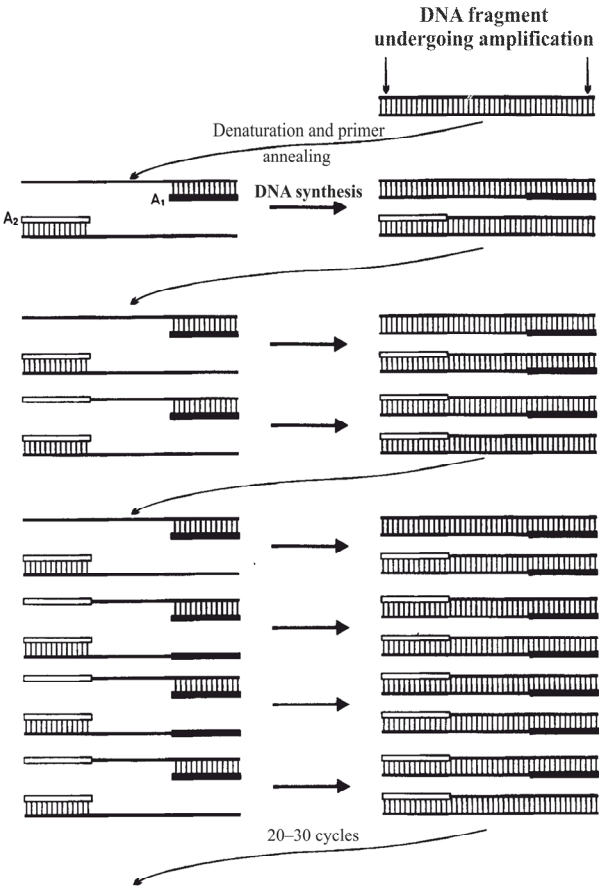

Fig. 1. A scheme of polymerase chain reaction. A1, A2 – oligonucleotide primers.

In case of absolute specificity the PCR product is a copy of the locus which is amplified, with no other nucleotide sequences present in the final product.

The precision of amplification is of crucial importance if the nucleotide sequence of the PCR product is critical for further experiments. Amplification errors are unacceptable if the primary structure of the amplified locus is to be determined or the PCR product is to be used for recombinant expression of a protein encoded by the DNA sequence in question.

The final yield of PCR product after the completion of the reaction is used to assess PCR efficiency quantitatively. Theoretically, the amount of product in the reaction mixture is doubled after each PCR cycle; however, this never occurs in real reactions.

PCR amplification of a trnT-trnL intergenic spacer fragment from chloroplast genome resulted in heteroduplex formation when a mixture of DNA molecules extracted from orange and tangerine juice was used as a template. Amplification of DNA from a mixture of orange and tangerine juice resulted in heteroduplex formation. PCR analysis of genuine juices was conducted simultaneously. The size of the PCR amplicon obtained from tangerine juice was 18% lower than that of the PCR amplicon derived from orange juice. Thus, a simple and reliable method for the identification and detection of falsified tangerine juice containing orange juice was discovered.

An international group of molecular systematics recently formed the "Barcode of life" consortium in order to create a database of genetic variation within a target gene (cytochrome c oxidase I [COI]) for fast and accurate identification of species. However, genetic variation within the COI gene of plants was not sufficient for species identification, and therefore the intergenic region psbA-trnH was suggested for use as a DNA target. The ultimate goal of this study was to create a database of PCR-RFLP profiles of the psbA-trnH intergenic region. Primers used for PCR are shown in Table 1.

Table 1. Sequence of primers designed for psbA-trnH and trnF-trnL intergenic regions

Primer name	Application	Sequence (5'-3')	Source
psbA3'f	*psbA-trnH* chloroplast	TCA ACC AAC CAC AAA GAC ATT GGC AC	114
trnHf	*psbA-trnH* chloroplast	CGC GCA TGG TGG ATT CAC AAT CC	114
Taberlet-E	trnF-trnL chloroplast	GGT TCA AGT CCC TCT ATC CC	115
Taberlet-F	trnF-trnL	ATT TGA ACT GGT GAC ACG AG	115

However, PCR with primers designed by Kress yielded no amplification products when heat-treated foodstuffs were tested. Analysis of PCR amplicons obtained with Taberlet's primers showed that elderberry (560 bp) could be qualitatively differentiated from other types of fruits and berries; apple (392 bp) and pear (411 bp) differed from other species, but not from each other; and blueberries (465 bp), grapes (459 bp), and pomegranate (463 bp) could be distinguished from other species, but not from each other. This allows for the assumption that in some cases a single set of primers can be used to identify several fruits and berries present in the sample.

PCR products were treated with restriction enzymes *ApoI* and *DdeI* and RFLP profiles were analyzed for a more precise differentiation of the amplification products.

Restriction enzymes cut double-stranded DNA at specific sites. Each restriction enzyme has a specific target sequence, which usually consists of 4–6 base pairs. The enzyme cuts DNA at each point where the target sequence is found. Different restriction enzymes have different target sequences. The results of the study are shown in Table 2 [2].

Table 2. PCR amplicon size and PCR-RFLP profiles obtained for a variety of fruit and berries

Species		PCR fragment size (bp)		
Name	Latin name	PCR amplicon	*Apo*I	*Dde*I
Apple	*Malus* spp.	392	137, 265	96, 293, 314
Blueberry	*Vaccinium* spp.	465	187, 289	471
Elderberry	*Sambucus nigra*	560	142, 254, 396	95, 111, 342
Grapes*	*Vitis vinifera*	459	110, 361	a) 80, 96, 107, 182 b) 95,105, 255
Pear*	*Pyrus* spp.	411	138, 157	a) 96, 318, 330 b) 396
Pomegranate	*Punica granatum*	463	95, 178, 202	100, 353

* The two profiles (a) and (b) were obtained with *DdeI* for different varieties of this species

High probability of restriction site loss caused by point mutations (nucleotide substitutions), inversions, deletions, and insertions in the genomic DNA sequence can present a problem for plant species identification employing this method [1].

The 5S DNA sequence proposed as an alternative is a convenient target, since it is highly conserved among species and the copy number for the 5S rRNA genes is high. Species-specific internal spacers are used for phylogenetic studies and species identification [6].

The 5S rRNA is a component of all ribosomes, except for those found in the mitochondria of certain plant species, and is transcribed from hundreds to thousands of genes in all higher eukaryotes. Genes encoding 5S rRNA are located separately from the 18S-26S rRNA gene clusters and arranged in the form of tandem repeats, with alternating arrays of 5S rRNA-coding sequences and non-transcribed spacers (NTSs) at one or more sites in the genome. Clusters of genes may be connected and therefore localize to the same chromosome or show independent localization in the genome. 5S rRNA genes of higher eukaryotes are organized in tandem repeats of the basic unit of 200-900 bp in length, with a copy number ranging from 1000 to 50000.

The gene consists of 120 bp and is connected with spacers of different sizes. The 120-bp sequence of 5S rRNA is conserved within a species, while the structure of NTS domain clusters, as well as their length (100–800 bp), varies from species to species due to a weaker selection pressure compared to that acting on the coding region. The high degree of conservation characteristic of 5S rRNA gene is due to the function of 5S rRNA, a part of the large ribosomal subunit in all eukaryotic organisms. Some regions of the gene exhibit a higher level of conservation than others due to their role in the regulation of 5S rRNA transcription [11].

Sequence conservation in the coding regions and considerable variety in the spacer regions provide a good model for investigation of the organization and evolution of multigenic system in different plant species. In view of these assumptions, changes in the NTS region were used for the study and visualization of 5S rDNA arrays in the genome, evolution studies, and phylogenetic reconstruction in some plant species [5].

Experiments on the qualitative and quantitative identification of raw materials derived from fruit and berries by real-time PCR with 5S rRNA and ANS (anthocyanidin synthase) as target DNA sequences were conducted by American researchers in 2009.

Nine new primers designed for rbcL sequence region in accordance with the rbcL sequences for apple (X69749.1), blackcurrant (L11204.2), blueberries (L12625.2, AF419837.1, AF419836.1, AF419835.1, AF421107.1, AF124576.1), raspberry (U06825.1), strawberry (U06805.1), orange (58678-60105), pineapple (L19977.1), and pomegranate (L10223.1) are presented in Table 3.

Table 3. rbcL primer sequences

Primer name	Nucleotide sequence
rbcL1forward	5'-TTGGCAGCATTYCGAGTAACTCC-3'
rbcL2forward	5'-TGGCAGCATTYCGAGTAACTC-3'
rbcLAreverse	5'- CCTTTRTAACGATCAAGRC-3'
rbcLBreverse	5'- AACCYTCTTCAAAAAGGTC-3'
rbcLCreverse	5'- TTCSGCACAAAATAMGAAACGG-3'
rbcLDreverse	5'- TAGTATTTGCDGTGAATCCC-3'
rbcLEreverse	5'- TGATCTCCACCAGACAKACG-3'
rbcLFreverse	5'- ATATGCCAAACRTGRATACC-3'
rbcLHreverse	5'- ATATGCCAAACRTGRATACC-3'

Amplification profiles of some DNA samples extracted from juice and assayed with EMFxaANS primers are shown in Fig. 2. The length of amplification products ranged from 250 to 1000 bp. Strawberry, raspberry/blackberry, apple, and orange could be distinguished using these primers.

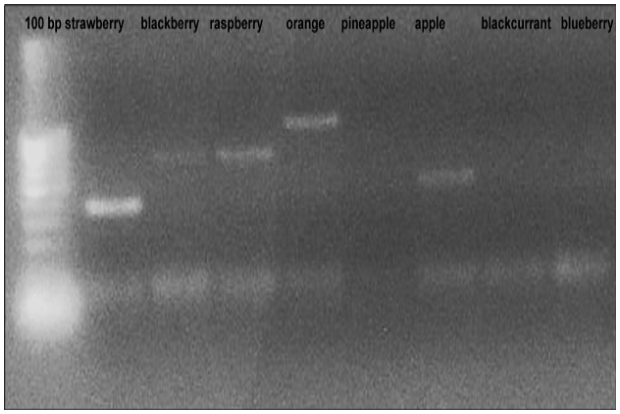

Fig.2. Amplification profile of a set of DNA samples assayed with EMFxa ANS primers.

Polymorphic bands were obtained for pineapple, orange, apple, strawberry, raspberry, and blackberry in a similar experiment employing primers for 5S rRNA sequences. Raspberries, apple/orange, pineapple, and blackberries could be identified unambiguously. Amplification profiles for fragments encoding 5S rRNA are.shown.in.Fig.3.

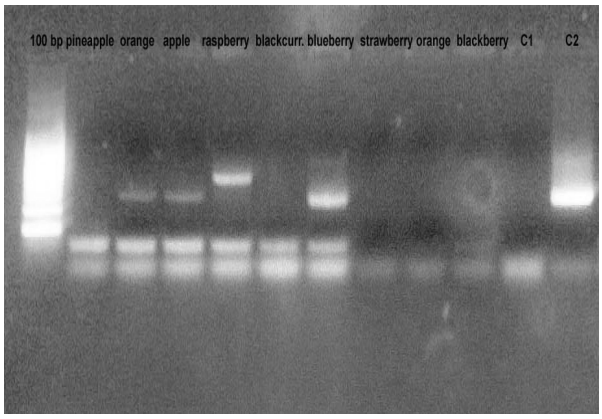

Fig. 3. Amplification profile of some DNA samples assayed with primers for 5S rRNA.

Thus, the use of 5S rRNA and anthocyanidin synthase as target DNA sequences allowed for intrageneric and intraspecific differentiation of amplification products obtained from samples of fruits and berries. Practical application of this technique for laboratory testing of incoming raw materials and product quality control is difficult because a large database must be created to enable fast analysis of the data produced. The behavior of the primers in question during the analysis of GM products is hard to predict.

Chinese researchers have proposed to use a complex containing a non-coding and variable ITS (internal translation spacer) region and the gene encoding 5.8S rRNA to identify raw materials in food, since the profiles obtained using these sites are much more varied due to lower intraspecific polymorphism and significantly higher interspecific variability of the ribosomal area in question compared to 18S rRNA and 25S rRNA regions.

About 20 types of common food allergens, including scallops, squid, shrimp, crab, salmon, mackerel, chicken, pork, red caviar, meat, gelatin, orange, kiwi, walnuts, soybeans, matsutake, peach, sweet potato, apple, and banana were used as the objects of study. DNA from raw material derived from other fruit was not analyzed. Primers designed for this system are shown in Table 4 [7].

Table 4. Sequences of primers designed for the ITS1-5.8S rRNA-ITS2 cluster

Primer name	Nucleotide sequence
ITS5A(F)	CCTTATCATTTAGAGGAAGGAG
ITS4(R)	TCCTCCGCTTATTGATATGC

Further studies were focused on searching for a DNA sequence allowing for more sensitive generic and interspecific differentiation. The ribosomal gene rp116 from chloroplast genome was suggested as the DNA target. Analysis of nucleotide sequences listed in the database for the ribosomal gene rp116 from plants of the genus Prunus (peach) was used to design new primers which are shown in Table 5 [9].

The method suggested allowed for unambiguous identification of members of the genus Prunus, but intraspecies differentiation (of peach and apricot, for example) remained impossible.

Table 5. Sequences of primers designed for gene rp116

Primer name	Nucleotide sequence
–	5'-GTT TCT TCT CAT CCA GCT CC-3'
–	5'-GAA AGA GTC AAT ATT CGC CC-3'

Analysis of data published in Russia and abroad during the recent years showed that a method of qualitative identification and quantification of fruit-derived raw material in foodstuffs has not been developed yet. However, the number of PCR procedures for species identification of fruit-derived raw material in food is increasing due to the increase of the volume of data on the composition of the genome of fruit and berry plants allowing for the use of new genes and nucleotide sequences as DNA targets.

The use of short oligonucleotide primers allows for differentiation between various samples of fruit materials; multiple annealing sites (and consequently, PCR initiation sites) for such primers are found in large genomes. The shorter the primers, the higher the number of putative annealing sites. One of the limitations of further involvement of such primers in PCR is the distance between the two annealing sites for oppositely directed primers. Reliability of the entire genotyping system decreases if the formation of long PCR products is expected when such primers are used.

The length of a PCR product obtained with random primers usually ranges from 0.2 to 2.0 kb. Two types of PCR products can be formed in this case; the type depends on the localization of primer annealing sites on the target DNA (Fig. 4).

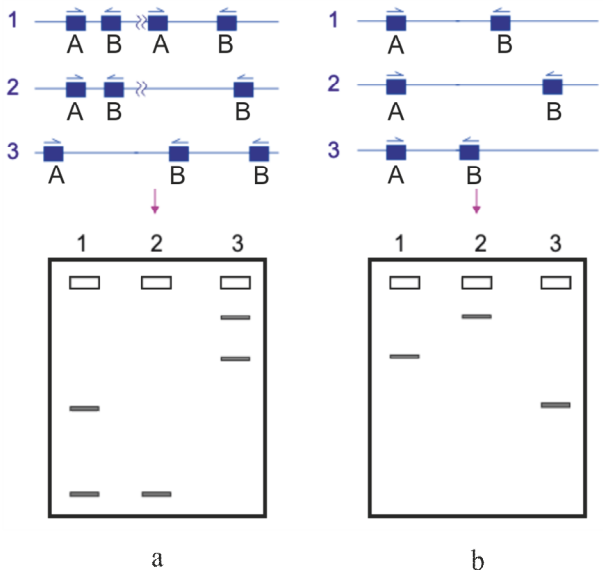

Fig. 4. Scheme of microorganism genotyping based on PCR with random primers: a – strain-specific differences related to different localization of primer annealing sites on DNA. A DNA fragment present in the genome of strain 1 is missing from the genomes of strains 2 and 3, and therefore the band corresponding to the PCR product is not found in the electrophoregram; b – the results of amplification of DNA samples containing the same set of primer annealing sites. DNA of strains 2 and 3 contain deletions of varying length between primer binding sites; alternatively, the DNA of strains 1 and 2 can contain insertions which are missing from the DNA of strain 3. Therefore, PCR products of different lengths are formed, as evident from the results of electrophoresis. A, B – primer annealing sites on DNA.

In the first case, the PCR products differ in length due to the different number of annealing sites for one or both primers in the template DNA (Fig. 4-a); in the second case, the number of annealing sites in specific genetic loci is similar and the differences are due to the difference in lengths of DNA fragments framed by the annealing sites (Fig. 4-b). Both these phenomena can be observed simultaneously in real samples [4, 9].

Genotyping of eukaryotes sometimes results in simultaneous formation of up to 100 amplicons. The resulting pattern of amplification is species- and strain-specific despite the use of random primers. The number of primer annealing sites on a certain DNA template can vary considerably for primers of the same length having different primary structures.

Formation of PCR products of the first type requires the creation a huge database of passports for all the research objects, which is extremely challenging due to the large number of varieties characteristic of many plant species under investigation. Therefore, design of primers providing for the formation of products of the second type was attempted. Calculation of the length of the representative DNA segments was the first step of the design procedure.

CONCLUSIONS

Molecular genetic markers have priority in the set of modern molecular genetic tools that help plant selection breeders in solving practical problems, making the selection process more sophisticated and efficient.

Molecular markers are important in biotechnology, since the use of these markers allows for detection and control of loci determining quantitative and qualitative features of organisms. Identification of a range of important features of plants, such as the weight of grains and seeds, plant height, starch concentration, diameter, length and weight of seeds or fruit, and others, employed these markers [4, 9].

Methods of molecular genetics provide a powerful solution to many fundamental problems of evolution, genetics, selection breeding, preservation of genetic diversity, genotype identification, and precise determination of genotype origin. The development of new technology based on the use of PCR-generated markers for investigation of molecular genetic polymorphism has been extremely rapid.

Application of PCR to identify the source of plant DNA found in foodstuffs is currently only local in Russia, because the normative documents, test systems, and most importantly, the protocols themselves are mostly under development, while in other countries the research in this field of food industry has been going on for over 10 years.

REFERENCES

1. Altukhov, Yu.P., *Geneticheskie protsessy v populyatsiyakh: ucheb. posobiye* (Genetic processes in populations: a study guide) (Akademkniga, Moscow, 2003).
2. Biryukova, V.A., Zaitsev, V.S., Pankin, A.A., et al., DNK-genotipirovanie kartofelya i ego dikorastushchikh sorodichei na osnove polimorfizma umerennykh povtornostei semeistva R173 (DNA genotyping of potato and its wild relatives based on polymorphism of moderate repeats of the family R173), in *Materialy Mezhdunarodnoy yubileynoy nauchno-prakticheskoy konferentsii: Nauchnyye trudy.* (Materials of International jubilee scientific and practical conference: Proceedings), Minsk, 2003. Part 1. P. 313.
3. Kil', V.I., and Gronin, V.V., Geneticheskie markery chuvstvitel'nosti populyatsiy koloradskogo zhuka k transgennomu kartofelyu (Genetic markers of sensitivity of the Colorado potato beetle populations to transgenic potatoes), *Nauka Kubani* (Kuban' Science), 2005. № 4. P.126.
4. Komarova, I.N., Razrabotka PTsR–test–sistem dlya vidovoi identifikatsii i kolichestvennoi otsenki myasnogo syr'ya v sostave melkoizmel'chennykh polufabrikatov i gotovykh myasnykh produktov (Development of PCR test systems

for species identification and quantification of raw meat in of semi-finished and finely ground meat products, *Dis…kand.tekhn.nauk* (Diss.… Cand. Sci. (Tech.), Moscow, 2005.
5. Lewin, B., *Geny* (Genes); translated into Russian by Gintsburg, A.L. and Ilyin, T.S. (Mir, Moscow, 1987).
6. Palilova, A.N., Urbanowich, O.Yu., Dolmatovich, T.V., et al., Poisk molekulyarnykh markerov ustoychivosti rasteniy kartofelya k virusnoy infektsii (Search for molecular markers of potato plant resistance to virus infection), in *Materialy Mezhdunarodnoy yubileynoy nauchno-prakticheskoy konferentsii: Nauchnyye trudy*. (Materials of International jubilee scientific and practical conference: Proceedings), Minsk, 2003. Part 1. P. 316.
7. Politov, D.V., Primenenie molekulyarnykh markerov v lesnom khozyaistve dlya identifikatsii, inventarizatsii i otsenki geneticheskogo raznoobraziya lesnykh resursov (Application of molecular markers in forestry for identification, inventory and assessment of genetic diversity of forest resources), *Lesokhozyaystvennaya informatsiya* (Forestry Information), 2008. V. 34. P. 24.
8. Prosekov, A.Yu. and Babich, O.O., *Gennaya inzheneriya: uchebnoe posobie* (Genetic engineering: a tutorial) (Redaktsiya zhurnala “Dostizheniya nauki i tekhniki APK”, Moscow, 2010).
9. Romanova, O. V., Identifikatsiya sortov kostochkovykh kul'tur s pomoshch'yu PTsR-analiza. (Identification of the varieties of stone fruit plants by PCR analysis), *Dis. ... kand. sel'.-khoz. nauk* (Diss. Cand. Sci (Agriculture)), Moscow, 2007.
10. Coyne, V.E., James, M.D., and Reid, Sh.J., *Molecular biology techniques manual: standard PCR protocol.* 1994.
11. Frey, J.E., Genetic flexibility of plant chloroplasts, *Nature*, 1999. V. 398. P.115.
12. Fulcrand, N., Cheynier, V., and Oszmianski. J., An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan- 3 -ol condensation, *Phytochemistry*, 1997. № 46. P. 223.
13. James, S.A., Collins, M.D., and Roberts, I.N., Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera Zygosaccharomyces and Torulaspora, *Int. J. Bacteriol.*, 1996. № 46. P. 189.

THEORY AND PRACTICE OF PRION PROTEIN ANALYSIS IN FOOD PRODUCTS

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Abstract: The article presents the results of the research on methods of identification and quantitative determination of prion proteins in biological samples and multicomponent mixtures based on them. Analysis of nucleotide sequence of DNA encoding the PRNP gene of the prion protein, including phylogenetic and comparative analysis of nucleotide sequences of normal and pathogenic prion protein in cattle, was performed. Oligonucleotide primers for amplification of the PRNP gene of pathogenic prion protein were designed and synthesized. The high specificity of the developed test system was confirmed.

Keywords: prion, protein, encephalopathy, safety, quality, PCR, analysis

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INTRODUCTION

Prions (proteinaceous infectious particles) are a special class of purely protein agents, free of nucleic acids, causing severe diseases of central nervous system in human and a number of higher animals [1–3].

Prion protein can exist in two forms: a non-infectious vitally important protein present in the organism of mammals, including human, and an infectious protein, which is a mutation of the normal prion protein causing prion diseases of animals and man.

Prion diseases are a group of transmissive neurodegenerative diseases of animals and humans. The diseases are characterized by prolonged incubation periods, but rapid progression from the moment of clinical onset of the disease. All prion diseases are lethal and there is no efficient methods of treatment so far. In 1997, Stenly B. Prusiner won the Nobel Prize for the outstanding discovery of prions.

Spongiform encephalopathy in cattle was registered in Great Britain, Switzerland, Ireland, Portugal, France, Germany, the Netherlands, Italy, Denmark, and Falkland Islands. The reported cases of disease were caused by the import of infectious animals or diseased meat-and-bone meal tankage produced from the killing products and used for breeding of the young stock in these countries [4, 5].

Prophylaxis of prion diseases is based on prohibition of the infected meat products or other killing products on food market. In this connection, in the Enactment of the Chief State Medical Officer of the Russian Federation no. 15 of 15.12.2000 «On the Measures for Prevention of Creutzfeldt–Jacobs Disease Spreading on the Territory of the Russian Federation», preventing measures aimed at prohibition of import of diseased meat and meat products were defined for the first time.

Taking this into account, improvement and development of new methods for identification of prion proteins in biological material is of scientific and practical interest.

OBJECTS AND METHODS OF THE STUDY

Whole milk, whole beef blood, blood plasma, cheese, beef muscle tissue, stromal fractions, gelatin, and samples of cattle meat were used. Samples of meat and blood were collected from animals having passed the veterinary control; the carcasses were proven fit for human consumption. The following nucleotide sequences corresponding to the PRNP gene of the prion protein deposited in the GenBank database were analyzed: *Equus caballus* (house horse), *Equus asinus* (house donkey), *Sus scrofa* (pig), *Bos taurus* (cow), *Bos javanicus* (Javan bull), *Bubalus bubalis* (buffalo), *Syncerus caffer caffer* (African buffalo), *Capra hircus* (goat), *Ammotragus lervia* (jubate sheep), *Ovis aries* (urial), *Rangifer tarandus granti* (northern deer), *Capreolus capreolus* (roedeer), *Alces alces alces* (elk), *Cervus elaphus nelsoni* (northamerican elk), *Cervus dama* (fallow deer), and *Homo sapiens* (human).

In the work, we used standard, common, and original methods, including the phylogenetic analysis of the protein gene nucleotide sequences, differential amplification of specific sequences and real-time polymerase chain reaction (PCR). The experiments involving PCR were performed following the requirements on determination of pathogenic microorganisms in cattle processing products.

Prior to the studies, independently of the analysis method, primary treatment of the samples was performed. In the case of analysis of soft and easily grinded materials (meat, cheese, etc.), averaged sample of the product weighing 1 g was collected, grinded using a sterile scalpel, scissors, and disposable spatula, and homogenized using a porcelain pistil in a ceramic mortar, with thorough mixing of the content.

For samples of dry particulate materials (gelatin) and liquid or semi-liquid materials (milk, blood, etc.), which require no grinding and are homogeneous, disposable spatula or a pipette was used to introduce 100–150 μL of bulk volume of a sample to an

Eppendorf tube (5–7 mm from the tube bottom). To prevent cross-contamination, the grinding instruments were used once, washed carefully, and sterilized.

For isolation of different protein fractions of animal origin, the samples were pretreated as follows: muscles of different animals were thoroughly freed from fat and connective tissue; weighed amount (3–4 g) of the tissue was cut by a knife on a watch glass. Distilled water was added at the ratio of 1 : 6 (by mass) and extraction was performed on cold at 0°C for 30 min. Then, the sediment was separated by centrifugation at 83 s^{-1} for 5 min. The supernatant was carefully decanted and used for quantitative protein determination.

Liquid samples were prepared by dilution in distilled water, so that the protein content in a gel pocket would not exceed 5 μg per 20 μL solution.

Determination of the total protein in samples was performed according to a technique of total and protein nitrogen fractions determination in meat, meat products, and protein-containing food products by the burning method of Duma [6].

Protein identification was performed by fingerprinting of peptide masses. Proteins were identified by the mass spectrum of amino acid sequences upon hydrolysis with trypsin in polyacrylamide gel.

To perform the mass spectrometry analysis, 0.5–2 μL of sample solution and 0.3 μL of a 20 mg/mL 2,5-dihydroxybenzoic acid solution in 20% acetonitrile aqueous solution with 0.5% TFA (Aldrich) were mixed on a ground steel support. Mass spectra were recorded on an Ultraflex II (Bruker, Germany) tandem MALDI time-of-flight mass spectrometer in the mass range of 700–4500 *m/z* under laser power optimal for the best resolution and registration of trypsin autolysis peaks, which were further used for internal calibration.

Mass spectra were processed with a FlexAnalysis 2.4 (Bruker Daltonics, Germany) software. If needed, fragmentation spectra of individual peptides were registered under tandem mode. Possible amino acid sequences were indexed in successfully fragmented peptides.

The accuracy of the average [MH+] measured mass in the linear mode was 5 Da. The accuracy of the monoisotopic measured masses in the reflecto-mode without internal calibration was 0.01% and after an additional calibration using trypsin autolysis peaks, 0.005%. Accuracy of the monoisotopic measured masses of fragments was 1 Da.

Molecular mass distribution of the proteins in the samples was evaluated by protein electrophoresis according to Laemmli [7].

Proteins were separated in denaturing 12% separating and 4% concentrating polyacrylamide gel supplemented with 0.1% sodium dodecyl sulfate. Electrophoresis was performed in a separating buffer supplemented with 0.1% sodium dodecyl sulfate under 15 mA. Gel was stained with 0.2% Coomasie Brilliant Blue R250 dye, prepared using glacial acetic acid, at high temperature for 7–10 min and then washed three times with distilled water.

Gels were viewed and imaged using a TCP-20M (Vilber Lourmat, United States) UV-transilluminator at the wavelength of 312 nm. Data storage and processing were performed with a DOC-it-LS gel-documenting system.

Gel calibration was performed using a set of protein markers by SibEnzyme containing 12 highly purified recombinant proteins of molecular mass between 10 and 250 kDa. For quantitative evaluation of normal prion protein content, gel was calibrated using human serum albumin protein solutions of known concentration.

Protein concentration in a sample was calculated according to the formula:

$$C = (C_p \cdot C_f)/100,$$

where C_p is the mass fraction of the total protein in a sample, g/100 g, and S_f is the mass fraction of a protein fraction to the total protein content in a samples, g/100 g protein.

In the course of the study, 17 nucleotide sequences of the PRNP prion protein gene deposited in the GenBank were used. To elucidate the differences and search for homologous sequences, NCBI database was used [8, 9]. Nucleotide acid sequences were aligned using the ClustalW software.

For comparative analysis of the DNA nucleotide sequences encoding the PRNP gene, OligoCalc software was used. Phylogenetic tree was designed using the ClustalW software.

Computer-based primer selection analysis for amplification of specific sequences of the pathogenic prion protein was performed using the following software: NCBI Blast2 for determination of homology upon sequencing of relevant primers and Primer3 Output for selection and evaluation of the primers.

Immuno-PCR was performed using the reaction mixtures presented in Table 1.

Table 1. Composition of the PCR reaction mixture

Component	Final concentration	Component content per 25 μL of the mixture
10× PCR buffer	0.1 μM	2.5 μL
10 mM dNTP mixture	0.2 mM	0.5 μL
Primer 1 (50 μL)	1 μM	0.5 μL
Primer 2 (50 μL)	1 μM	0.5 μL
Taq DNA polymerase	1.25 un.	0.25 μL
25 mM $MgCl_2$	1.5 mM	1 μL
DNA template	0.1–1 μg	Varies in function of concentration in a sample
Deionized water	-	Adjusted to 25 μL

RESULTS AND DISCUSSION

Total protein content in the samples is reported in Table 2.

Table 2. Total protein content in the samples

Subject of the study	Mass, mg	Total nitrogen content, %	Coefficient for calculations	Total protein, %	Measurement error, ±δ,%
Whole milk	195.60	0.656	4.64	3.02	0.31
	192.40	0.648			
Gelatin	188.12	15.123	5.55	84.32	0.89
	180.72	15.538			
Whole blood	214.50	3.462	6.25	21.97	1.32
	254.80	3.571			
Cheese	189.70	4.686	4.64	21.46	0.95
	229.60	4.653			
Beef	126.50	3.222	5.62	18.46	1.11
	110.00	3.347			
Water-soluble beef proteins	97.40	1.148	5.62	6.48	0.41
	96.50	1.167			
Salt-soluble beef proteins	136.30	1.520	5.62	8.52	0.53
	142.25	1.528			
Stromal beef proteins	186.80	0.675	5.62	3.78	0.23
	171.20	0.679			

Based on the data presented in Table 2, one may conclude that the total protein content in samples was 84.32 g/100 g for gelatin, 21.97 g/100 g for blood, 21.46 g/100 g for cheese, and 18.46 g/100 g for beef, with 6.48 g/100 g of water-soluble beef proteins, 8.52 g/100 g salt-soluble beef proteins, and 3.78 g/100 g stromal proteins.

We did not succeed to optimize the conditions for separation of cattle whole blood electrophoretic separations, therefore, we analyzed the blood plasma which was obtained by centrifugation at 3000 rpm for 5 min. The supernatant containing light fractions of blood proteins was used for analysis. The results of protein fraction distribution in the samples are presented in Fig. 1 and Table 3.

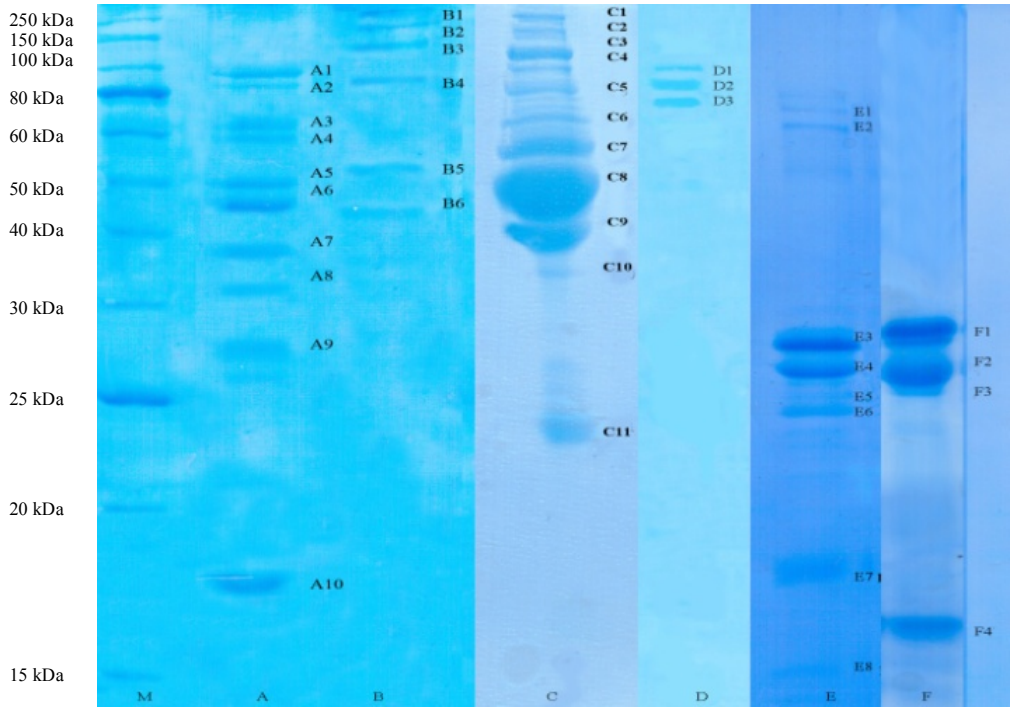

Fig. 1. PAGE in a 12% separating and 4% concentrating gel: M, molecular weight marker; A, beef protein water-soluble fraction; B, beef protein salt-soluble fraction; C, blood plasma; D, gelatin; E, whole milk; F, cheese.

Table 3. Mass fraction of the total protein and fraction distribution of the proteins

Sample	Number of samples	Total protein, g/100 g	Number of protein fractions in a range		
			15–30 kDa	30–40 kDa	40–250 kDa
Beef protein water-soluble fraction	20	6.48	1	2	6
Beef protein salt-soluble fraction	20	8.52	0	0	6
Beef protein stromal fraction	20	3.78	0	0	2
Blood plasma	20	9.73	1	2	8
Gelatin	10	84.32	0	0	3
Whole blood	20	3.02	6	0	2
Cheese	20	21.46	4	0	1

In the course of the study, we found that in fractions of stromal and salt-soluble beef proteins there are no low molecular weight protein fractions, which is in good agreement with the literature data, while in the fraction of water soluble proteins there are two protein fractions with masses from 30 to 40 kDa. The indicated protein mass is within the range of normal prion protein mass.

The obtained electrophoresis diagrams of blood plasma samples indicate the presence of two protein fractions with masses from 30 to 40 kDa.

Electrophoresis separation of industrial samples of gelatin, which is produced by partial hydrolysis of collagen obtained from cattle nails, jacket and skin, strings, and tendons, demonstrated high grade of purity. No low molecular weight protein fractions were observed.

Milk and cheese proteins were also fractionated (Table 4). Analysis of electrophoresis diagrams indicates the presence of traditional milk proteins in all samples. Caseins are characterized by molecular masses of ~22–32 kDa; β-lactoglobulin, ~18 kDa; α-lactalbumin, ~14 kDa; lactoferrin, 80 kDa; and serum albumin, ~66 kDa. No alien protein fractions weighing from 30 to 40 kDa was detected in the samples.

Table 4. Fraction composition of whole milk and cheese proteins

Band number	Molecular weight, kDa	Protein	%, to the total casein content	%, to the total serum protein content	%, to the total protein content
E 1	73.82	lactoferrin	-	17.76	3.39
E 2	68.11	blood serum albumin (SA)	-	17.04	43.24
E 3	29.63	αs1-casein	53.99	-	23.84
E 4	27.83	β-casein	29.77	-	4.14
E 5	26.09	αs2-casein	5.17	-	8.87
E 6	25.28	κ-casein	11.07	-	8.93
E 7	18.62	β-lactoglobulin	-	44.85	4.05
E 8	15.88	α-lactalbumin	-	20.34	3.39
D1	32.11	αs1-casein	37.31	-	37.31
D2	30.34	αs2-casein	11.23	-	11.23
D3	28.03	β-casein	41.50	-	41.50
D4	26.51	κ-casein	9.96	-	9.96

The absence of protein with molecular mass corresponding to that of normal prion protein (30–40 kDa) evidences the low possibility of prion protein presence in samples of whole milk, cheese, and salt-soluble and stromal beef proteins and therefore, the low level of infectiveness of the samples under study. The standard procedure of veterinary control, that is the certificate of fit for human consumption, is sufficient.

Further on, for unambiguous identification of the protein fractions in the above-indicated samples as normal prion proteins, one-dimensional electrophoresis was followed by the protein in-gel cleavage with trypsin and identification by peptide mass fingerprinting.

Samples selected for studies are presented in Table 5.

Table 5. Protein samples for investigation

Sample name	Protein mass, kDa
Beef protein water-soluble fraction	32.38
Blood plasma	34.89

Mass spectrum of amino acid sequences upon in-gel hydrolysis with trypsin was used for protein identification.

Quantitative content of the normal prion protein in samples was estimated by electrophoresis according to Laemmli followed by staining of the gel with Coomasie Brilliant Blue R250 (Table 6).

Table 6. Protein fraction composition of samples under study

Sample name	Molecular weight, kDa	%, to the total protein content	%, to the total protein content in a sample	Mass fraction, %
Beef protein water-soluble fraction	37.42	18.19	10.78	0.74
	32.38		7.41	0.51
Blood plasma	39.17	22.06	21.03	2.05
	34.89		1.03	0.1

Relative content of protein fractions from 30 to 40 kDa in fractions of beef water-soluble proteins was 18.19% to the total amount. Electrophoresis diagrams of blood plasma indicate that the relative content of protein fractions from 30 to 40 kDa in blood plasma was 22.06%. Therefore, our study evidences that the fractions of water-soluble proteins from beef and blood plasma under study indeed are normal prion proteins of the cattle.

Phylogenetic connections between the organisms may be elucidated by comparison of sequences of whole genes or their fragments encoding ribosomal RNA. The data on completely or partially sequenced rRNA genes of different organisms are deposited in international databases and are available in the internet. Today, methods based on determination of ribosomal gene nucleotide sequences are widely used for identification of different infection types [10].

We chose the following PRNP sequences: *Equus caballus* (house horse), *Equus asinus* (house donkey), *Sus scrofa* (pig), *Bos taurus* (cow), *Bos javanicus* (Javan bull), *Bubalus bubalis* (buffalo), *Syncerus caffer caffer* (african buffalo), *Capra hircus* (goat), *Ammotragus lervia* (jubate sheep), *Ovis aries* (urial), *Rangifer tarandus granti* (northern deer), *Capreolus capreolus* (roedeer), *Alces alces alces* (elk), *Cervus elaphus nelsoni* (northamerican elk), *Cervus dama* (fallow deer), and *Homo sapiens* (human) (see Fig. 2).

```
gi|27733849 Equus       ATGGTGAAAAGCCACGTAGGCGGCTGGATTCTGGTTCTCTTTGTGGCCAC 50
gi|119514511 Equus      ATGGTGAAAAGCCACGTAGGCGGCTGGATTCTGGTTCTCTTTGTGGCCAC 50
gi|119489983 Sus        ATGGTGAAAAGCCACATAGGTGGCTGGATCCTCGTTCTCTTTGTGGCCGC 50
gi|119489801 Bos        ATGGTGAAAAGCCACATAGGCAGTTGGATCCTGGTTCTCTTTGTGGCCAT 50
gi|54125480 Bos         ATGGTGAAAAGCCACATAGGCAGTTGGATCCTGGTTCTCTTTGTGGCCAT 50
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gi|119655282 Ammotragus ATGGTGAAAAGCCACATAGGCAGTTGGATCCTGGTTCTCTTTGTGGCCAT 50
gi|89160951 Ovis        ATGGTGAAAAGCCACATAGGCAGTTGGATCCTGGTTCTCTTTGTGGCCAT 50
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gi|50442307 Alces       ATGGTGAAAAGCCACATAGGCAGCTGGATCCTAGTTCTCTTTGTGGCCAT 50
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gi|308194928 Homo       ATGGCG------AACCTTGGCTGCTGGATGCTGGTTCTCTTTGTGGCCAC 44
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gi|119489801 Bos        GTGGAGTGACGTGGGCCTCTGCAAGAAGCGACCAAAACCTGGAGGAGGAT 100
gi|54125480 Bos         GTGGAGTGACGTGGGCCTCTGCAAGAAGCGACCAAAACCTGGAGGAGGAT 100
gi|54125508 Bubalus     GTGGAGTGACGTGGGCCTCTGCAAGAAGCGACCAAAACCTGGAGGAGGAT 100
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Fig. 2. *Beginning.* Alignment of the PRNP gene nucleotide sequences.

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gi|50442307 Alces       GGAACACTGGGGGGAGCCGATACCCGGGACAGGGAAGTCCTGGAGGCAAC 150
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gi|50442285 Cervus      GGAACACTGGGGGGAGCCGATACCCGGGACAGGGAAGTCCTGGAGGCAAC 150
gi|308194928 Homo       GGAACACTGGGGGCAGCCGATACCCGGGGCAGGGCAGCCCTGGAGGCAAC 141
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gi|54125508 Bubalus     CGTTATCCATCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGG 200
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gi|119655282 Ammotragus CGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTG- 199
gi|89160951 Ovis        CGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTG- 199
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Fig. 2. *Continued.* Alignment of the PRNP gene nucleotide sequences.

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gi|50442285 Cervus      GGGGTCAGCCCCATGGTGGTGGCTGGGGACAGCCACATGGTGGTGGAGGC 276
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                * ********.* ** **********.**********. **.** .*
gi|27733849 Equus       ATTCATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 470
gi|119514511 Equus      ATTCATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 470
gi|119489983 Sus        ATACACTTTGGCAGTGACTATGAGGACCGTTACTATCGTGAAAACATGTA 476
gi|119489801 Bos        ATACATTTTGGCAGTGACTATGAGGACCGTTACTATCGTGAAAACATGCA 497
```

Fig. 2. *Continued.* Alignment of the PRNP gene nucleotide sequences.

```
gi|54125480 Bos          ATACATTTTGGCAGTGACTATGAGGACCGTTACTATCGTGAAAACATGCA 497
gi|54125508 Bubalus      ATACATTTTGGTAATGACTATGAGGACCGTTACTATCGTGAAAACATGCA 497
gi|54125464 Syncerus     ATACATTTTGGTAATGACTATGAGGACCGTTACTATCGTGAAAACATGCA 497
gi|119514499 Capra       ATACATTTTGGCAATGACTATGAGGACCGTTACTATCATGAAAACATGTA 473
gi|119655282 Ammotragus  ATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|89160951 Ovis         ATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|73697718 Rangifer     ATACATTTTGGCAACGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|50442265 Rangifer     ATACATTTTGGCAACGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|50442321 Capreolus    ATACATTTTGGCAACGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|50442307 Alces        ATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|158714095 Cervus      ATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|50442285 Cervus       ATACATTTTGGCAATGACTATGAGGACCGTTACTATCGTGAAAACATGTA 473
gi|308194928 Homo        ATACATTTCGGCAGTGACTATGAGGACCGTTACTATCGTGAAAACATGCA 464
                         **.** ** ** *.********************.********** *

gi|27733849 Equus        CCGTTACCCCAACCAAGTGTACTACAGGCCGGTAAGTGAGTACAGCAACC 520
gi|119514511 Equus       CCGTTACCCCAACCAAGTGTACTACAGGCCGGTAAATGAGTACAGCAACC 520
gi|119489983 Sus         CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTACAGCAACC 526
gi|119489801 Bos         CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAGTAACC 547
gi|54125480 Bos          CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAGTAACC 547
gi|54125508 Bubalus      CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAGTAACC 547
gi|54125464 Syncerus     CCGTTACCCCAACCAAGTATACTACAGGCCAGTGGATCAGTATAGTAACC 547
gi|119514499 Capra       CCGTTACCCCAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACC 523
gi|119655282 Ammotragus  CCGTTACCCCAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACC 523
gi|89160951 Ovis         CCGTTACCCCAACCAAGTGTACTACAGACCAGTGGATCAGTATAGTAACC 523
gi|73697718 Rangifer     CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|50442265 Rangifer     CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|50442321 Capreolus    CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|50442307 Alces        CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|158714095 Cervus      CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|50442285 Cervus       CCGTTACCCCAACCAAGTGTACTACAGGCCAGTGGATCAGTATAATAACC 523
gi|308194928 Homo        CCGTTACCCCAACCAAGTGTACTACAGGCCCATGGATGAGTACAGCAACC 514
                         ******************.********.**.*...* **** *.****

gi|27733849 Equus        AGAACAACTTTGTGCACGACTGCGTCAACATCACGGTCAAGCAGCACACA 570
gi|119514511 Equus       AGAACAACTTTGTGCACGACTGCGTCAACATCACGGTCAAGCAGCACACG 570
gi|119489983 Sus         AGAACAGTTTTGTGCATGACTGCGTCAACATCACCGTCAAGCAGCACACA 576
gi|119489801 Bos         AGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGGAACACACA 597
gi|54125480 Bos          AGAACAATTTTGTGCATGACTGTGTCAACATCACAGTCAAGGAACACACA 597
gi|54125508 Bubalus      AGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGGAACACACA 597
gi|54125464 Syncerus     AGAACAGCTTTGTGCATGACTGTGTCAACATCACAGTCAAGGAACACACA 597
gi|119514499 Capra       AGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|119655282 Ammotragus  AGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|89160951 Ovis         AGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|73697718 Rangifer     AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|50442265 Rangifer     AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|50442321 Capreolus    AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|50442307 Alces        AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|158714095 Cervus      AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|50442285 Cervus       AGAACACCTTTGTGCATGACTGTGTCAACATCACAGTCAAGCAACACACA 573
gi|308194928 Homo        AGAACAACTTTGTGCACGACTGCGTCAATATCACAATCAAGCAGCACACG 564
                         ******  ******** ***** ***** *****.*****.*.*****.

gi|27733849 Equus        GTCACCACCACCACCAAGGGGGAGAACTTCACCGAGACCGACGTCAAGAT 620
gi|119514511 Equus       GTCACCACCACCACCAAGGGGGAGAACTTCACCGAGACCGACGTCAAGAT 620
gi|119489983 Sus         GTGACCACGACCACCAAGGGGGAGAACTTCACCGAGACGGACGTCAAGAT 626
gi|119489801 Bos         GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 647
gi|54125480 Bos          GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 647
gi|54125508 Bubalus      GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 647
gi|54125464 Syncerus     GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACGTCAAGAT 647
gi|119514499 Capra       GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 623
gi|119655282 Ammotragus  GTCACCACCACCACCAAGGGGGAGAACTTCACTGAAACTGACATCAAGAT 623
gi|89160951 Ovis         GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 623
gi|73697718 Rangifer     GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATTAAGAT 623
gi|50442265 Rangifer     GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATTAAGAT 623
gi|50442321 Capreolus    GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATTAAGAT 623
gi|50442307 Alces        GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATTAAGAT 623
gi|158714095 Cervus      GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 623
gi|50442285 Cervus       GTCACCACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGAT 623
gi|308194928 omo         GTCACCACAACCACCAAGGGGGAGAACTTCACCGAGACCGACGTTAAGAT 614
                         ** ***** ********************** **.** ***.* *****
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Fig. 2. *Continued.* Alignment of the PRNP gene nucleotide sequences.

```
gi|27733849 Equus       CATGGAGCGCGTGGTGGAGCAGATGTGCATCACCCAGTACCAGAAAGAGT 670
gi|119514511 Equus      CATGGAGCGCGTGGTGGAGCAGATGTGCATCACCCAGTACCAGAAAGAGT 670
gi|119489983 Sus        GATAGAGCGCGTGGTGGAACAGATGTGCATCACCCAGTACCAGAAAGAGT 676
gi|119489801 Bos        GATGGAGCGAGTGGTGGAGCAAATGTGCATTACCCAGTACCAGAGAGAAT 697
gi|54125480 Bos         GATGGAGCGAGTGGTGGAGCAAATGTGCATTACCCAGTACCAGAGAGAAT 697
gi|54125508 Bubalus     GATGGAGCGAGTGGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 697
gi|54125464 Syncerus    GATGGAGCGAGTGGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 697
gi|119514499 Capra      AATGGAGCGAGTGGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|119655282 Ammotragus AATGGAGCGAGTGGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|89160951 Ovis        AATGGAGCGAGTGGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|73697718 Rangifer    GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|50442265 Rangifer    GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|50442321 Capreolus   GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|50442307 Alces       GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|158714095 Cervus     GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|50442285 Cervus      GATGGAGCGAGTTGTGGAGCAAATGTGCATCACCCAGTACCAGAGAGAAT 673
gi|308194928 Homo       GATGGAGCGCGTGGTTGAGCAGATGTGTATCACCCAGTACGAGAGGGAAT 664
                  **.*****.** ** **.**.***** ** ********* ***..**.*
gi|27733849 Equus       ACGAGGCTTTTCAACAAAGAGGGGCGAGCGTGGTCCTCTTCTCCTCCCCG 720
gi|119514511 Equus      ACGAGGCTTTTCAACAAAGAGGGGCGAGCGTGGTCCTCTTCTCCTCCCCG 720
gi|119489983 Sus        ACGAGGCGTACGCCCAAAGAGGGGCCAGTGTGATCCTCTTCTCCTCCCCT 726
gi|119489801 Bos        CCCAGGCTTATTACCAACGAGGGGCAAGTGTGATCCTCTTCTCTTCCCCT 747
gi|54125480 Bos         CCCAGGCTTATTACCAACGAGGGGCAAGTGTGATCCTCTTCTCTTCCCCT 747
gi|54125508 Bubalus     CCCAGGCTTATTACCAACGAGGGGCAAGTGTGATCCTCTTCTCTTCCCCT 747
gi|54125464 Syncerus    CCCAGGCTTATTACCAACGAGGGGCAAGTGTGATCCTCTTCTCTTCCCCT 747
gi|119514499 Capra      CCCAGGCTTATTACCAAAGGGGGGCAAGTGTGATCCTCTTTTCTCCCCCT 723
gi|119655282 Ammotragus CCCAGGCTTATTACCAAAGGGGGGCAAGTGTGATCCTCTTTTCTTCCCCT 723
gi|89160951 Ovis        CCCAGGCTTATTACCAAAGGGGGGCAAGTGTGATCCTCTTTTCTTCCCCT 723
gi|73697718 Rangifer    CCCAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|50442265 Rangifer    CCCAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|50442321 Capreolus   CCCAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|50442307 Alces       CCCAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|158714095 Cervus     CCGAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|50442285 Cervus      CCGAGGCTTATTACCAAAGAGGGGCAAGTGTGATCCTCTTCTCCTCCCCT 723
gi|308194928 Homo       CTCAGGCCTATTACCAGAGAGGATCGAGCATGGTCCTCTTCTCCTCTCCA 714
                  .  **** *:  ..**..*.**. * ** .**.******* **  * **
gi|27733849 Equus       CCTGTGGTCCTCCTCATCTCTT---------------------------- 742
gi|119514511 Equus      CCTGTGGTCCTCCTCATCTCTTTCCTCATTTTCCTCATAGTGGGCTGA 768
gi|119489983 Sus        CCTGTGATCCTCCTCATCTCTTTCCTCCTTTTCCTCATAGTGGGCTGA 774
gi|119489801 Bos        CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 795
gi|54125480 Bos         CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 795
gi|54125508 Bubalus     CCTGTGATCCTCCTCATCTCTTTGCTCATTTTTCTCATAGTAGGATAG 795
gi|54125464 Syncerus    CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 795
gi|119514499 Capra      CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|119655282 Ammotragus CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|89160951 Ovis        CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|73697718 Rangifer    CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|50442265 Rangifer    CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|50442321 Capreolus   CCTGTGATCCTCCTCATATCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|50442307 Alces       CCTGTGATCCTCCTAATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|158714095 Cervus     CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|50442285 Cervus      CCTGTGATCCTCCTCATCTCTTTCCTCATTTTTCTCATAGTAGGATAG 771
gi|308194928 Homo       CCTGTGATCCTCCTGATCTCTTTCCTCATCTTCCTGATAGTGGGATGA 762
                  ******.******* **.****
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Notes:
** indicates identical nucleotide sequences;*
−, shift of the nucleotide sequences for a more efficient alignment;
. or :, nucleotide substitution.

Fig. 2. *Ending.* Alignment of the PRNP gene nucleotide sequences.

As follows from Fig. 2, there are point differences at positions 5, 7–12, 16, 21–22, 24, 30, 33, 47, 50, 61, 63, 66, 94, 108, 110, 114, 126, 135, 138, 153, 156, 160, 162, 171, 174, 190, 193, 196, 201–223 (numbered as in *Bos taurus* protein) between *Bos taurus*, *Bos javancus, Bubalus bubalis*, and *Syncerus caffer caffer*; positions 225, 231, 237, 240, 246, 255, 261, 264, 270, 273, 283, 294, 296–298, 318, 320, 324, 339, 348, 366, 375, 378, 381, 399, 408, 411, 414, 438, 444, 447,453, 456, 459, 462, 496, 528, 532, 540, 543, 554–555, 564, 570, 576, 582, 589, 600, 606, 630, 636, 642, 648, 660, 663, 676, 686, 698–699, 705, 708–709, 721, 723, 726, 738, 741–742, 744, 747, 762, 769–795 are different only in *Equus caballus*.

To better visualize the level of evolutionary relatedness of the prion protein sequences, a phylogenetic tree presented in Fig. 3 was built in the ClustalW software (see Fig. 2 for designations).

Also, gene sequences of pathogenic and normal prion protein from *Ovis aries* was performed (Fig. 4). It demonstrated that the nucleotide sequences of PrP^{c} and PrP^{sc} are identical.

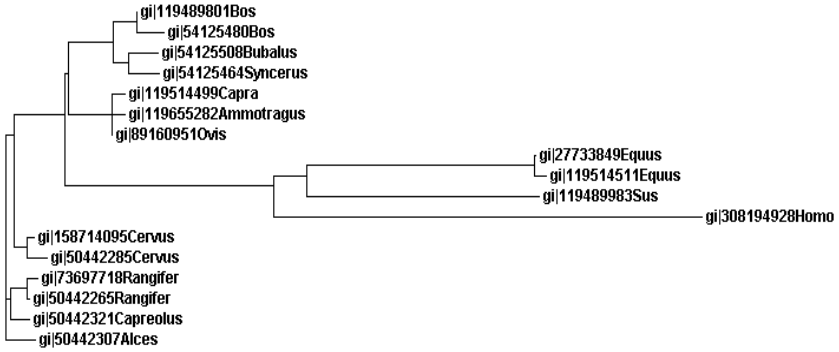

Fig. 3. Phylogenetic tree of the PRNP protein gene sequences.

```
   gi|341942290PrPsc
GGGTCAAGGTGGTAGCCACAGTCAGTGGAACAAGCCCAGTAAGCCAAAAACCAACATGAA 60
   gi|47028553PrP  -
GGTCAAGGTGGTAGCCACAGTCAGTGGAACAAGCCCAGTAAGCCAAAAACCAACATGAA 59
            ************************************************************
   gi|341942290PrPsc
GCATGTGGCAGGAGCTGCTGCAGCTGGAGCAGTGGTAGGGGGCCTTGGTGGCTACATGCT 120
   gi|47028553PrP
GCATGTGGCAGGAGCTGCTGCAGCTGGAGCAGTGGTAGGGGGCCTTGGTGGCTACATGCT 119
            ************************************************************
   gi|341942290PrPsc
GGGAAGTGCCATGAGCAGGCCTCTTATACATTTTGGCAATGACTATGAGGACCGTTACTA 180
   gi|47028553PrP
GGGAAGTGCCATGAGCAGGCCTCTTATACATTTTGGCAATGACTATGAGGACCGTTACTA 179
            ************************************************************
   gi|341942290PrPsc
TCGTGAAAACATGTACCGTTACCCCAACCAAGTGTACTACAGACCAGTGGATCAGTATAG 240
   gi|47028553PrP
TCGTGAAAACATGTACCGTTACCCCAACCAAGTGTACTACAGACCAGTGGATCAGTATAG 239
            ************************************************************
   gi|341942290PrPsc
AACCAGAACAACTTTGTGCATGACTGTGTCAACACCACAGTCAAGCAACACACAGTCAC 300
   gi|47028553PrP
TAACCAGAACAACTTTGTGCATGACTGTGTCAACATCACAGTCAAGCTACACACAGTCAC 299
            ********************************** ***********:************
   gi|341942290PrPsc
CACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGATAATGGAGCGAGTGGT 360
   gi|47028553PrP
CACCACCACCAAGGGGGAGAACTTCACCGAAACTGACATCAAGATAATGGAGCGAGTGGT 359
            ************************************************************
   gi|341942290PrPsc GGAGCAAATGTGCATCACCCAGTACCAGAGAGAATCCCAGGCTT 404
   gi|47028553PrP    GGAGCAAATGTGCATCACCCAGTACCAGAGAGAATCCCAGGCT- 402
            ****************************************
```

Fig. 4. Alignment of normal (PrP^{c}) and pathogenic (PrP^{sc}) forms of PRNP prion protein from *Ovis aries*.

The phylogenetic analysis confirmed that prion protein sequences are rather conserved and differ only by conformation and relative stability to proteolysis it associates with. This does not allow choosing a DNA target among the prion sequences for further analysis with PCR. Therefore, here we chose a variety of the PCR method, i.e. the real-time immuno-PCR, where DNA molecule is used as a marker, to detect infectious prion proteins. Immuno-PCR allows to detect pathogenic prion protein using specific antibodies labeled with double-strand DNA. Immuno-PCR combines the universality of the enzyme-linked immunosorbent assays with sensitivity of PCR. The method allows for protein detection at the level of several hundred molecules.

To choose an appropriate antibody reacting with pathogenic prion proteins, we analyzed commercial antibodies. Because of the high inter-species homology noted for the PrP protein, antibodies against peptide conjugates are the most feasible.

Therefore, we chose a mouse monoclonal antibody 15B3 (Prionics) obtained using 3 different sequences (epitopes) of human PrP: 15b3-1 includes amino acid residues 142–148 GSDYEDR(YY); 15b3-2, residues 162–170 YYRPVDQYS; and 15b3-3, residues 214–226 CITQYQRESQAYY (Fig. 5).

```
   gi|56180813Sus
MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGG- 59
   gi|119514512Equus MVKSHVGGWILVLFVATWSDVGLCKKRPKPGG-
WNTGGSRYPGQGSPGGNRYPPQGGGG- 58
   gi|6110615Ovis
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGG- 59
   gi|1149617Capra
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGG- 59
   gi|34334038Bos
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGR 60
   gi|89160954Homo  --MANLGCWMLVLFVATWSDLGLCKKRPKPGG-
WNTGGSRYPGQGSPGGNRYPPQGGGG- 56
                 :::* *:****** ***:********** *************************

   gi|56180813Sus  -------
WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQWNKPSKPKTN 112
   gi|119514512Equus -------WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGG-
SHGQWNKPSKPKTN 110
   gi|6110615Ovis  -------WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGG-
SHSQWNKPSKPKTN 111
   gi|1149617Capra  -------WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGG-
SHSQWNKPSKPKTN 111
   gi|34334038Bos  GQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGG-
THGQWNKPSKPKTN 119
   gi|89160954Homo  -------WGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGG-
WGQGGGTHSQWNKPSKPKTN 108
                 ******************************** ***** :*.**********

   gi|56180813Sus
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYYRENMYRYPNQVYYRPVDQ 172
   gi|119514512Equus
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVNE 170
   gi|6110615Ovis
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQ 171
   gi|1149617Capra
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLMHFGNDYEDRYYRENMYRYPNQVYYRPVDQ 171
   gi|34334038Bos
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYYRENMHRYPNQVYYRPVDQ 179
   gi|89160954Homo
MKHMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQVYYRPMDE 168
             ***:************************..***.**********:**********:::

   gi|56180813Sus
YSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDVKMIERVVEQMCITQYQKEYEAYAQR 232
   gi|119514512Equus
YSNQNNFVHDCVNITVKQHTVTTTTKGENFTETDVKIMERVVEQMCITQYQKEYEAFQQR 230
```

Fig. 5. *Beginning.* Amino acid sequence of the PRNP prion protein.

```
    gi|6110615Ovis
YSNQKNFVHDCVNITVKQHTVTTTTKGENFTETDIKIMERVVEQMCITQYQRESQAYYQR 231
    gi|1149617Capra
YSNQNNFVHDCVNITVKQHTVTTTTKGENFTETDIKIMERVVEQMCITQYQRESQAYYQR 231
    gi|34334038Bos
YSNQNNFVHDCVNITVKEHTVTTTTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQR 239
    gi|89160954Homo
HSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMMERVVEQMCITQYERESQAYYQR 228
             :***:.*********:*:*****************:*::***********::* :*: **

    gi|56180813Sus    GASVILFSSPPVILLISFLLFLIVG 257
    gi|119514512Equus GASVVLFSSPPVVLLISFLIFLIVG 255
    gi|6110615Ovis    GASVILFSSPPVILLISFLIFLIVG 256
    gi|1149617Capra   GASVILFSPPPVILLISFLIFLIVG 256
    gi|34334038Bos    GASVILFSSPPVILLISFLIFLIVG 264
    gi|89160954Homo   GSSMVLFSSPPVILLISFLIFLIVG 253
             *:*::***.***:******:*****
```

Fig. 5. *Ending.* Amino acid sequence of the PRNP prion protein.

15B3-1 and 15B3-2 bind beta-sheets that accumulate in PrP^{sc}, and 15B3-3 recognizes amino acid residues near the C-terminus.

15B3 is an antibody specifically recognizing an aberrantly folded PrP^{sc} protein, and not the normal PrP molecules (PrP^{c}).

The Prionics company proved experimentally that 15B3 reacts with pathogenic PrP^{sc} prions of man, cattle, sheep, deer, mouse, and hamster, but does not react with the normal prions. Therefore, 15B3 can be used as a detecting antibody for further analysis.

The procedure goes as follows. Antigen (prion protein) is introduced into a 96-well polysterene plate, 100 μL per well, and the plate is incubated at 37°C for 60 min. To choose the optimal conditions for antigen adsorption on the plastics, the antigen was incubated at concentrations of 0.5, 1, 2.5, 5, 10, 25, and 50 μg/mL for 30, 60, 90, 120, 150, or 180 min. Unbound material is removed from the wells with a simple shaking followed by three washes (washing buffer: 50 mM Tris, 150 mM NaCl, and 0.5 mL/L Tween 20). Non-specific binding sites are blocked by a 30-min incubation with PBS supplemented with bovine serum albumin, 100 μL per well. After removal of the blocking solution (by washing), biotinylated monoclonal antibody to pathogenic prion protein 15B3 is added, 100 μL per well, to determine the adsorbed material, and the plate is incubated for 2 h at 18°C. Unbound antibodies are removed by a triple washing of the wells with PBS containing 1 mL/L Tween and triple washing with PBS containing 15 g/L bovine serum albumin. To prepare DNA reporter agent, streptavidin–biotin complex was chosen as a binding unit between the antibody and the DNA reporter.

A molecule of streptavidin comprises four identical subunits and is capable of binding four biotin molecules, which allowed using it as a binding unit between two biotin-containing compounds. In this case, DNA tail is also biotinylated, and streptavidin functions as a bridge binding the two molecules containing biotin residues.

Preparation of the conjugates of antibodies and DNA with biotin is accompanied by minimal changes in their immunological activity.

Recombinant streptavidin is pre-incubated for 45 min at 4°C with biotinylated DNA reporter in the molar ratio of 1 : 2. Then, the streptavidin–DNA complex is added to the wells and the plate is incubated for 30 min at room temperature. The wells are washed 5 times with PBS and 10 times, with distilled water, and then subjected to PCR.

By this stage of the study, we have already aligned the PRNP gene nucleotide sequences and have built the phylogenetic tree. Alignment of gene sequences of pathogenic and normal prion proteins from *Ovis aries* has demonstrated that the nucleotide sequences of PrP^{c} and PrP^{sc} are identical.

To choose a high-performance DNA target, we performed the analysis of GenBank, Sol Genomic Network, and EMBL-EBI databases, which proved that the prion protein gene sequences are rather conserved; therefore, it is not possible to choose a DNA target among the prion sequences for further analysis with PCR. Therefore, we have chosen the real-time immuno-PCR method for detection of infectious prion proteins, where DNA molecule is used as a marker.

Mouse monoclonal antibody 15B3 obtained using three different sequences (epitopes) of the human PrP peptide (15b3-1 includes amino acid residues 142–148 GSDYEDR(YY); 15b3-2, residues 162–170 YYRPVDQYS and 15b3-3, residues 214–226 CITQYQRESQAYY) was chosen for the work.

It has been shown experimentally that 15B3 reacts with pathogenic PrP^{sc} prions of man, cattle, sheep, deer, mouse, and hamster, but does not react with the normal prions. Therefore, 15B3 can be used as a detecting antibody for further analysis.

The primer design as such is preceded by the construction of a detailed model of the target gene or another nucleotide sequence to be amplified.

To perform the immuno-PCR analysis, a DNA (or a DNA tail) template was needed.

To decrease the risk of false response due to exogenous contamination of DNA in the assay, we designed a DNA tail which does not exist in nature. A synthetic random 194 bp long sequence (fragment length in the range of 150–300 bp is considered optimal) was prepared (see Fig. 6) [11, 12].

AGGAGGTGGCCACGACTGCGAAGGAGGTGGCGTAGGATAGAGT-
CAGTCCTTGGCCTCCTTGGCCCAGTTAAGAAGTTGCAGCCACA-
CACGCTGTTGTTGGGTTCGGGGCGGAGTTGCAGCCATCTACACAAACGA-
TACCCTCGTGCAGCTGGAGAAGCAGCACGGCCTATTACCTGGAGGAGGATCGAAACTGA

Fig. 6. DNA template sequence.

The created sequence was analyzed in GenBank using the BLAST software to confirm that there are no homologs of the sequence.

One of the key factors in the reaction are the primers, synthetic oligonucleotides 20–30 nucleotide long. Primers are complementary to DNA chains in regions at the boarder of a chosen DNA fragment and are oriented with their 3'-ends facing each other and along the chosen DNA sequence to be amplified. The length of the amplified fragment is determined by the distance between the primers.

In the PCR amplification, two oligonucleotide primers are used. Primers are chosen so that the synthesis by polymerase would proceed only between them, doubling the number of copies of this DNA region. As a result, the amount of a specific fragment grows exponentially.

Primer construction, probably, is the most critical parameter for a successful PCR analysis. Primer sequence determines a whole number of parameters, such as the position and length of the product, its melting temperature, and yield of the product. Poorly constructed primer may lead to small amount of the product, its absence due to non-specific amplification and/or dimer formation by a primer, which may become a competitive process inhibiting the product formation [11].

Taking into account the above-mentioned issues, the following two 20-nucleotide long primers were selected for the synthesized DNA tail:

>>>>>> left primer – starting from 41 bp-AGTCAGTCCTTGGCCTCCTT;

<<<<<< right primer – starting from 193 bp-CAGTTTCGATCCTCCTCCAG.

Using the Primer3 software, melting temperature (t_m) and other parameters of the primers were chosen (Table 7).

The melting temperature of the left primer t_m = 59.8°C and the right primer t_m = 60.25°C.

Annealing temperature is set 4–5°C below the melting temperature.

Therefore, the optimal annealing temperature in the amplification program will be t_a = 56°C for the left primer and t_a = 55.8°C, for the right one.

Table 7. Parameters of the primers

Primer type	Starting position, bp	CG, %	Length, bp	t_m,°C	Sequence
Left primer	41	55	20	60.25	AGTCAGTCCTTGGCCTCCTT
Right primer	193	55	20	59.80	CAGTTTCGATCCTCCTCCAG

Table 8. Final characteristics for primer construction

Main requirements to the primers	Values	Comparison of the characteristics of chosen primers with the requirements
Primer length	from 15 to 30 nucleotides	20 bp, fits
GC content	from 45% to 55%	55%, fits
Melting temperature (t_m)	from +55°C to +75°C	t_m = 60.25°C and t_m =59.80°C fits
Annealing temperature (t_a)	4–5 degrees below the melting temperature	t_a =56°C и t_a =55.80°C, fits
Secondary structure of the primer	Primer should not fold into a secondary structure with melting temperature equal to or above the t_m of the primer	Fits (verified using the *Mfold* 3.2 software package)
Secondary structure of the target site	Target site should not fold into a secondary structure with melting temperature equal to or above the t_m of the primer	Fits (verified using the *Mfold* 3.2 software package)
Homo- and heterodimerization of the primers	Excluded, especially at the 3'-end	Fits (verified using the Hybrid software package)
Primer specificity	The degree of complementarity to the target site is close to 100%; less than 70% homology with other nucleotide sequences	Fits (verified using the BLAST software)

Therefore, at this stage of the study, random synthetic target DNA sequence (DNA tail) 194 bp long has been created. Analysis of the GenBank using the BLAST software demonstrated that the created sequence has no homologs among the sequences of the database.

Two 20-bp primers were synthesized for the DNA tail. Using the Primer3 software, primer parameters were chosen.

Studies of the specificity of the developed PCR system were performed by the example of meat chop containing the mixture of muscle tissues of beef and pork and supplemented with 1.0, 2.0, 5.0, 10.0, and 15.0 pork meat infected with a pathogenic prion protein. Each stage of DNA isolation was accompanied by the addition of an internal standard. The presence of the pathogenic prion protein in pork tissues was confirmed using the commercial TeSeE^{TM} ELISA test-system.

The analysis demonstrated high specificity of the developed PCR system: no non-specific response to 100-% fish flour or chicken chop, as well as their mixtures, was registered. The results are presented in Fig. 7.

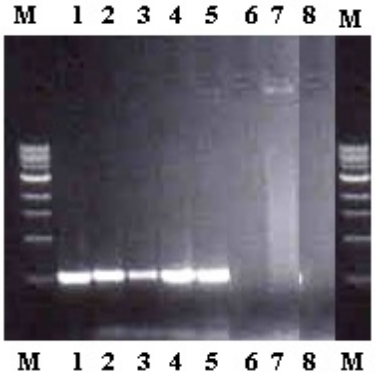

Fig. 7. Evaluation of the specificity and sensitivity of the test-system: M, marker; 1, 1.0% infected pork meat in the meat chop; 2, 2.0% infected pork meat in the meat chop; 3, 5.0% infected pork meat in the meat chop; 4, 10.0% infected pork meat in the meat chop; 5, 15.0% infected pork meat in the meat chop; 6, 100% meat chop; 7, fish flour; 8, chicken chop.

Besides, the specificity of the developed test-system was studied based on the comparative analysis of the results on determination of the pathogenic prion protein obtained using the proposed PCR test-system and a commercially available ELISA assay TeSeE^{TM}. Over 200 samples of clinical material were tested in parallel. Test results of the commercially available TeSeE^{TM} assay and the proposed test-system matched in 198 out of 200 cases. Sensitivity of the reference (commercially available) method was 96.5%. In five of the positive samples not detected by the TeSeE^{TM} ELISA assay, initial DNA target concentration did not exceed 100 copies/mL. Therefore, the higher stability of detection of low DNA concentrations in the PCR method, if compared with other methods, is confirmed by the results of clinical samples study (Table 9).

Table 9. Comparison of the results of pork testing using the PCR test-system and the TeSeE^{TM} ELISA assay

	Number of analyzed samples					
	PCR test-system			TeSeE^{TM} ELISA assay		
	«+»	«–»	«inh»	«+»	«–»	«inh»
«+» n = 60	58	1	1	57	2	1
«–» n = 140	1	139	0	2	134	4
Relative specificity	96,5			96,5		

Notes: «+», positive samples; «–», negative samples; «inh», inhibited samples.

Comparison of the results obtained with the proposed PCR test-system and the reference method evidence real-time high specificity of the developed PCR method.

Therefore, high specificity of the developed test-system and oligonucleotide primers was confirmed by three ways: 1) using the Primer3 software; 2) by electrophoretic separation of the meat chop samples with different percent content of pork tissues infected with a pathogenic prion protein; and 3) by comparative analysis of the results of pathogenic prion protein determination using the proposed PCR test-system and a commercial ELISA assay TeSeE^{TM}.

REFERENCES

1. Wickner, R.B., Edskes, H.K., Roberts, B.T., Baxa, U., Pierce, M.M., Ross, E.D., and Brachmann, A., Prions: Proteins as Genes and Infectious Entities, *Genes Dev.*, 2004. V. 18, no. 5, P. 470–485.
2. Heikenwalder, M., Julius, C., Aguzzi, A., Prions and Peripheral Nerves: A Deadly Rendezvous, *J. Neurosci.*, 2007. V. 85, P. 2714–2725.
3. Weissmann, C., and Aguzzi, A., Approaches to Therapy of Prion Diseases, *Annu. Rev. Med.*, 2005. V. 56, P. 321–344.
4. Bartz, J.C., Dejoia, C., Tucker, T., Kincaid, A.E., and Bessen, R.A., Extraneural Prion Neuroinvasion without Lymphoreticular System Infection, *J. Virol.*, 2005. V. 79, P. 11858–11863.
5. Vana, K., Zuber, C., Nikles, D., and Weiss, S., Novel Aspects of Prions, Their Receptor Molecules, and Innovative Approaches for TSE Therapy, *Cell. Mol. Neurobiol*., 2007. V. 27, no. 1, P. 107–128.
6. *Elementnyi ekspress analizator Rapid N cube. Kratkoe rukovodstvo* (Express elemental analyzer Rapid N-cube. Brief manual) (Moscow, 2009).
7. Prosekov, A.Yu., Babich, O.O., and Sukhikh, S.A., *Sovremennye metody issledovaniya syr'ya i biotekhnologicheskoi produktsyi: Laboratornyi praktikum* (Modern methods of investigation of raw materials and biotechnology products: Laboratory course), (Kemerovo Institute of Food Science and Technology, Kemerovo, 2012).

8. http://www.ncbi.nlm.nih.gov/genbank/
9. Prusiner, S.B., Prions, *Proc. Natl. Acad. Sci. USA*, 1998. V. 95, no. 23, P. 13363–13383.
10. Apetri, A.C., and Surewicz, A.C., Kinetic Intermediate in the Folding of Human Prion Protein, *J. Biol. Chem.*, 2002. V. 277, P. 44589–44592.
11. Patrushev, L.I., *Iskusstvennye geneticheskie sistemy. T. 1. Gennaya i belkovaya inzheneriya* (Artificial genetic systems. V. 1. Gene and protein engineering) (Nauka, Moscow, 2004).
12. Aguzzi, A., Sigurdson, C., Heikenwalder, M., Molecular Mechanisms of Prion Pathogenesis, *Annu. Rev. Pathol. Mech. Dis.*, 2008. V. 3, P. 11–40.

PROCESSES, EQUIPMENT, AND APPARATUSES FOR THE FOOD INDUSTIRY

SIMULATING THE REFRIGERATION OF BATCH DAIRY PRODUCTS IN A MULTIZONE COLD SUPPLY SYSTEM

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Abstract: Methods that simulate the fast refrigeration of foods on the basis of a model of adjustable heat sink according to the principle of programmed freezing are considered. In this case, a fast freezer is seen as a system of modules, each of which can independently ensure the necessary heat-sink conditions for the fast refrigeration process. The focus is made on the analysis of physicochemical processes that form the water crystallization front at the first freezing stage, taking into account the thermophysical specifics of organizing a multizone combined system of refrigeration supply. Test-bench studies were conducted to obtain the main regularities of fast freezing of single-piece packaged dairy products by the nitrogen + air combined method in a wide range of heat-exchange conditions. The fast freezer has two freezing zones with various temperatures, allowing an efficient distribution of energy costs and creating the optimal conditions for freezing and for the continuity of the technological cycle. A mathematical model has been developed on the basis of experimental data analysis to determine the main technological parameter, the duration of food refrigeration in a nitrogen + air combined two-zone fast freezer with adjustable heat sink. The integral characteristics of the mathematical model have been determined. The model's adequacy to the real freezing process has been proved.

Keywords: combined method, refrigeration, dairy products, fast freezer, nitrogen, temperature, zone, duration, calculations

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INTRODUCTION

The simulation of refrigeration processes used for various foodstuffs and raw materials with precise goal setting and results obtained has been conducted by many authors, such as I.G. Alyamovskii, A.M. Brazhnikov, K.P. Venger, D.G. Ryutov, G.B. Chizhov, I.G. Chumak, and A.P. Sheffer. These studies were based on the theories of Planck, Stefan, Lamé, and Clapeyron, and the solution of the problem was reduced to determining the duration of food freezing to a preset volume-averaged or final temperature in the middle of the body in a criterion, dimensionless, or classical form.

The simplest dependence for determining the duration of the refrigeration process was developed by Planck. This solution is considered classical, being notable for its simplicity and ease of use. It is built on the following assumptions:

– a homogeneous moisture-containing body is cooled to the cryoscopic temperature before refrigeration;

– ice formation occurs without overcooling and isothermally at the cryoscopic temperature, and the thermophysical properties of the frozen part of the body's total volume do not depend on temperature, the thermal capacity of the frozen part being equal to zero, and

– refrigeration occurs by removing heat from the body surface, the heat transfer coefficient and the temperature of the heat-sinking media being constant.

The first analytical solution to the problem of the duration of freezing a flat plate from the initial temperature, which is higher than the cryoscopic one, to the final temperature of the middle, which is below the cryoscopic one, was obtained by D.G. Ryutov and was widely recognized in refrigeration technology [1].

In order to take into account the duration of the plate's temperature decline after the convergence of phase boundaries, a linear temperature change is admissible along the thickness of the frozen layer. Further heat exchange in the plate is analyzed on the basis of the regularities of simple cooling. The time during which the temperature of the plate center decreases from the cryoscopic to the finial preset temperature is summed to the duration calculated by the Planck formula.

In order to take into account the influence of the initial temperature of the product plate on the duration of refrigeration to the heat amount removed from the mass unit during freezing, a binomial multiplier was introduced $(1 + 0.0053\ t_i)$.

Alyamovskii adjusted Ryutov's formula by assuming that the temperature distributed parabolically at the initial moment of freezing [2].

V.E. Kutsakova proposed a model for calculating the duration of refrigeration of an infinite plate, characterized by a simple mathematical formula and

reduced to the Planck formula, taking into account the period of further freezing and the process of crystallization front movement. The model assumes a linear approximation of the temperature field of the material along the axis of temperature front distribution at the stages of refrigeration and the period of relaxation of the temperature field [3].

It is assumed that during refrigeration the heat transfer coefficient changes insignificantly in the course of the process. At the same time it is known that the linear and volume rates of refrigeration increase sharply as the heat transfer coefficient increases at a small thickness of the frozen layer, and, as the thickness increases, this effect is smoothed. However, under the conditions of adjustable heat sink, the heat transfer coefficient changes significantly in a two-zone nitrogen + air combined fast freezer, which should be taken into account during the process organization.

A new approach to solving the problem of determining the duration of refrigeration of biological objects was proposed by V.M. Stefanovskii, holding that, during cold treatment, it is important to know not the thickness of the frozen layer but the mass of unfrozen water by time point τ [4].

Thus, the considered analytical dependences for determining refrigeration duration have both an advantage in the relative simplicity of equations and general drawbacks, inherent in the Planck formula and its modifications: excessive simplifications in the problem statement and the representation of the temperature profile as a linear function.

When solving frost penetration problems according to Stefan, the phase boundary is represented as a line dividing the frozen and unfrozen zones of a product, which is usually called the crystallization front, which moves from the periphery to the center during refrigeration.

The statement of the Stefan problem for freezing a product plate in the interpretation of A.M. Brazhnikov considers a plate with a thickness of $2l$, initial temperature t_i, and cryoscopic temperature t_{cr} situated in an environment with temperature t_{en} ($t_{en} \leq t_{cr} \leq t_i$). The thermophysical characteristics of the plate are assumed to be constant within each zone and change in discrete steps at phase transformations. The refrigeration process consists of two stages. At the first stage, the product is cooled from its initial temperature to the cryoscopic temperature at the product surface, and, at the second stage, the crystallization front moves from the object surface to the object center. It is assumed that practically all moisture freezes up at the end of the second stage. The problem under consideration is nonlinear. It has no exact solution. Various approximate methods are used. One of them is the selection of a temperature function from experimental data, the search for a coefficient from boundary conditions, and the solution of a differential equation resulting from the Stefan condition [5].

Academician L.S. Leibenzon has developed a method that makes it possible to introduce values that characterize the boundary conditions on the object surface instead of derivates at the phase boundary [6].

The best results in determining the duration of the fast freezing of a large group of products were obtained by Russian scientists, in particular, by Venger, E.V. Semenov, I.E. Lobanov, B.S. Babakin, and M.I. Voronin, where the concerned problem of heat transfer in the conditions of continuous heat sink is based on an approximate method, the method of Leibenzon's integral relations, developed by A.M. Pirvedyan, V.A. Karpychev, and Brazhnikov [7, 8, 9].

Despite the abundance of theories and methods developed for solving this problem, in practice it is difficult to decide on a single model that would describe and take into account with a high accuracy all factors present in the problem statement. Therefore, various options and methods of freezing any biological object should take into account the geometric shape of a product, its structural heterogeneity, the specific changes of its thermophysical characteristics during the phase transition from moisture to ice, the availability of packaging, the quality of a product ready for processing, etc. The latest publications of Russian and foreign scientists, A.A. Tvorogova, P.B. Chizhova, V.O. Buyanov, and N.G. Craiver, are dedicated to the most important characteristic for frozen products, structure assessment by the condition of ice crystals depending on thermophysical and technological factors [10, 11, 12]. Buyanov has found out that intensive heat exchange, owing to high process velocities and low air temperatures, forms a finely crystalline ice structure in cheese mass, preserving at the same time the microstructure and consistency in a satisfactory condition. The consequence of fast freezing is almost full homogeneity of ice composition.

Changes in the consistency of cheeses during refrigeration and subsequent storage are associated with the loss of relatively free water. Moreover, changes in the consistency of frozen products can be avoided by adjusting the pH value.

Some of these factors are easy to take into account, while others are in a complex dependence on process stages. Therefore, it is rational to use the existing ready-made solutions to the theory of thermal conductivity during the analytical description of refrigeration processes; such solutions make it possible to reduce the problem of freezing products of complex geometric shapes to the refrigeration of products that are represented by equivalent bodies of simple forms, such as a plate, a cylinder, or a sphere [13].

Thus, the analysis of the kinetics of the fast refrigeration process in Buyanov's studies used mathematical methods of experimental design, and an object under study was represented as an equivalent body, i.e., an indefinite plate [11]. The knowledge of control mechanisms of these processes will make it possible to directionally regulate the composition and properties of frozen products and the degree of their intensity, preserving the initial nutritive and biological values. The effect of low temperatures on the microorganisms and general microflora of frozen cheese was studied by Russian scientists Buyanov, I.O. Larina, I.V. Buyanova, and O.V. Kriger [14].

At present, foreign specialists in creative collaboration with Russian scientists have developed a number of units to freeze products in liquid nitrogen:

from small units for 50–100 kg/h, made by Messer (the United States), to large continuous action units, made by Linde (the United States), Cryo-Quick by Air Products, Union Carbide by AGA, etc. [7, 15, 16]. The CER Chachak factory (Yugoslavia) produces cryogenic trizonal tunnel fast freezers that use liquid and gaseous carbon dioxide or liquid nitrogen. The specialists of CER Chachak note a number of technical and economic advantages of a machineless refrigeration system [7].

Foreign multizone cryogenic apparatuses have a capacity of 150–1500 kg/h; the tunnel length varies within 7–15 m; and the consumption of liquid nitrogen for the refrigeration of 1 kg of products is from 1.2 to 1.5 kg, the temperature of outgoing nitrogen vapors being from –50 to –70ºC.

The first study of dumpling refrigeration using liquid nitrogen was conducted in Russia by N.D. Abramov. Subsequent studies by N.A. Aleksandrova, G.D. Shabetnik, O.V. Anistratova, and B.N. Semenov showed the economic efficiency of liquid nitrogen for the refrigeration of endocrine–enzymatic special raw materials, as well as curds [7, 8, 9].

Li Ruixia, Wang Weicheng, and D. Coulomb studied the long-term development of refrigeration technology, including the modeling of cooling and frost-formation processes for cold accumulators [15, 16].

Of increasing interest lately has been a technology that includes two systems of air and cryogenic freezing and combines the advantages of both methods. The combined method uses combinations of flow-through systems with traditional machine systems.

Venger's works [7] describe the findings concerning heat exchange during the refrigeration of poultry carcasses by the combined method. The analysis has also shown that the nitrogen + air refrigeration reduces the process duration practically by 2–3 times compared to the air method and excludes product mass loss through shrinkage, because the instantly formed frozen layer hinders moisture evaporation from the product surface.

The comparative analysis of prospects for the development of refrigeration engineering and technology, conducted by Buyanov, showed the practicality of introducing multizone fast freezers with a combined cold supply system into the industry. Here he distinguished two main interrelated priorities: increasing the energy efficiency of freezers and their environmental safety [17]. He found out that high process velocities reduce the duration of refrigeration of batch dairy products and their mass.

Of interest is a freezer in which cold air is supplied to the initial refrigeration stage at a temperature of –50ºC, and then the vapors of boiling liquefied nitrogen at a temperature of –120ºC are supplied through the lower channels in the containers for final product freezing [18].

A two-module fast freezer efficiently solves the problems of fast refrigeration kinetics, the rational operating characteristics of the modules, the formation of a continuous technological process, and the resulting use of the cooling potential of the refrigerating environment [19].

Several tunnel freezers for the fast refrigeration of batch foods have been patented in the United States.

The purpose of this work is the development of a mathematical model for determining the duration of the fast refrigeration of foods in a two-zone modular fast freezer based on a nitrogen + air combined cold supply system with adjustable heat sink.

OBJECTS AND METHODS OF RESEARCH

For refrigeration modeling, a mathematical model developed by the Russian scientist Venger was taken as the basis [7]. In order to verify the adequacy of the developed mathematical model to the real refrigeration process, experiments were conducted at a special bench, designed at the Kemerovo Institute of Food Science and Technology, Russia (Fig. 1).

The experiments were conducted at the research laboratories of the departments of Heat and Cold Engineering, Technology of Milk and Dairy Products, and Technology of Fats, Biochemistry, and Microbiology at the Kemerovo Institute of Food Science and Technology, Russia.

The study of the specifics of the combined refrigeration method based on nitrogen and air systems of cold treatment was conducted at an experimental bench, the principal diagram of which is given in Fig. 1.

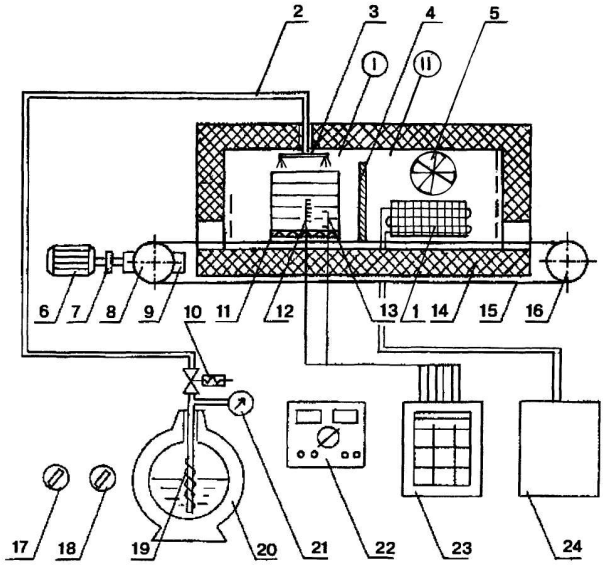

Fig 1. Principal diagram of the experimental bench: (1) Evaporators; (2) a liquid nitrogen pipeline; (3) a liquid nitrogen header with nozzles; (4) a partition; (5) a fan; (6) a direct-current motor; (7) a coupling; (8) a driving drum; (9) a reducer; (10) a solenoid valve; (11) trays; (12) a thermocouple unit; (13) heat meters; (14) a heat-insulated tunnel; (15) a cable; (16) a driven drum; (17) a controller of voltage fed to the heating element; (18) a controller of voltage fed to the fan; (19) a heating element; (20) a Dewar vessel, ADS-15; (21) an electric-contact manometer; (22) a voltage rectifier; (23) an interface unit; and (24) a refrigerating machine. I is the liquid nitrogen spraying (active effect) zone; II is the air cooling zone (temperature equalization by the product volume).

The main component of the bench is a heat-insulated tunnel (14), consisting of two zones.

The first zone (I) is a module of the nitrogen vapor effect (active zone). The second zone (II) is a module of the cold air flow effect. A sprayer (3) is installed in zone I. The nozzles are connected by a pipeline (2) to a system of cryogenic liquid supply, which consists of a Dewar vessel, ADS-15 (20), an electric-contact manometer (21), which is connected in series with a heating element (19) and which ensures an automatic, with the help of a solenoid valve (10), constant pressure within 0.02÷0.1 MPa in the Dewar vessel. The latter two are fed through a control, RKO-250-2A (17).

Nitrogen vapors enter zone II through windows in the upper part of heat-insulated partition 4. Evaporators I and fans 5 are installed in the partition, checkered one opposite another, creating a double-sided symmetrical air circulation. The number of the revolutions of the fans changes by the voltage control (18). Cold is supplied to zone II by refrigerating machine 24, which runs on Freon 404.

A system that transports the product to and through the tunnel consists of a carrier with meshed trays (11), which are connected by a cable (15) through the driving (8) and driven (16) drums, the bolt coupling (7), and the reducer (9) with the DC motor (6), the number of revolutions of which is regulated by the voltage rectifier (22).

The bench is equipped with two heat flow probes 13 and thermocouple unit 12, from which the signal is sent to interface unit 23, consisting of a microcontroller (which controls a measuring complex), an interface for data exchange with a PC, and an analog-to-digital circuit.

Chromel–copel thermocouples with a junction-point diameter of 0.3 mm were used as sensors for measuring the ambient temperature in the modules and in the sample under study during refrigeration. The rates of the nitrogen and air flows in the tunnel were measured by a thermal anemometer T-3 with a scale range of 0.1÷15 m/s.

During the experiments, measurement errors were observed and assessed by relative error values expressed in percent.

The freezer design makes it possible to maintain temperatures down to –120°C in zone I and down to –42°C in zone II and to create an air flow rate up to 8 m/s. During the experiments, the following parameters were measured and controlled:

– nitrogen and air vapor temperatures in product zones,
– the temperature field of the product under study,
– the density of the heat flow from the product to the cooling environment,
– the rate of air flow circulation in zone II, and
– the thickness of the portions of products under study.

The objects of refrigeration were packaged dairy products represented by the following collective groups: Dutch cheese portions and packaged curds of various fat contents. The product thicknesses were 0.025, 0.03, and 0.05 m, weighing from 0.1 to 0.5 kg.

The objects of research were placed on the bench transporter, and refrigeration was conducted from the initial temperature of 15°C to the preset volume-averaged product temperature of –20°C, which was equal to the subsequent storage temperature. The junction point of the thermocouples was introduced into a sample of a given experimental series all through its thickness at equal lengths. The thermocouple readings were recorded by the microcontroller every 4 s.

The refrigeration experiments were conducted under the following conditions: fast refrigeration at the temperature of nitrogen vapors in zone I from –50 to –90°C and in zone II at air temperatures from –20 to –40°C and an air circulation rate of 5 m/s. In zone I of the freezer, nitrogen vapors actively affected the product surface, and in zone II the process heat exchange continued between the product and the air flow but less intensively until the preset final temperature was reached.

The experimental studies of heat exchange processes during refrigeration were conducted using mathematical methods of experiment design. The main experimental material in the heat exchange studies was thermograms of the combined refrigeration process and the averaged-integral values of the heat flow density, which served as the basis for determining the duration and average rate of refrigeration.

The average rate of refrigeration was calculated in line with the recommendations of the International Academy of Refrigeration as a ratio of the distance from the product surface to its thermal center to the time lag between a temperature of 0°C reached on the surface and that in the thermal center 10°C lower than the cryoscopic temperature.

The density of the heat flow was measured with heat meters DPTP. The true value of the heat flow density was derived from an expression:

$$q = A \cdot K \cdot K_t, \text{ W/m}^2, \quad (1)$$

where A is the value of a signal from the heat meter, mV;
K is the operating coefficient of the heat meter, W/(m^2·mV);
K_1 = 165.26 W/(m^2·mV), K_2 = 160.73 W/(m^2·mV);
K_t is a dimensionless temperature correction coefficient, which takes into account the signal error of the heat meter at low temperatures:

$$K_t = 0.000017t^2 + 1.005,$$

where t is the heat meter's temperature at a certain time point,°C.

RESULTS AND DISCUSSION

The situation of heat exchange during fast refrigeration under consideration is represented as a system of modules, each of which can independently provide the necessary heat sink conditions for the process. In addition, the specifics of a multizone combined system of cold supply are taken into account; i.e.:

– in zone (module) I, the nitrogen vapors freeze the product to a volume-averaged temperature, which is equal to the product's cryoscopic temperature;
– in zone II (which may consist of several modules) the product is affected by cold air, cooled by nitrogen vapors escaping from module I (partially), and by the

(main) machine refrigeration system to the preset final temperature of the product.

The model under development was based on the method of Leibenzon's integral relations with account for the following assumptions:

– the product has the shape of an indefinite plate;
– the heat exchange conditions are symmetrical;
– the temperature of the cooling environment is constant within each stage; and
– the thermophysical characteristics of the product change in discrete steps during the water phase transfer, and they are constant within one phase of the water condition.

For the analytical description of the fast refrigeration process, conventional simplifications were used, and the problem was solved by dividing the whole process into three stages, which were considered sequentially:

– the first stage is refrigeration to the cryoscopic temperature on the product surface;
– the second stage is refrigeration to the cryoscopic temperature in the product's thermal center; and
– the third stage is refrigeration of the frozen product to the preset temperature in the thermal center.

First stage. The known solution is based on the hypothesis of the presence of the "temperature front," which proliferates from the surface to the central layers of the object with the final velocity.

The accurate problem statement consists in solving a thermal conductivity equation:

$$\frac{\partial t}{\partial \tau} = a \cdot \frac{\partial^2 t}{\partial x^2}, \qquad (2)$$

that satisfies the initial:

$$t(x,0) = t_i = \text{const}, \qquad (3)$$

and boundary conditions:

$$\left(\frac{\partial t}{\partial x}\right)_{x=r} = 0; \quad \left[\frac{\partial t}{\partial x} - \frac{\alpha}{\lambda}\cdot\left(t - t_{av}\right)\right]_{x=0} = 0, \qquad (4)$$

where $t_{av} < t_{cr} < t_i$, t_{av} is the ambient temperature, °C; t_i is the initial product temperature, °C; t_{cr} is the product cryoscopic temperature, °C;
$\delta = 2r$ is the thickness of the product plate, m;
a is the thermal conductivity coefficient, m^2/s;
and λ is the thermal conductivity coefficient of the product, W/(m·K).

In order to solve the problem under consideration, dimensionless variables were introduced.

$$\theta = \frac{t_i - t}{t_i - t_{av}}; \quad \xi = \frac{x}{r}; \quad Fo = \frac{a\cdot\tau}{r^2}; \quad Bi_2 = \frac{\alpha}{\lambda_2}\cdot r, \qquad (5)$$

and moreover:

$$\theta_{cr} = \frac{t_i - t_{cr}}{t_i - t_{av}}. \qquad (6)$$

The duration of the first phase of the refrigeration stage:

$$Fo_I^a = \frac{1}{12Bi_2}\cdot\left[Bi_2^2 + 4Bi_2 - 8\ln\cdot\left(1 + 0{,}5Bi_2\right)\right]. \qquad (7)$$

The duration of the second phase of the refrigeration stage:

$$Fo_I^b = \frac{Bi_2 + 3}{3Bi_2}\cdot\ln\frac{2}{\left(Bi_2 + 2\right)\cdot\left(1 - \theta_{cr}\right)}. \qquad (8)$$

The full duration of the first stag refrigeration to the cryoscopic temperature on the plate surface, is determined as follows:

$$Fo_I = Fo_I^a + Fo_I^b. \qquad (9)$$

The calculation diagram of the product at the refrigeration stage is given in Fig. 2.

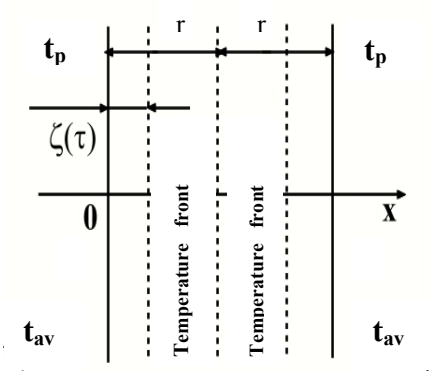

Fig. 2. Formation of the temperature front at the refrigeration stage.

Second stage. The plate is split into two zones: frozen and unfrozen. It is assumed that the frozen zone is $0 \le x \le \tilde{x}(\tau)$, and the unfrozen zone is $\tilde{x}(\tau) \le x \le r$, where $x = \tilde{x}(\tau)$ is the boundary of the crystallization front (Fig. 3).

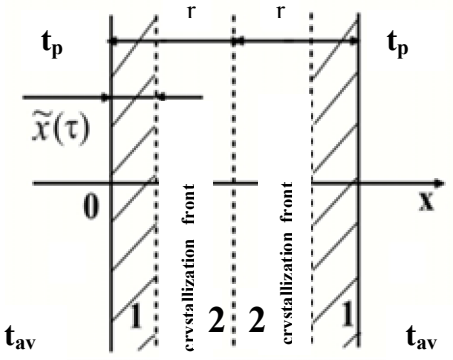

Fig. 3. The calculation diagram of the product at the refrigeration stage.

The solution of the above equations should be subject to the following conditions:

$$t(x,0) = t_{cr} + \frac{\alpha}{2 \cdot r \cdot \lambda_2} \cdot \left(t_{av} - t_{cr}\right) \cdot \left(x^2 - 2 \cdot r \cdot x\right), \quad (10)$$

$$\left[\frac{\partial t_1}{\partial t} - \frac{\alpha}{\lambda} \cdot (t_1 - t_{av})\right]_{x=0}; \left(\frac{\partial t}{\partial x}\right)_{x=r} = 0, \quad (11)$$

$$\left(t_2 - t_1\right)_{X=\tilde{X}} = 0; \quad (t)_{X=\tilde{X}} = t_{cr}. \quad (12)$$

In addition, functions t_1 and t_2 must satisfy the Stefan condition:

$$\left(\lambda_1 \cdot \frac{\partial t_1}{\partial x} - \lambda_2 \cdot \frac{\partial t}{\partial x}\right)_{X=\tilde{X}} = L \cdot W \cdot \omega \cdot \rho \cdot \frac{d\tilde{x}}{d\tau}, \quad (13)$$

where λ_1 is the coefficient of thermal conductivity of the frozen zone,
W/(m·K);
L is the heat of the phase transfer of crystallization, J/kg;
W is the product's relative moisture content, %; and
ω is the share of frozen-out moisture, unit.

Then the problem at hand is reduced to a dimensionless form:

$$\theta_1 = \frac{t_i - t_1}{t_i - t_{av}}; \xi = \frac{x}{r}; \xi_{(\tau)} = \frac{\tilde{x}(\tau)}{r};$$

$$\theta_{cr} = \frac{t_i - t_{cr}}{t_i - t_{av}}; Fo = \frac{a \cdot \tau}{r^2}; Bi_1 = \frac{\alpha}{\lambda_1} \cdot r. \quad (14)$$

The formula for determining the duration of the second stage has the following form:

$$Fo_{II} = \frac{\beta}{2\eta} \cdot \frac{Bi_1 + 2}{Bi_1\left(1 - \theta_{cr}\right)} + \frac{1}{3} \cdot \left[\left(\frac{Bi_1 + 1}{Bi_1}\right)^2 \cdot \ln\left(Bi_1 + 1\right) - \frac{2Bi_1 + 1}{Bi_1}\right], \quad (15)$$

where $\beta = \frac{L \cdot \omega \cdot \rho \cdot a_2 \cdot W}{\lambda_2 \cdot \left(t_i - t_{av}\right)}$; $\eta = \frac{\lambda_1}{\lambda_2}$.

The total duration of the product stay in zone (module) I is calculated by summarizing $Fo_I + Fo_{II}$.

As was noted above, in terms of the production process, by the time the product transfers from zone I to zone II of the multizone combined fast freezer, the volume-averaged temperature of the product must be equal to the cryoscopic temperature. In order to determine the time of transfer, it is necessary to calculate the volume-averaged temperature of the product at this refrigeration stage. To this end, let us use Venger's methodology, according to which the product plate in this situation is split into three zones: frozen zone I with a thickness of ε_1, unfrozen zone II, and zone III, frozen from the other side of the product, with a thickness of ε_2 [7].

Since various thicknesses of the frozen layers are assumed, the intensities of heat exchange also differ. Therefore, an asymmetry coefficient k is introduced.

Then, the formula for determining the volume-averaged dimensionless temperature is as follows:

$$\theta_V = \frac{Bi_2}{4}\left(\frac{\varepsilon_1^2}{Bi_2\varepsilon_1 + 1} + \frac{\varepsilon_2^2}{Bi_2\varepsilon_2 + k}\right) + \frac{\theta}{6}\left(2 - \varepsilon_1 - \varepsilon_2\right)^2. \quad (16)$$

If the symmetrical conditions of heat sink are ensured, then $\varepsilon_1 = \varepsilon_2 = 1$ and k = 1. Then:

$$\theta_V = \frac{Bi_2}{4}\left(\frac{\varepsilon^2}{Bi_2 + 1}\right). \quad (17)$$

After the transfer to zone (module) II of the multizone freezer, the refrigeration stage still continues but under different heat-sink conditions, i.e., under the different values of criterion Bi. Here it is important to note that, while in zone I the product is cooled by nitrogen vapors at temperature t_{av1} (by the data of experiment $t_{av1} = -70$°C), in zone II it is cooled by the air flow at temperature t_{av2} (by the experimental data, $t_{av2} = -30$°C). In addition, the volume-averaged temperature of the product in zone II continues to decrease until the crystallization fronts meet, which will finally mean the completion of the refrigeration stage, after which the further freezing stage begins until the preset temperature is reached: either the volume-averaged temperature or that in the thermal center of the product, depending on the process conditions.

In order to determine the span of refrigeration completion in zone II, we have to determine the temperature in the thermal center of the product, θ_c, at the transition time, as well as the thickness of the frozen layer, ε. Taking into account the symmetry of the problem under consideration, apparently, $\theta_c = \theta/2$. Then the sought time interval is

$$Fo = \frac{\Delta\varepsilon}{\beta\theta_{cr}\theta_c} - \frac{\eta}{2\beta\theta_{cr}\theta_c^2}\ell n\left(\frac{2\theta_c Bi\varepsilon + 2\theta_c - \eta Bi}{2\theta_c Bi\Delta\varepsilon + 2\theta_c - \eta Bi}\right). \quad (18)$$

Third stage. Here the refrigeration of the frozen product to the preset final volume-averaged temperature of the body is considered (Fig. 4).

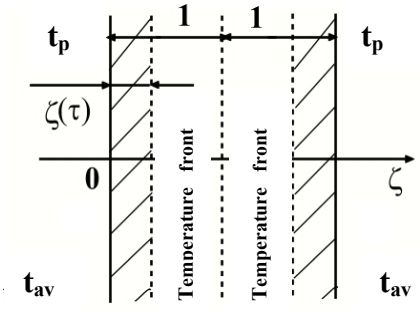

Fig. 4. Calculation diagram of the product at the further freezing stage.

The following equation is solved:

$$\frac{\partial T_1}{\partial Fo} = \frac{\partial^2 T_1}{\partial \xi^2}, \quad (19)$$

at the following boundary conditions:

$$\left(\frac{\partial T_1}{\partial \xi}\right)_{\xi=1} = 0; \quad \left(\frac{\partial T_1}{\partial \xi} - Bi_1 \cdot T_1\right)_{\xi=0} = -Bi_1. \quad (20)$$

The result of the solution is a formula for determining the duration of the plate's further freezing down to volume-averaged temperature t_v:

$$Fo_{III} = \frac{Bi_1 + 3}{3Bi_1} \cdot \ln \frac{2(1-\theta_{kp}) \cdot (Bi_1 + 3)}{3(Bi_1 + 2) \cdot (1-\theta_{1v})}. \quad (21)$$

The total duration of the refrigeration of the product plate is determined as the sum of the durations of individual stages:

$$Fo = Fo_I + Fo_{II} + Fo_{III}. \quad (22)$$

Formula 15 includes the value of a share of frozen-out moisture, which is associated with the volume-averaged temperature of the product plate when the center reaches the cryoscopic temperature. In order to determine the share of frozen-out moisture of real objects of research, Ryutov's formula has been used, which takes into account the relative amount of nonfreezing bound moisture (σ), as a share of the amount of dry substances:

$$\omega = \left(1 - \sigma \frac{1 - \omega_p}{W}\right)\left(1 - \frac{t_{kp}}{t_v}\right), \quad (23)$$

where W is a mass share of moisture, unit share;

ω_p is the mass share of freezing-out moisture, unit share.

Table 1 presents the results of calculating the share of frozen-out moisture in the objects of research in a wide temperature range.

Table 1. Calculation results of the share of frozen-out moisture

Refrigeration temperature, minus °C	Curds of 5% fat content, W = 74.5%	Dutch cheese bar, W = 40.5%
-10	0.699	0.600
-15	0.799	0.730
-20	0.849	0.800
-25	0.879	0.840
-30	0.899	0.870
-35	0.913	0.880
-40	0.924	0.882
-45	0.932	0.888
-50	0.939	0.890
-55	0.945	0.893
-60	0.949	0.900
-70	0.956	0.920

When studying the thermophysical processes of refrigeration technology, the volume-averaged temperature is viewed as a very important value. In nonstationary processes, characteristic of fast freezing, it changes in time because the temperature field of a body changes, as well as its thermophysical characteristics. Therefore, the problem of determining this temperature becomes more complicated.

Simplifying the problem and assuming that the thermophysical characteristics within one phase are constant, we may represent the dimensionless volume-averaged temperature of the product by an integral:

$$\theta_{v1} = \int_0^1 \theta_1(\xi, Fo_{II}) d\xi. \quad (24)$$

From the integral relation we have:

$$\theta_1(\xi, Fo_{II}) = \chi_1(Fo)\xi + \chi_2(Fo), \quad (25)$$

where $\chi_1 = -\frac{Bi_1(1-\theta_k)}{1+Bi_1\xi}$; $\chi_2 = \frac{\theta_1 + Bi_1\xi}{1+Bi_1\xi}$. (26)

At time point Fo_{II}, considered in the problem, $\xi = 1$, then:

$$\chi_1 = -\frac{Bi_1(1-\theta_k)}{1+Bi_1}; \qquad \chi_2 = \frac{\theta_1 + Bi_1}{1+Bi_1}. \quad (27)$$

Inserting expression 27 into 25, we obtain:

$$\theta_1(\xi, Fo_{II}) = -\frac{Bi_1(1-\theta_k)}{1+Bi_1}\xi + \frac{\theta_1 + Bi_1}{1+Bi_1}, \quad (28)$$

We introduce expression 28 into integral 24 and find:

$$\theta_{v1} = \frac{\theta_k(2+Bi_1) + Bi_1}{2(1+Bi_1)}. \quad (29)$$

Taking into account $\theta_k = \frac{t_{cr} - t_k}{t_{cr} - t_{av}}$, we obtain:

$$t_v = t_i - \frac{\theta_k(2+Bi_1) + Bi_1}{2(1+Bi_1)}(t_i - t_{av}). \quad (30)$$

Then the final temperature in the product center that corresponds to the preset volume-averaged temperature will be equal to:

$$t_k = \frac{2 \cdot t_v \cdot (1+Bi_1) - Bi_1 \cdot t_{av}}{2+Bi_1}. \quad (31)$$

Analysis of the findings shows that, at the fast refrigeration of the research objects in nitrogen vapors in zone I of the combined fast freezer, the temperature

of the product surface reaches very quickly the cryoscopic value. As a result, the duration of the first stage of the process tends to zero. In this case, the heat front hypothesis, which underlies the model, is inapplicable because the product surface reaches the cryoscopic temperature much earlier than the temperature front reaches the thermal center. Analytically, this leads to the fact that the second summand Fo_I^6 (15) becomes negative, which contradicts the criterion of formula applicability for calculating Fo. Consequently, if this criterion is inapplicable, the mathematical formula for calculating the dimensionless time of the whole refrigeration process will have the following form:

$$Fo = Fo_{II} + Fo_{III} \ . \quad (32)$$

Tables 2 and 3 show the thermophysical characteristics of dairy products used as the research objects and for calculations.

Table 2. Thermophysical characteristics of the research objects

Product name	Mass share of moisture, %	Density, kg/m^3	Thermal capacity, kJ/(kg·K)	Thermal conductivity, W/(m·K)	Temperature conductivity, 10^7 m/s^2
Dutch cheese, F_{ds}= 45%	40.5 ± 0.2	1070	2.5	0.35	1.31
Curds, 5% fat content	74.5 ± 0.2	962	3.27	0.43	1.37

Table 3. Thermophysical characteristics of the frozen products [20]

Product name	Cryoscopic temperature, °C	Density, kg/m^3	Thermal capacity kJ/(kg·K)	Thermal conductivity, W/(m·K)	Temperature conductivity, 10^7 m/s^2
Dutch cheese, F_{ds} = 45%	Minus 6.2	1025	1.28	1.10	8.38
Curds, 5% fat content	Minus 3.0	960	2.18	1.15	5.49

The predicted results for the duration of refrigeration compared to the experimental data given in Buyanov's works [19] show that the maximum value of the maximum relative error does not exceed 14%, which indicates the adequacy of the newly developed mathematical model to the real refrigeration process. Thus, a mathematical model has been developed for determining the duration of the fast refrigeration of foods in a two-zone modular fast freezer based on a nitrogen + air combined cold supply system with adjustable heat sink.

REFERENCES

1. Ryutov, D.G. and Khristodulo, D.A., *Bystroe zamorazhivanie myasa* (Fast Freezing of Meat) (Pishchepromizdat, Moscow, 1956). 140 p.
2. Alyamovskii, I.G., Geints, R.G., Golovkin, N.A., et al., *Analiticheskoe issledovanie tekhnologicheskikh protsessov obrabotki myasa kholodom* (Analytical Study of Processing Meat with Cold) (TsNIITEImyasomolprom, Moscow, 1970). 183 p.
3. Kutsakova, V.E., Frolov, S.V., Yakovleva, M.I., et al., About freezing time of foods. *Kholodil'naya tekh.* (Refrigeration Equipment), 1997. № 2. P. 16–17.
4. Stefanovskii, V.M., A new method of calculating the duration of meat refrigeration, *Kholodil'naya tekh.* (Refrigeration Equipment), 1989. № 11. P.15–19.
5. Brazhnikov, A.M., *Teoriya termicheskoi obrabotki myasoproduktov* (Theory of Thermal Processing of Meat Products) (Agropromizdat, Moscow, 1987). 270 p.
6. Leibenzon, L.S., On the problem of solidification of the terrestrial globe from its molten state, *Tr. Akad.Nauk SSSR*, 1965. V. 4.
7. Venger, K.P. and Antonov, A.A., *Azotnye sistemy khladosnabzheniya dlya proizvodstva bystrozamorozhennykh pishchevykh productov* (Nitrogen Cold-Supply Systems for the Production of Fast-Frozen Foods). (Uzorech'e, Ryazan', 2002). 207 p.
8. Lobanov, I.E., Babakin, B.S., Aitikeev, R.B., Voronin, M.I., and Babakin, S.B., Mathematical model of the ice buildup process on a spherical surface for cold accumulators, *Vestn. Int. Acad. Refrigeration*, 2013. № 4. p. 12–15.
9. Semenov, E.V., Babakin, Voronin, M.I., B.S., Vygodin, V.A., and Babakin, S.B., Modeling a process of pneumocryoelectroseparation of raw materials of a biological origin, *Vestn. Int. Acad. Refrigeration*, 2013. № 2. P. 62–66.
10. Tvorogova, A.A. and Chizhova, P.B., Objective assessment of the structure of frozen whipped fruit desserts by the condition of ice crystals, *Kholodil'naya tekh.* (Refrigeration Equipment), 2013. № 2. P. 69–72.
11. Buyanov, V.O., Freezing hard cheeses in the conditions of adjustable heat sink, *Syrodelie maslodelie* (Cheesemaking Buttermaking), 2009. № 4. P. 46–48.
12. Craiver, N.G. and Zartzky, N.E., Viscoelastic behavior of refrigerated and frozen low moisture Mozzarella cheese, *J. Food Sci.*, 2004. V. 69. № 3. P. 123–128.

13. Voskoboinikov, V.A., Developing process parameters for freezing preshaped foodstuffs, *Vestn. Int. Acad. Refrigeration*, 2012. № 1. P. 28–30.
14. Buyanov, V.O., Larina, I.O., Buyanova, I.V., and Kriger, O.V., The role of low temperatures in assessing the microbiological condition of frozen cheeses, *Syrodelie maslodelie* (Cheesemaking Buttermaking), 2008. № 3. P. 25–26.
15. Li, Ruixia and Wang, Weicheng, Qinghua daxue xuebao. Ziran kexue ban, *J. Tsinghua Univ. Sci. and Technol.*, 2006. V. 46. № 5.
16. Coulomb, D., World tendencies and priorities in development of low-temperature engineering, *Vestn. Int. Acad. Refrigeration,* 2012. № 4. P. 3–7.
17. Buyanov, O.N. and Buyanova, I.V., Current technologies of freezing and storing dairy products, *Pererabotka moloka* (Milk Processing), 2010. № 4. P.36–38.
18. Antonov, A.A. and Venger, K.P., Technical and economic assessment of the operation of fast freezers, *Myasnaya industriya* (Meat Industry), 2002. № 6. P. 40–42.
19. Buyanov, V.O., Development of a technology of the combined fast freezing of foods. *Extended Abstract of Cand. Sci. (Eng.) Dissertation*. (Kemerovo, 2009). 18 p.
20. Latyshev, V. and Tsiryul'nikova, N., Standardization of the properties of foods, *Kholodil'naya tekh.* (Refrigeration Equipment), 1990. № 2. P. 33–34.

KINETICS OF THE VACUUM DRYING OF CHEESES

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Abstract: Cheeses are analyzed as vacuum drying objects. An experimental vacuum drier and its elements are schematized. The operating principle of the experimental setup is described. Moisture is demonstrated to be among the most important components of cheese. The physicochemical composition of cheeses is considered. The forms and energy of moisture binding in cheese are discussed. The hygroscopic and thermophysical properties of cheeses are reported. The kinetics of the vacuum drying of cheeses has been investigated. The vacuum drying of cheeses includes two stages: the drying rate is constant at the first stage and decreases at the second stage. The temperature curves of cheeses have been plotted in the temperature–moisture weight fraction coordinates. Drying curves in the heat load–time, temperature–time, and moisture weight fraction–time coordinates have been obtained and analyzed for various cheeses. Cheese drying rate curves have been constructed by graphical differentiation. The maximum cheese drying rates have been determined. Equilibrium moisture content values for cheese drying have been found. The cheese shrinkage ratio has been correlated with the thickness of the cheese bed being dried and with the shape and size of cheese pieces. Cheese shrinkage at both stages of vacuum drying proceeds uniformly. Raising the drying temperature above the prescribed temperature reduces the shrinkage ratio.

Keywords: kinetics, vacuum drying, cheeses, temperature, shrinkage, moisture, dryers, heat, drying curves

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INTRODUCTION

When considering cheese as a vacuum drying object, note that the variation of the properties of cheese during drying depends on the physicochemical properties, structure, and binding forms of moisture in the material and on the thermophysical characteristics of the cheese, including specific features of mass and energy transfer. The basic structural elements of cheese are macrograins, interlayers between macrograins, microvoids, and micrograins. The main element of each macrograin is a protein network, whose cells contain numerous included micrograins as fat drops, lipoid drops, and crystals.

The passage of fat from milk into cheese depends on many factors. Other conditions being equal, medium-size fat globules primarily pass into cheese, followed by small and large ones [1, 2]. Milk fat is viewed as the most valuable constituent of milk, even though milk proteins are still more valuable from the standpoint of nutrition physiology. The following four factors contribute to the particular significance of milk fat in milk and dairy products: economic attractiveness, nutritional value, taste, and the physical properties of dairy products that are due to the presence of fat [3].

During cheese ripening, all cheese components undergo profound changes and, as a result, the given cheese brand acquires its characteristic texture and pattern [4].

The moisture content of cheese depends on processing conditions, namely, renneting temperature and time, second-heating temperature, partial salting of cheese curds, water addition in the second heating, and cheese curds processing time. As the curdling temperature and second-heating temperature are decreased, the moisture capacity of the cheese curds and the water content of the finished product increase. As the temperature is raised, the water content of the cheese decreases. Moisture is lost at the salting stage due to osmotic water transfer and at the ripening stage via evaporation. The intensity of the microbiological and biochemical processes occurring in a cheese depends on the initial moisture content of the cheese after pressing [4, 5].

For most hard and semihard cheeses, the weight fraction of fat in dry matter is 45–50% and the weight fraction of moisture is 40–44%.

Fat in cheese is mainly in the form of micrograins 10–15 μm in diameter. There are also larger fat inclusions, which are referred to as fat drops and are uniformly distributed throughout the cheese bulk. The fat drops and lipoid micrograins in cheese are milk fat destabilized during cheese making and ripening. This is true because, above 20°C, cheese fat can melt out of cheese curd, and this is the main obstacle in thermal cheese dehydration.

OBJECTS AND METHODS OF THE STUDY

The objects of our study were the Sovetskii, Shveitsarskii, Altaiskii, Gornyi, Moskovskii, Gollandskii, Kostromskoi, Poshekhonskii, Yaroslavskii, and Ozernyi brands of cheese. The study was carried out using standard, commonly accepted, and modified physicochemical, rheological, microbiological, and biochemical methods.

The drying techniques and drier designs employed in cheese making are very diverse. First of all, any drier design should ensure uniform heating and drying of the product and reliable control of its temperature

and moisture content. The driers should have a sufficiently high output capacity, but at the same time they should be economical in terms of heat and electricity requirements and should be metal-intensive to the least possible extent. Present-day driers should be multipurpose, capable of drying various materials [6, 7].

Only a few types and sizes of driers are manufactured at present. Many of the various driers operated by cheese makers are single-copy models designed by the manufacturer itself or, much more frequently, by a branch research institute or education institution, and many have been purchased from foreign companies.

Drier designers should take into consideration that drying is a unit operation involved in manufacturing, so the design of the apparatus should ensure establishing optimal processing conditions and obtaining a high-quality dry product. Although each product as an object of drying has its specific properties, for organizing full-scale drier production at machine-building plants it is rational to develop versatile apparatuses intended for a group of similar materials and fitted with up-to-date automated monitoring and process control systems [8–10].

When selecting a rational drier design and drying method, one should be guided by the following requirements:

– high quality indexes of the product (reconstitutability, unaffected odor);

– minimum heat, steam, air, and electric power consumption per kilogram of moisture evaporated or per ton of finished product;

– high process intensity minimizing the dimensions of the apparatus (in terms of the amount of water removed from 1 m^3 of the apparatus volume or 1 m^2 of the working area);

– maximum possible automatability and mechanizability of the drying process.

In all cases, it is necessary to thoroughly study the effects of the main parameters of the drying agent and the material to be dried on the dehydration process. In particular, it is necessary to understand whether it is possible to increase the drying temperature, the air flow rate, and the relative humidity of the air and to use oscillating drying modes, combined heat supply and drying methods, and feedstock pretreatment. It is also necessary to take care of the chemical composition of the finished product (retention of vitamins, proteins, and fat) [11–14].

Taking the aforesaid into account, we designed the cheese vacuum drying unit schematized in Fig. 1.

This drying unit is versatile and can be employed in the drying of practically any feedstock of vegetable and animal origins. The drying chamber *2*, shown in Fig. 2, is a horizontal cylinder with a wall thickness of 15 mm, an inner diameter of 290 mm, and a length of 530 mm.

The chamber is heat-insulated from the outside to prevent heat dissipation to the environment. The heat-insulating material is sheet Termafleks, whose heat transfer coefficient is below 0.034 W/(m^2 K) at 25°C (RF State Standard GOST 7076-99). One end of the chamber is convex; it is made from steel, like the cylindrical part of the chamber, and welded to the latter. The second end is removable, made from a 40-mm-thick organic glass sheet.

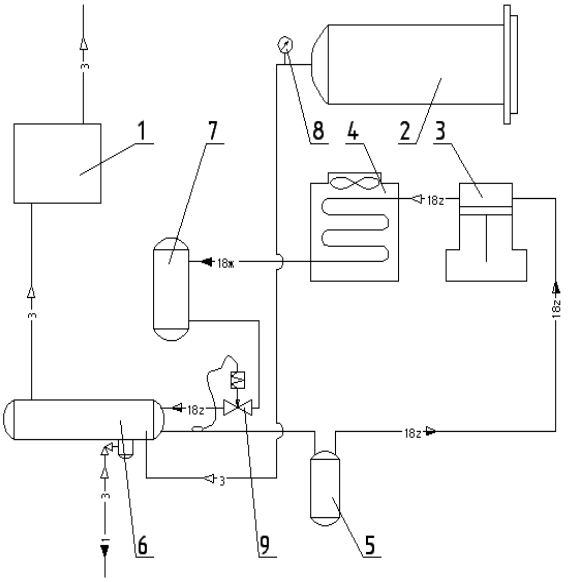


Fig. 1. Schematic of the experimental vacuum drier: (*1*) vacuum pump, (*2*) vacuum drying chamber, (*3*) compressor, (*4*) condenser, (*5*) liquid separator, (*6*) desublimator, (*7*) receiver, (*8*) vacuum gage, and (*9*) thermal expansion valve.

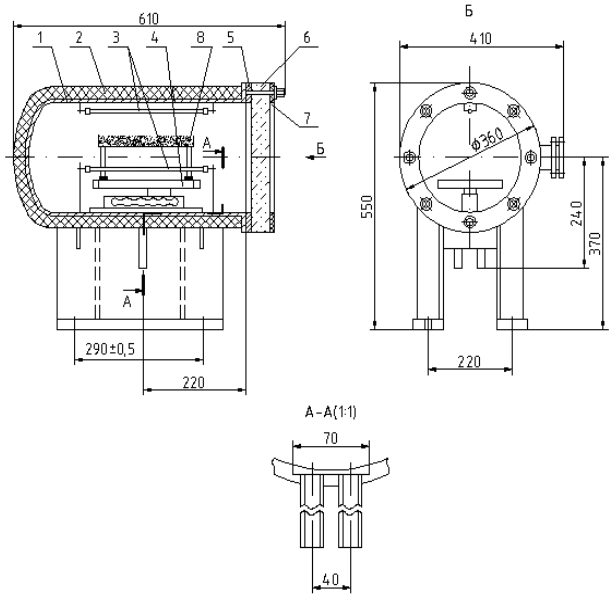


Fig. 2. Drying chamber: (*1*) body, (*2*) heat insulation, (*3*) heaters, (*4*) balance, (*5*) vacuum-tight rubber, (*6*) organic glass, (*7*) sealing ring, and (*8*) product.

The heat supply system should ensure uniform heating of the product being dried. The heaters should allow the applied power to be regulated and should have a low thermal inertia. The sources of heat in the unit are two KGT 220-1000 infrared lamps *3*, each having a power of 1 kW. The IR heaters are in the upper and lower parts of the chamber, 50–70 mm away from the pan on which product *8* is placed.

The design of the vacuum chamber allows the distance between the product pan and the heaters to be varied. The product bed is heated by IR radiation pulses to the preset temperature. A specific feature of the IR lamps is their low thermal inertia. This feature enables one to fairly precisely maintain the necessary temperature during vacuum drying. In the lower part of the drying chamber, there is a pipeline connecting the chamber with a desublimator. The desublimator is a shell-and-coil, in-tube boiling, heat exchanger serving as the evaporator in the refrigeration unit shown in Fig. 3.

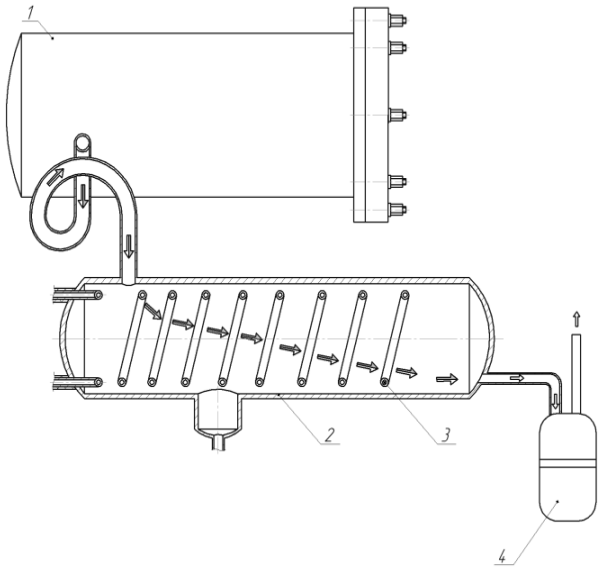


Fig. 3. Schematic of the desublimator: (*1*) vacuum chamber, (*2*) desublimator, (*3*) evaporator coil, and (*4*) vacuum pump.

The desublimator is intended for removing, from the vacuum chamber, the water vapor released by the product being dried. The cold surface of the coil condenses the moisture removed from the product, intensifying the drying process by generating a water vapor partial pressure drop between the drying chamber and the condenser. This difference between the partial pressures makes the vapor move from the product to the evaporator surface. Throughout the drying process, the moisture evaporated from the product freezes progressively on the coil surface. In the lower part of the desublimator, there is a valve for unsealing the system and for removing the ice that will have frozen on the evaporator surface by the completion of the drying process.

The vacuum in the system is maintained with a 2TW-1C two-stage vacuum pump. The evaporated moisture and noncondensable gases are removed in the following way: the moisture evaporated from the product moves through a pipeline to the desublimator, where it passes through the evaporator and freezes on its surface; the unfrozen water vapor and noncondensable gases are pumped out by the vacuum pump and are discharged into the environment.

A block diagram of the control and measurement system of the vacuum drying unit is presented in Fig. 4.

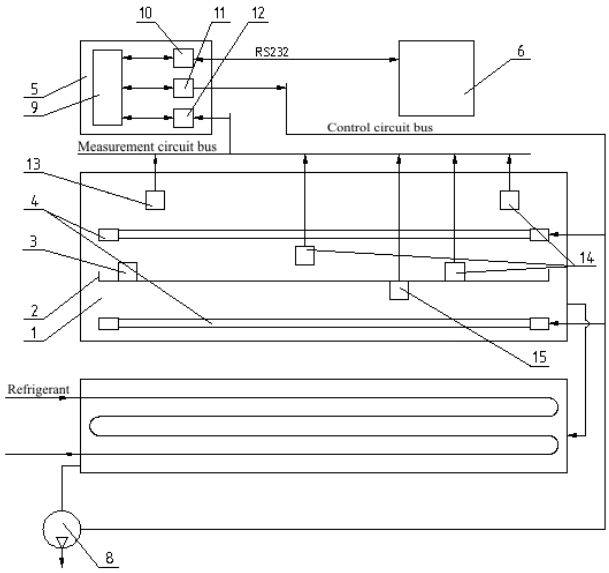


Fig. 4. Block diagram of the experimental bench: (*1*) vacuum chamber, (*2*) pan, (*3*) material being dried, (*4*) IR heaters, (*5*) interface block, (*6*) PC, (*7*) condenser, (*8*) vacuum pump, (*9*) microcontroller, (*10*) PC interface, (*11*) executor control unit, (*12*) analog-to-digital converter, (*13*) pressure sensor, (*14*) temperature sensor, and (*15*) load cell.

The drying parameters and algorithm are set by the researcher from the console of PC 6 and are input in the interface block 5 through an RS 232 serial port. The interface block 5 consists of a microcontroller 9 controlling the operation of the measurement unit, a data exchange interface 10 between the microcontroller and the PC, an executor control circuit 11, and an analog-to-digital converter circuit 12. The signal from the executor control block 11 is directed through a control bus to the heaters 4 and vacuum pump 8 for maintaining the preset temperature, heat load, and residual pressure.

The weight of the material being dried is monitored using an electronic load cell (strain gage) 15 mounted inside the drying chamber, and the residual pressure in the system is measured with a pressure sensor 13. The temperature sensors 14 are used to monitor the temperature inside the chamber, in the cheese bulk, and on the cheese surface. The amount of heat supplied by the IR heaters is also recorded. The temperature and pressure, and weight sensors are connected with the PC through the interface block. The signals received from the temperature and pressure sensors and load cell (strain gage), which are proportional to the change in the corresponding process parameters, are digitized by the analog-to-digital converter 12 and are input into the PC to be stored and processed.

RESULTS AND DISCUSSION

Moisture is among the most important components of cheese. The moisture content of cheese has an effect on the ripening process, cheese structure, and the thermophysical properties of cheese. We quantified different forms of moisture and their bonding energies in some cheeses (Tables 1, 2).

Table 1. Amounts of different forms of moisture in cheeses, %

Cheese brand	Physicochemical binding			Physicomechanical binding
	adsorbed moisture		osmotically bound moisture and microcapillary moisture	wetting moisture and macrocapillary moisture
	monomolecular adsorption	polymolecular adsorption		
Sovetskii	7.0	11.0	12.0	10.0
Gollandskii	5.0	8.0	19.0	12.0
Ozernyi	4.0	6.0	21.0	17.0

Table 2. Moisture binding energies in cheeses 10^{-5}, J/kg

Moisture–material binding form	Cheese brand		
	Sovetskii	Gollandskii	Ozernyi
Physicochemical binding			
Monomolecular adsorption	4.20–2.70	3.90–2.50	3.40–2.50
Polymolecular adsorption	2.20–0.50	2.30–0.70	2.20–0.70
Osmotically bound moisture	0.45–0.12	0.65–0.10	0.60–0.10
Physicomechanical binding			
Microcapillaries	0.45–0.12	0.65–0.10	0.60–0.10
Wetting and macrocapillary moisture	<0.10	<0.10	<0.10

The total weight fraction of moisture is 40% in Sovetskii cheese, 44% in Gollandskii, and 48% in Ozernyi. Sovetskii cheese has the largest proportion of bound moisture, specifically 18.0%; the bound water content of Gollandskii is 13.0%, and that of Ozernyi is 10.0%. Sovetskii cheese, which contains the smallest total amount of moisture among the three cheeses considered, contains the largest proportion of bound moisture. The proportion of high-energy bonds in cheeses depends on the production technology and on the ripening time. P.F. Krasheninin and V.P Tabachnikov established that cheeses gain water-holding capacity as they ripen. That is, in the first approximation, the ripening time can be viewed as a factor in the amount of bound water in cheese: the longer the ripening process, the higher the bound water content of the cheese. This correlation is in full agreement with our data.

Our studies demonstrated that the forms of bound moisture differ in their energetic characteristics and, on passing from free moisture (wetting and macrocapillary moisture) to bound moisture (mono- and polymolecular adsorption), the moisture–dry cheese matter binding energy increases markedly. The binding energy ($\times 10^{-5}$, J/kg) is <0.10 for wetting and macrocapillary moisture, 0.45–0.12 for osmotically bound and microcapillary moisture, 2.30–0.50 for polymolecularly adsorbed moisture, and 4.20–2.50 for monomolecularly adsorbed moisture. Therefore, monomolecularly and polymolecularly adsorbed moisture is most strongly bound owing to its highest binding energy. In view of this, monomolecularly adsorbed moisture is the main index of the hydration of cheese components and is essential for dried foods to be reconstitutable.

Dry foods are known to absorb water from ambient air during storage until they come to the equilibrium state. The equilibrium moisture content of dairy products has been investigated by R.I. Ramanauskas.

We studied the hygroscopic properties of a number of cheeses (Table 3).

Table 3. Hygroscopic properties of cheeses

Relative air humidity, %	10	20	30	40	50	60	70	80	90
Sovetskii cheese									
Equilibrium moisture content, %	7.0	10.0	11.5	13.5	15.5	17.5	19.5	22.0	26.5
Gollandskii cheese									
Equilibrium moisture content, %	5.0	6.5	8.0	9.5	11.0	13.0	17.0	25.0	33.0
Ozernyi cheese									
Equilibrium moisture content, %	4.0	5.0	6.0	7.0	8.0	10.0	15.0	22.0	31.0

The equilibrium moisture content of cheese decreases with a decreasing relative humidity of ambient air. As the equilibrium air humidity decreases, the energy of binding between moisture and the dry matter of the product increases.

Table 4 lists thermophysical characteristics of cheeses. In order to find appropriate drying conditions for any product, including cheese, it is necessary to know not only its physicochemical properties, but also its thermophysical characteristics. Knowledge of thermophysical characteristics is essential for finding both processing conditions and technological parameters. When choosing processing conditions (temperature, heat flux density, and residual pressure), it is necessary to know the heat capacity and thermal diffusivity of the product for gaining insight into the temperature profile across the bed and into the temperature variation rate. In the determination of technological parameters (thickness of the bed to be dried and degree of comminution), it is necessary to know the heat conductivity of the product, because the optimum thickness of the bed being dried depends on this property.

Table 4. Thermophysical properties of cheeses

Cheese	Thermophysical property			
	density, kg/m^3	heat conductivity, W/(m K)	eat capacity, J/(kg K)	thermal diffusivity, 10^{-6} m^2/s
Sovetskii	1070	0.34	2570	0.135
Gollandskii	1060	0.35	2530	0.133
Ozernyi	1040	0.35	2540	0.132

Drying kinetics is commonly understood as the variation of the volume-average moisture content of the material being dried (φ) and its temperature (*t*) with time τ. The drying process is most precisely described by drying curves in the time–moisture content coordinates, by drying rate curves in the moisture content–drying rate coordinates, and by temperature curves in the moisture content–material temperature coordinates. The performances of driers differing in output capacity cannot be compared in terms of material weight variation during drying. This is done by plotting the moisture content of the material versus time (φ versus τ curves).

Data required for constructing these curves are usually obtained in the laboratory by recording the weight and temperature of the material during its drying. Here, drying is commonly carried out using hot air under fixed conditions. For vacuum drying, fixed conditions mean a constant material temperature and residual pressure. Naturally, the laboratory measurements need to be corrected prior to be carried over to the industrial process, in which drying is typically conducted under variable conditions. The time variation of the volume-average moisture content of the material, $\varphi = f(\tau)$, is graphically represented as the so-called drying curve. In the general case, the drying curve consists of several segments corresponding to different drying stages. Fi-gure 5 shows vacuum drying curves (heat load versus time, temperature versus time, and moisture weight fraction versus time) for Shveitsarskii cheese.

For 9–15 min, until the preset residual pressure (2–3 kPa) is reached, no heat is supplied from the heaters (Fig. 5a) and the cheese temperature decreases from 17–15 to 12–10°C.

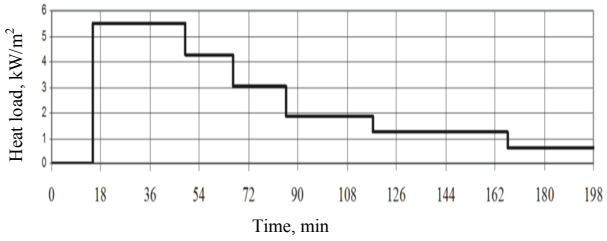
This decrease in temperature is due to the intensive evaporation of moisture from the cheese surface. The decrease in the moisture weight fraction during the period of time required to bring the drier to the preset operating conditions is 2–3%. The segment A–B indicates the time required to reach the preset residual pressure (2–3 kPa). This period is followed by the first drying period, specifically, the constant-rate drying period, represented by the segment B–K_1 of the moisture weight fraction curve. In the first period, the moisture weight fraction decreases at a practically constant rate; that is, equal amounts of moisture are removed in equal periods of time.

The cheese temperature increases owing to the heat supplied from the heaters. The cheese temperature in the first period reaches the preset value and is maintained at this level (Fig. 5b). By the end of the first period, the temperature distribution in the bulk of the cheese being dried becomes uniform. The heat load at the beginning of the first period has the maximum allowable value. Once the cheese has reached the preset temperature, the heat load is reduced. This decrease in heat load is necessary to prevent the cheese drying temperature from exceeding the preset value.

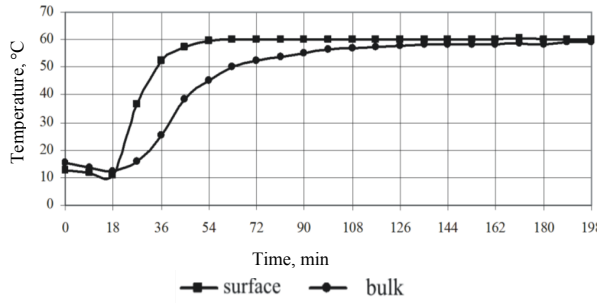
In the first drying period, the amount of moisture removed from the cheese is the largest. In this period, the moisture weight fraction decreases by 24% in Shveitsarskii cheese, by 23% in Gollandskii cheese, and by 34% in Poshekhonskii cheese. The duration of the first drying period is 74 min for Shveitsarskii cheese, 83 min for Gollanskii cheese, 92 min for Kostromskoi cheese, and 80 min for Poshekhonskii cheese. The constant drying rate period lasts until the first critical moisture content point. The point K_1 in the moisture weight fraction versus time curve indicates the instant the straight line BK_1 turns into the K_1C curve. The critical moisture content point is the boundary between the constant drying (first) period and the decreasing drying rate (second) period.

In the constant drying rate period, the intensity of the process is determined only by the parameters of the drying agent and is independent of the moisture content (moisture weight fraction) and physicochemical properties of the material. At a certain value of the moisture weight fraction, the moisture removal rate starts decreasing and the second, decreasing drying rate period begins. The onset of the second period corres-

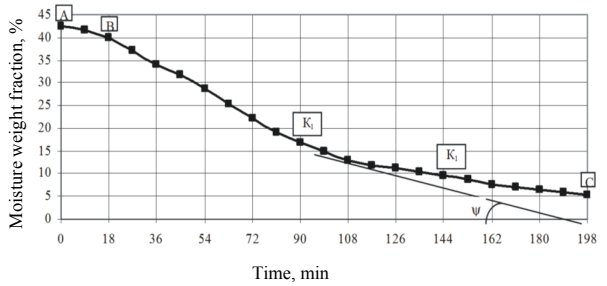
ponds to the critical moisture content of the material. In the second period, the moisture that is most strongly bound to the product is removed. Here, the evaporation rate decreases, the drying process slows down, and the temperature equalizes throughout the cheese bulk.



(a)



(b)



(c)

Fig. 5. Drying curves for Shveitsarskii cheese: (a) heat load, (b) surface and bulk temperatures, and (c) moisture weight fraction. Drying conditions: $t = 60°C$, $q = 5.52$ kW/m^2, $P = 2–3$ kPa, and $h = 10$ mm.

In the decreasing drying rate period, the drying rate falls as the moisture content of the material decreases. In this period, bound moisture is removed and the gradual decrease in the drying rate is due to the increasing moisture–material binding energy.

In the decreasing drying rate period (segment K_1C), the moisture weight fraction of Shveitsarskii cheese decreases by 12%; that of Gollandskii cheese, by 15%; that of Kostromskoi cheese, by 24%; that of Poshekhonskii cheese, by 12%. The duration of this period is 108 min for Shveitsarskii cheese, 100 min for Gollandskii cheese, 17 min for Kostromskoi cheese, and 100 min for Poshekhonskii cheese.

The curve segment representing the decreasing drying rate period (K_1C) can be divided into segments corresponding to the first and second phases of this period (K_1K_2 and K_2C). The junction point K_2 for the two phases of the decreasing drying rate period indicates the second critical moisture content. By the second critical point, the evaporation zone reaches deep layers of the product. At this point, moisture is transferred only as vapor and adsorbed moisture is mainly evaporated.

At the end of drying, the moisture weight fraction versus time curve asymptotically approaches the equilibrium moisture content for the given drying conditions. Once the equilibrium moisture content is attained, drying stops; that is, the drying rate becomes zero.

By taking the first derivative of the $\varphi = f(\tau)$ function, we obtain the drying rate understood as a change in the moisture content of the material per unit time ($d\varphi/d\tau$, %/min). The drying rate curves were constructed by graphical differentiation of drying (moisture weight fraction) curves: the drying rate at a given point in time is defined as the slope of the tangent to the drying curve at the corresponding moisture content point (Fig. 5c):

$$\tan\psi = \frac{\mathrm{d}\varphi}{\mathrm{d}\tau}. \quad (1)$$

The maximum drying rate N in the constant drying rate period is

$$\tan\psi = \left(\frac{d\varphi}{d\tau}\right)_{\max} = N,\ \%/\mathrm{h}\ \text{or}\ \%/\mathrm{min}. \quad (2)$$

At the end of the process, when the equilibrium moisture content is reached, the drying rate is

$$\frac{d\varphi}{d\tau} = 0.$$

Figure 6 plots the drying rate curves for the cheeses examined. At the beginning of the drying process, as the residual pressure decreases to the preset value, the drying rate increases from zero to its maximum value. The maximum drying rate is 0.62 %/min for Shveitsarskii cheese, 0.71 %/min for Gollandskii cheese, 0.88 %/min for Kostromskoi cheese, 0.78 %/min for Poshekhonskii cheese, 0,92 %/min for Rizhskii cheese, and 0.75 %/min for Rossiiskii cheese.

In the constant drying rate period, the drying rate takes its maximum value. In this period, the moisture content of Shveitsarskii cheese decreases by 18%; that of « Gollandskii cheese, by 17%; that of Kostromskoi cheese, by 22%; that of Poshekhonskii cheese, by 28%; that of Rizhskii cheese, by 48%; that of Rossiiskii cheese, by 32%.

The drying rate begins to decrease at the first critical point. The run of the curves in the decreasing drying rate period is typical of colloidal, capillary porous gels.

The second critical point K_2 indicates the second critical value of moisture content. It corresponds to the moisture content limit at which the mechanism of moisture transfer in the material changes. This point indicates the onset of the removal of polymolecularly adsorbed moisture.

The second critical point occurs at the following moisture weight fractions in the cheeses: Shveitsarskii, 10%; Gollandskii, 10%; Kostromskoi, 13%; Poshekhonskii, 8%; Rizhskii, 10%; Rossiiskii, 9%. The moisture weight fraction in the dry cheeses is 4–5%. The difference between the moisture weight fraction at the second critical point and the moisture weight fraction in the dry cheeses is the amount of polymolecularly adsorbed moisture; therefore, the weight fraction of polymolecularly adsorbed moisture in the cheeses is 4–9 %.

The temperature curves, $t = f(\varphi)$, are very informative. These curves, introduced by A.V. Lykov, are of high significance for drying analysis. Figure 7 presents the temperature curves for the vacuum drying of the cheeses.

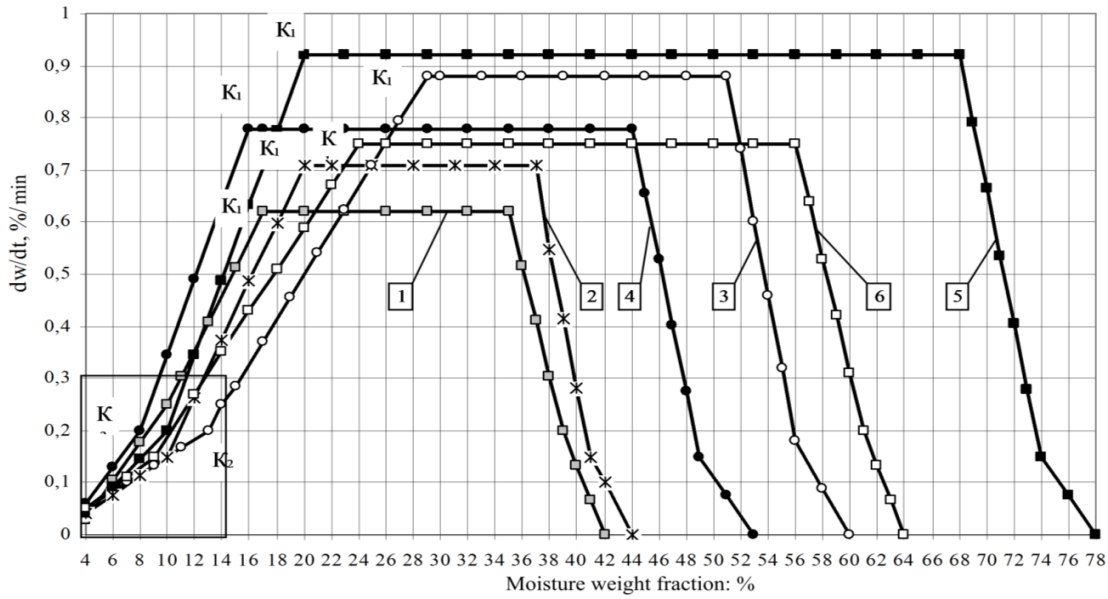


Fig. 6. Cheese drying rate curves: (*1*) Shveitsarskii, (*2*) Gollandskii, (*3*) Kostromskoi, (*4*) Poshekhonskii, (*5*) Rizhskii, and (*6*) Rossiiskii.

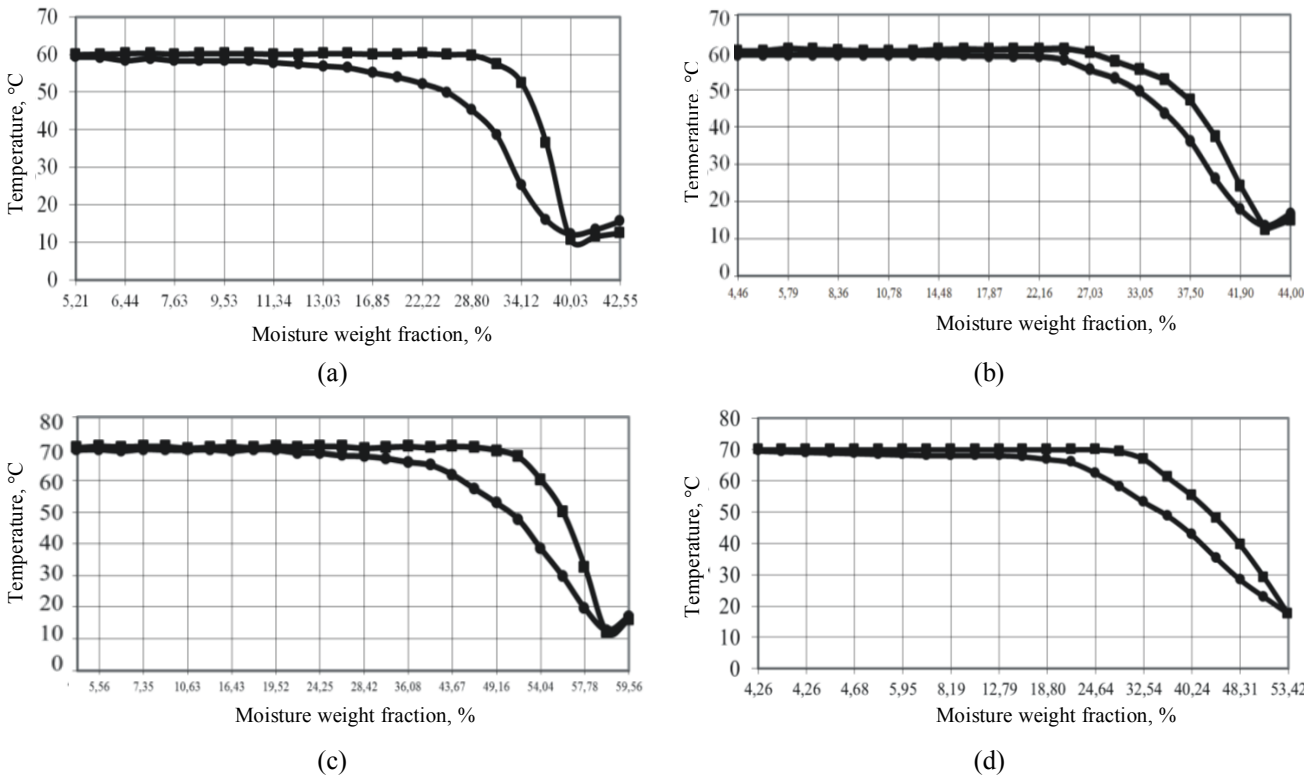


Fig. 7. Temperature curves for the vacuum drying of cheeses: (a) Shveitsarskii, (b) Gollandskii, (c) Kostromskoi, and (d) Poshekhonskii.

At the early stages of drying, the cheese temperature decreases, because no heat is supplied from the heaters. At the beginning of the first period of drying, once the heaters are switched on, the surface tempera-

ture of the material begins to rise to reach the wet bulb temperature. In this period, heat removal is most intensive and almost all of the heat supplied to the material is spent on moisture evaporation. By the end of this period, the temperature equalizes throughout the bulk of the cheese bed.

Starting at the first critical point, the moisture removal rate decreases. Once the moisture content of the cheese has reached its equilibrium value, the drying process is complete. The equilibrium moisture content of Shveitsarskii cheese is 5.21%; that of Gollandskii cheese if 4.46%; that of Kostromskoi cheese is 5.46%; and that of Poshekhonskii cheese is 4.26%.

Most materials (peat, grain, leather, dough, bread, etc.) shrink throughout the drying process. However, some materials (clay, ceramic masses, and some others) shrink during the constant drying rate period and their shrinkage ceases near the critical moisture content if the moisture content gradient in the material bulk is not large. Other materials (wood, coal) shrink only in the decreasing drying rate period, starting at approximately critical moisture content.

The lowest shrinkage is observed for cheese processed at a residual pressure of 2–3 kPa. It was established that the shrinkage ratio of Gollandskii, Kostromskoi, and Poshekhonskii cheeses increases with an increasing size of cheese pieces and with an increasing thickness of the cheese bed being dried. As the bed thickness is increased from 10 to 30 mm, the shrinkage ratio changes from 3 to 14%. At a bed thickness of 40 mm, the shrinkage ratio is 15–24%. Cheese drying at appropriate regime and technological parameters minimizes moisture weight fraction drops and the shrinkage ratio and leaves the shape of cheese pieces unchanged.

Figure 8 plots the shrinkage ratio of cheese as a function of the initial moisture weight fraction.

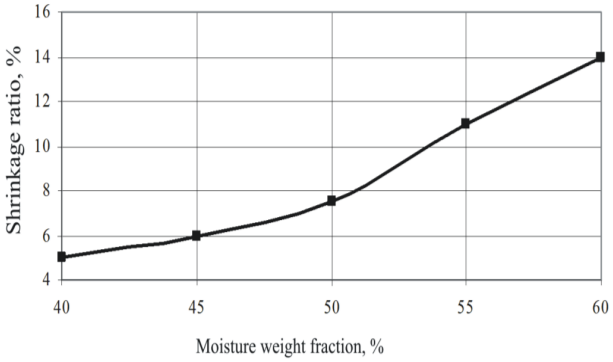


Fig. 8. Shrinkage ratio of cheeses as a function of moisture weight fraction.

The shrinkage ratio increases with an increasing moisture weight fraction in cheese. The largest increase in the shrinkage ratio is observed at moisture weight fractions over 50%. As the moisture weight fraction in cheese changes from 40 to 50%, the shrinkage ratio increases by 2.5%; and, as the moisture weight fraction increases from 50 to 60%, the shrinkage ratio increases by 6.5%.

The surface layers, which have an effect on the particle size of the material being dried, tend to shrink in proportion to the moisture content of the surface rather than the average moisture content. This is the reason why almost no shrinkage is observed starting at some moisture content (moisture weight fraction), as is shown in Fig. 9.

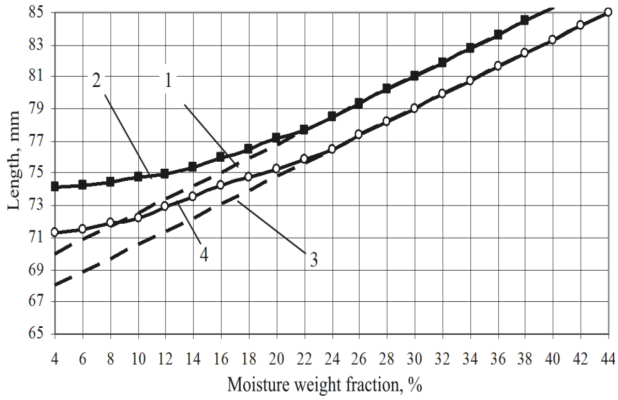


Fig. 9. Shrinkage curves for (*1*, *2*) Sovetskii and (*3*, *4*) Gollandskii chesees:
(*1*) t = 60°C, q = 5.52 kW/m^2, P = 2–3 kPa;
(*2*) t = 80°C, q = 5.52 kW/m^2, P = 2–3 kPa;
(*3*) t = 60°C, q = 7.36 kW/m^2, P = 2–3 kPa;
(*4*) t = 80°C, q = 7.36 kW/m^2, P = 2–3 kPa.

The shrinkage curves for Sovetskii cheese (curve *1*) and Gollandskii cheese (curve *3*) were recorded at the prescribed drying temperature, 60°C. Shrinkage curves *2* and *4* were obtained at 80°C, above the prescribed temperature. At this elevated temperature, surface layers dry up rapidly. Inner layers have a rather large moisture weight fraction. The shrinkage ratio at the increased temperature is smaller, but the dry cheese has a large moisture weight fraction.

As the drying temperature is raised, the shrinkage ratio decreases because of the increasing moisture weight fraction gradient inside the material. When there is a moisture weight fraction gradient, the surface layers tend to shrink to a larger extent than the inner layers. However, the shrinkage of the surface layers is impeded by the inner layers, which are richer in moisture than the former. As a consequence, the actual shrinkage of the surface layers is smaller than the shrinkage that is equivalent to the amount of moisture removed from them. Therefore, an increase in the moisture weight fraction drop between the inner and surface layers leads to an increase in the difference between the actual shrinkage and the theoretical shrinkage corresponding to the amount of liquid removed.

Thus, the shrinkage ratios of cheeses were correlated with the thickness of the bed being dried and with the shape and size of cheese pieces. At a bed thickness of 10 to 30 mm, the shrinkage ratio of the cheeses is 3 to 14%, depending on the shape and size of cheese pieces. The shrinkage ratio increases with an increasing moisture weight fraction in cheese. Shrinkage was observed to proceed uniformly in both periods of vacuum drying. As the drying temperature is raised over the prescribed value, the shrinkage ratio decreases because of the increasing moisture weight

fraction gradient in the bulk of the material.

The shrinkage of a wet material at a uniform moisture content and temperature distribution is a physical property of the material, its response to loss of liquid, and does not induce any detrimental stresses. However, the shrinkage of a material at a nonuniform moisture content distribution does bring the material into a stressed state that can lead to cracking of the body and to breaking of its structure. Therefore, the main obstacle to the rapid drying of many materials is their cracking. This cracking (local disruption) and complete breakup (structure disintegration) are caused by the development of bulk stresses in the material being dried that exceed the stress limit set by the strength of the material.

This stressed state of the material results from a nonuniform moisture content and temperature distribution in the material bulk [15–18].

The existing method of investigating shrinkage stresses does not exclude use of a phenomenological approach in the study of the shrinkage of moist bodies. Note that the capillary and disjoining pressures of a liquid phase in a solid are functions of moisture content. Accordingly, the field of capillary contractions under isothermal conditions will be similar to the moisture content field. Therefore, a nonuniform moisture content distribution (nonuniform moisture content field) is the most significant characteristic of the bulk-stressed state of a moist body being dried.

A similar situation is observed for thermal stresses. In the phenomenological approach, the bulk-stressed state of a body being heated is considered to be unambiguously determined by the nonuniform temperature distribution, or by the temperature field. The main cause of drying-induced cracking is the existence of high-gradient moisture content and temperature fields.

Figure 10 shows moisture content profiles along the thickness of cracky and noncracky cheese beds.

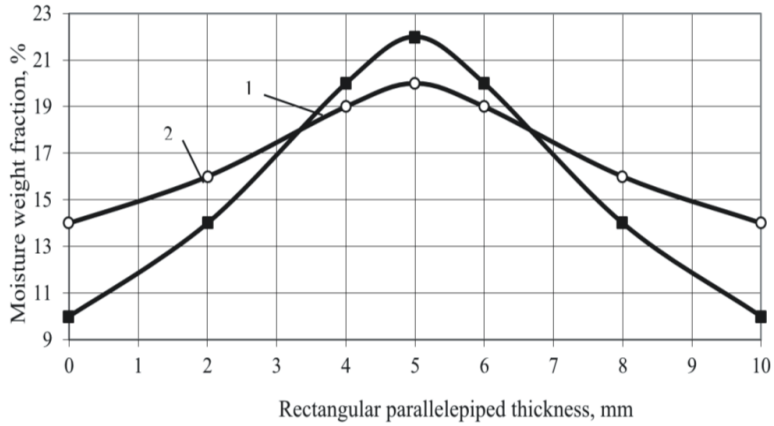


Fig. 10. Moisture content profiles along the thickness of (1) cracky and (2) noncracky cheese beds.

The drying of cheese as 10-mm-thick rectangular parallelepipeds at a moisture weight fraction drop of 12% causes cracking, but there are no cracks at a moisture weight fraction drop of 6%.

CONCLUSIONS

Cheese was investigated as an object of drying. The physicochemical composition of a number of cheeses was studied, binding forms and binding energies of moisture in the cheeses were considered, the hygroscopic properties of the cheeses were investigated, and the thermophysical characteristics of the cheese were analyzed.

The kinetics of the vacuum drying of the cheese was investigated. It was found that the vacuum drying of the cheeses includes two periods, namely, constant and decreasing drying rate periods. Drying curves (heat load versus time, temperature versus time, moisture weight fraction versus time) for various cheeses were recorded and examined. Drying rate curves for the cheeses were constructed by graphical differentiation of drying curves. The maximum drying rates were determined for some cheeses: Shveitsarskii, 0.62 %/min; Gollandskii, 0.71 %/min; Kostromskoi, 0.88 %/min; Poshekhonskii, 0.78 %/min. Using the same method, the amount of polymolecularly adsorbed moisture in the cheeses was determined to be 4–9%. Cheese temperature curves (temperature versus moisture weight fraction) were investigated. Equilibrium values of moisture content were determined for vacuum-dried cheeses.

The shrinkage ratios of cheeses were correlated with the thickness of the bed being dried and with the shape and size of cheese pieces. At a bed thickness of 10 to 30 mm, the cheese shrinkage ratio is 3 to 14%, depending on the shape and size of cheese pieces. The shrinkage ratio increases with an increasing moisture weight fraction in the cheese. Cheese shrinkage in both vacuum drying periods takes place uniformly. Raising the temperature over the prescribed value diminishes the shrinkage ratio owing to the increase in the moisture weight fraction gradient in the bulk of the material.

REFERENCES

1. Ermolaev, V.A. and Prosekov, A.Yu., *Vakuumnye tekhnologii molochno-belkovykh kontsentratov* (Vacuum Technologies for the Production of Milk–Protein Concentrates), Kemerovo: Kuzbassvuzizdat, 2010.
2. Ermolaev, V.A. and Kushevskii, I.V., *Tekhnika i tekhnologiya pishchevykh proizvodstv (Engineering and technologies in food production),* 2010, no. 3, pp. 67–71.
3. Ermolaev, V.A., Sadovaya, T.N., and Kushevskii, I.V., *Khranenie i pererabotka sel'khozsyr'ya (Storage and processing of agricultural raw materials),* 2010, no. 10, pp. 28–30.
4. Kushevskii, I.V., *Cand. Sci. (Eng.) Dissertation,* Kemerovo: Kemerovo Institute of Food Science and Technology, 2011.
5. Popov, A.M., Gurin, V.V., Rasshchepkin, A.N., and Rasshchepkina, E.A., *Khranenie i pererabotka sel'khozsyr'ya (Storage and processing of agricultural raw materials),* 2007, no. 9, pp. 30–32.
6. Semenov, G.V. and Brazhnikov, S.M., *Vestnik MAKh (Bulleting of the International Academy of Refrigeration),* 2002, no. 3, pp. 43–46.
7. Semenov, G.V. and Kas'yanov, G.I., *Sushka syr'ya: myaso, ryba, ovoshchi, frukty, moloko* (Drying of Raw Materials: Meat, Fish, Vegetables, Fruit, and Milk), Rostov-on-Don: MarT, 2002.
8. Kharaev, G.I., Kotova, T.I., Komissarov, Yu.I., and Khanturgaeva, G.I., *Khranenie i pererabotka sel'khozsyr'ya (Storage and processing of agricultural raw materials),* 2007, no. 9, pp. 28–29.
9. Ermolaev, V.A., *Tekhnika i tekhnologiya pishchevykh proizvodstv (Engineering and technologies in food production),* 2011, no. 2, pp. 28–32.
10. Ermolaev, V.A., *Syrodelie i maslodelie (Cheesemaking and buttermaking),* 2009, no. 6, pp. 36–37.
11. Ermolaev, V.A., *Vestnik KrasGAU (Bulletin of Krasnoyarsk State Agrarian University),* 2010, no. 1, pp. 148–151.
12. Devahastin, S. and Suvarnakuta P., Superheated steam drying of food products, in *Dehydration of Products of Biological Origin,* Mujumdar, A.S., Ed., Enfield, N.H.: Science Publishers, 2004, pp. 493–512.
13. Devahastin, S., Suvarnakuta, P., Soponronnarit, S., and Mujumdar, A.S., *Drying Technology,* 2004, vol. 22, pp. 1845–1867.
14. *Mathematical Modeling and Numerical Techniques in Drying Technology,* Turner, I. and Mujumdar, A.S., Eds., New York: Marcel Dekker, 1997.
15. Mujumdar, A.S., *Handbook of Industrial Drying,* Boca Raton, Fla.: CRC, Taylor and Francis Group, 2005.
16. Pan, Z., Shih, C., McHugh, T.H., and Hirschberg, E., *Food Science and Technology,* 2008, vol. 41, pp. 1944–1951.
17. Prakotmak, P., Soponronnarit, S., and Prachayawarakorn, S., *Journal of Food Engineering,* 2010, vol. 96, pp. 119–126.
18. Serrano, J., Velazquez, G., Lopetcharat, K., Ramirez, J.A., and Torres, J.A., *Journal of Dairy Science,* 2004, vol. 87, no. 2, pp. 3172–3182.

STUDYING THE FOAMING OF PROTEIN SOLUTIONS BY STOCHASTIC METHODS

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Abstract: A stochastic model studying the formation and destruction of a dispersed protein gas–liquid system (foam) is proposed. The regularities governing the formation of dispersed systems strongly depend on the conditions of a chemical engineering or engineering process, and both the formation of a foam and the destruction of the obtained foam layer occur simultaneously in the process of foam generation. Since a necessary condition for the construction of a stochastic model is the availability of statistical data, which provide the estimation of the number of both forming and bursting bubbles, the method of such a calculation is of topical interest. The model enables the description of the process state at every time moment of the first cycle. One of the characteristics of a foam is its dispersion, so the random variable characterizing the number of bubble per unit volume is introduced to study the processes of foam formation. The mathematical expectation, dispersion, and also the foam destruction rate function are proposed as a basis for the calculation of foaming efficiency characteristics. Since the model is formalized by a set of differential equations, it can also be used in the simulation modeling of the foaming process. The first cycle of the formation and destruction of a protein foam has systematically been studied. The constructed stochastic model has allowed the mathematical expectation and dispersion of the number of protein foam bubbles per unit volume to be calculated at any time moment of gas saturation within the first cycle. It has been shown that the applied numerical solutions of the differential equations are in good agreement with the analytical solutions given by simple formulas convenient for engineering calculations. A method of estimating the model parameters has been developed. The proposed model has allowed the quantitative description of the foaming process both on average and by states. It has been established that the time of the formation of a protein foam in a rotor-stator device at specified process parameters is advisable to be limited by the moment, at which the highest foam destruction rate is attained.

Key words: dispersed protein based gas–liquid systems, stability, stochastic model, probability, random variable moments, differential equations, numerical and analytical solution

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INTRODUCTION

Dispersed gas–liquid systems (foams) in both the liquid and solid form find wide application in different industries (oil-and-gas, food, and metallurgical industries, firefighting, etc.). The mechanism of the foam formation process is complicated due to the combined effect of numerous physicochemical, physicotechnical, and other factors. The regularities governing the formation of dispersed systems strongly depend on the conditions of a chemical engineering or engineering process, and both the formation and destruction of an obtained gas–liquid layer occur simultaneously in the process of foam generation [1–14]. As a consequence, these features complicate to a great extent the mathematical description of the foaming process [3–5, 15–18].

Among the principal characteristics of a foam are the expansion factor (foam-to-solution volumetric ratio), the dispersion (air bubble size), and the stability (time period from the formation of a foam to its partial or complete destruction) [3–8]. The foam stability applicable to any foam independently of its purpose may be considered as a basic characteristic.

It is known that foams based on protein solutions, an increase in the concentration of which improves the foaming properties of a system as a whole, are highly stable [9, 10, 19–24]. The formation of bubbles generally depends on the composition of a foamed solution (foaming agent) and the intensity of a mechanical action, whereas their destruction proceeds under the action of both internal and external forces. For this reason, the entire foam generation process representing a process flow may be considered as a dynamic system of flows or a queueing system. This queueing system will be studied by the methods of stochastic processes and queueing theory [25–27].

The objective of this study is to create a stochastic model, which would systematically describe the processes of foaming in protein solutions and determine the time of the formation of a foam of specified quality.

OBJECTS AND METHODS OF STUDY

Foam formation regularities were studied via the gas saturation of a protein solution (skim milk protein concentrate; protein mass fraction, 4.4%) in a rotor-stator device (GID-100/1 hydrodynamic disperser, which was developed, manufactured, and mounted in

the All-Russian Research Institute of Dairy Industry) at a rotor revolution speed from 1750 to 3000 rpm, a working chamber filling coefficient of 0.3, a rotor-stator gap of 0.1 mm, and a processed solution temperature of 13 ± 2°C. Control measurements were performed each three minutes after the freezing of samples in a nitrogen atmosphere and their transmitted-light microscopy on an AxioVert.A1 microscope with an AxioCamERc5 camera and a photo recording block. The number of bubbles was calculated from digital images with the use of corresponding software (the comparison of automatic and manual calculation results for the number of bubbles in the frozen samples shows that the former were underestimated by 18% on average).

Mathematical models were constructed using the tools of probability and stochastic process theory and queueing theory (in combination with the methods of mathematical statistics, mathematical analysis, and differential equations) [25–28].

The effect of the time of the gas saturation of a protein solution was studied using the stochastic model describing the efficiency of the process on average [29–34]. The mathematical expectation (average value) $M_i(t)$ of the random value characterizing the number of particles (bubbles) in the dispersed phase of a foam were considered at a time moment t on condition that their number at the initial time moment was $M_i(0) = i$, and the dispersion of this number was $D_i(t)$, $D_i(0) = 0$, $t \in [0,\infty)$, $i = 0, 1, 2, \ldots$.

A foam generator was considered as an inexhaustible bubble (hereinafter, arrival) generation source characterized by the parameter α, and the bubble destruction (hereinafter, arrival service) was characterized by the parameter β.

Model. A queueing system, to which arrivals entered, was considered. The number of arrivals, which has entered the system for the time t, is a random value ξ satisfying the Poisson process

$$P(\xi = k) = \frac{(\alpha t)^k}{k!} e^{-\alpha t},$$

where α is the intensity determined as the average number of generated bubbles per unit time, $t \in [0,\infty)$, $k = 0, 1, 2, \ldots$.

An arrival that has just entered the system is immediately served. The service time is a random value η distributed in compliance with the exponential law

$$P(\eta < t) = 1 - e^{-\beta t},$$

where $\beta = 1/t_{av}$ is the intensity of service, and t_{av} is the average arrival service time determined as an average bubble life time.

The served arrival leaves the system. It is required to calculate the mathematical expectation $M_i(t)$ and the dispersion $D_i(t)$.

The model was formalized by a "birth-and-death" linear differential equation set, from which a differential equation set for the direct calculation of $M_i(t)$ and $D_i(t)$ [29–34] can be deducted in the form

$$\begin{cases} \frac{d}{dt} M_i(t) + \beta M_i(t) = \alpha \\ \frac{d}{dt} Q_i(t) + 2\beta Q_i(t) = 2\alpha M_i(t) \\ Q_i(t) = D_i(t) + M_i^2(t) - M_i(t), \end{cases} \quad (1)$$

with the initial conditions

$$M_i(0) = i,\ D_i(0) = 0. \quad (2)$$

The solution of set (1) with consideration for Eq. (2) has the form [34]

$$\begin{cases} M_i(t) = \frac{\alpha}{\beta}(1 - e^{-\beta t}) + ie^{-\beta t} \\ D_i(t) = \frac{\alpha}{\beta}(1 - e^{-\beta t}) + ie^{-\beta t}(1 - e^{-\beta t}). \end{cases} \quad (3)$$

If a steady-state regime is attained rather quickly, it is convenient to perform express analysis with the formulas

$$M = \lim_{t\to\infty} M_i(t) = \alpha / \beta,$$

$$D = \lim_{t\to\infty} D_i(t) = \alpha / \beta. \quad (4)$$

Further studies have shown that the formation of bubbles may be considered as a Poisson process [34], and their destruction is a Poisson process only in the first approximation.

Really, it is known that the foam destruction dynamics can conditionally be divided into the three stages: the initial stage is slight destruction, when destruction factors have a minimal effect on a foam, and the destruction rate gradually increases, the active stage is characterized by a considerable increase in the destruction rate up to its maximum value and the greatest effect of each destruction factors, including the effect of their combination, and the attenuation stage is characterized by a decrease in the destruction rate of the stage [3–5].

The stability of a foam depends on the destruction rate, so the parameter $\beta = y(t)$, which has a rate dimension and depends on the foaming time moment, may be considered as its characteristic in our model. As is has been turned out, the destruction of a protein foam is most efficiently described by the function [34]

$$y(t) = B + \frac{A_1}{b_1} \exp(-(t-a)^2 / b_1^2), \quad (5)$$

where A_1, B, a, and b_1 are non-negative numerical parameters found from statistical data, $B > 0$, $y(t) > 0$, $\forall t \in [0,\infty)$.

Moreover, it has been established that the boundaries between the foam destruction stages can be determined by the critical points found for the function $y(t)$ using the standard differential calculation methods. For the convenient application of the function $y(t)$ in

engineering calculations, it has been noted that it is similar to the normal distribution density tabulated in the normalized form, so it is written below for convenience of calculations (instead of $y(t)$ at $A_1 = A/\sqrt{\pi}$, $b_1 = \sqrt{2}b$) that

$$\beta(t) = B + A\left(\frac{1}{\sqrt{2\pi}b}\exp(-(t-a)^2/(2b^2))\right).$$

RESULTS AND DISCUSSION

The principal technical parameters of most foaming devices are the working body revolution speed, the working chamber filling coefficient, and the processing time and temperature. It is known that an increase in the revolution speed ν of the working body of a foam generator can intensify the foaming process (decrease the time of the action on a solution) [4, 5, 9, 10, 12–14, 16, 21], although an intense mechanical action on the formed system also causes its destruction in this case. All these factors in combination have allowed the selection of just this characteristic to study its effect on the dynamics of the formation and destruction of a foam. The values of ν = 1750, 2000, 2500, 3000 rpm were considered, and the model parameters corresponding to them were denoted as α_ν, β_ν. The results of observations at time moments $t = 3m$ min, m = 1, 2, 3, 4, 5, depending on the revolution speed are plotted in Fig. 1.

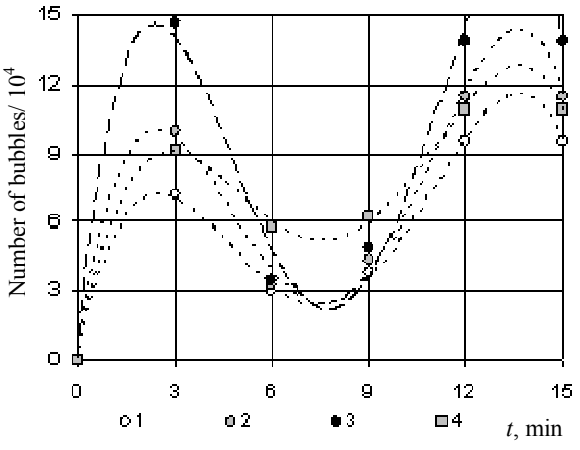

Fig. 1. Number of bubbles per unit volume at time moments $t = 3m$, m = 1, 2, 3, 4, 5 at ν of (*1*) 1750, (*2*) 2000, (*3*) 2500, and (*4*) 3000 rpm.

Assuming that there is almost no destruction at the first minute of generation, α_ν = 25000, 33250, 49500, 63700 1/min was determined from experimental data. The application of both the average value and the mean-square deviation enabled the estimation of the range of this parameter for each of the foaming processes. The obtained ranges of the parameter α_ν are 11500–38400, 12500–53900, 13200–77800, and 22000–105000 1/min, within which the values of this parameter fall during the repeated series of experiments, at ν = 1750, 2000, 2500, 3000, respectively.

It is obvious (Fig. 1) that foaming must be limited by 3 min. However, taking into account that just this interval was primarily selected as a time period between measurements in experimental studies, it might occur in reality that an "important moment" had merely been missed, and the repetition of all the series of studies with shorter intervals between measurements with consideration for necessary reproducibility would lead to considerable time, energetic, and material expenditures, it was decided to perform a theoretical study within the framework of already available empirical data and then repeated experimental studies of smaller series.

The formation of a foam was studied from the viewpoint of creating the conditions for its preservation. To accomplish this, it is necessary to minimize the effect of the factors, which lead to the destruction of a foam.

Let us study the foam destruction processes. The parameter (intensity) α in Eqs. (3) is determined as the average number of forming bubbles per unit time, and the parameter β was interpreted also as the average number of bubbles bursting for the same unit time.

The direct estimation of the number of bubbles bursting under the action of different factors presents a certain difficulty, so the values of the parameter β were fitted at fixed time moments. The differences between the generated number of bubbles and their actual number were taken into account. Then the fraction with respect to the actually generated flow of bubbles per unit time was calculated, e.g.,

$$\beta_{2500}(3) = \frac{49500 \cdot 3 - 146324}{146324} : 3 = 0.00496 \ \text{1/min}.$$

The values of the parameter β_ν. at time moments $t = 3m$ are plotted in Fig. 2. Their analysis makes it possible to say that the second oscillatory cycle characterized by the growth of the foam destruction rate after the period of descent begins after ≈12 min of the process. For this reason, it is inadvisable to study the foaming process for more than 12 min. Moreover, the highest values of empirical data for all the presented variants correspond to t = 6 min. Taking into consideration the time interval between measurements, it is possible to say that the greatest destructibility of a foam is really attained in the neighborhood of this point on the time axis with a radius of less than 3 min. At this stage, the interval (0, 9) with the center at t = 6 is taken for further studies.

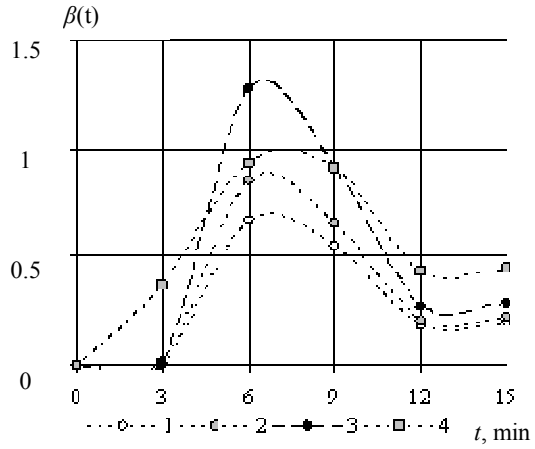

Fig. 2. Parameter β_ν versus time $t = 3m$ at ν of (*1*) 1750, (*2*) 2000, (*3*) 2500, and (*4*) 3000 rpm.

Since $\beta = 1/t_{av}$ is an averaged characteristic, this raises the question on what time interval should it be considered. Let us cite the values (Fig. 3) calculated for the average number of bubbles per unit foam volume by Eq. (3) for the parameter β calculated on the intervals to 3, 9, and 12 min. It is obvious that the consideration of the averaged constant parameter β enables the description of the number of foam bubbles generated by a rotor-stator device only on a limited time interval. To obtain a more precise description of the considered characteristic $M_i(t)$, it is necessary to express the parameter $\beta = \beta(t)$ in term of time, e.g., by function (5). Thus the specified functional dependence has allowed not only the study of trends in the change of the foam destruction rate, but also the determination of time moments important in this regard and, thereby, the recommended gas saturation time for a protein solution. In other words, the common regularities of the destruction of a protein foam are revealed from the results of studying a particular case.

Hence, taking into account the physical meaning of the parameter $\beta = \beta(t)$, the functional dependence determining it will enables the study of the foam destruction process within the first oscillatory cycle. The values of β_v were approximated by the function $\beta(t)$, the form of which enabled the detection of the time moments important for the foam formation and destruction processes (the physicochemical principles of which are explained in [3–5]) due to the presence of singular points (extrema, inflection points, etc.). Several variants of the approximation of the function $\beta_{2500} = \beta(t)$ in the form of Eq. (5) are shown in Fig. 4, and the error with consideration for the time moment of 12 min variates from 15 to 20%, respectively. When a shorter time interval is considered, the precision increases by nearly two times. Similar results were obtained for the other studied gas saturation processes (ν = 1750, 2000, 3000 rpm).

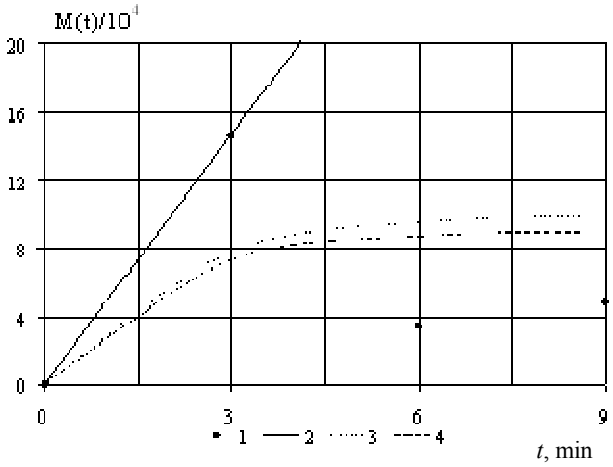

Fig. 3. Number of bubbles versus time t in the rotor-stator processing of a protein solution for (*1*) experimental data and β_{2500} of (*2*) 0.0050, (*3*) 0.4929, and (*4*) 0.5476 1/min.

Let us determine the time moment t0 (located within the interval from 2 to 5 min, as reflected by Fig. 4), from which the foam destruction rate begins to intensively grow.

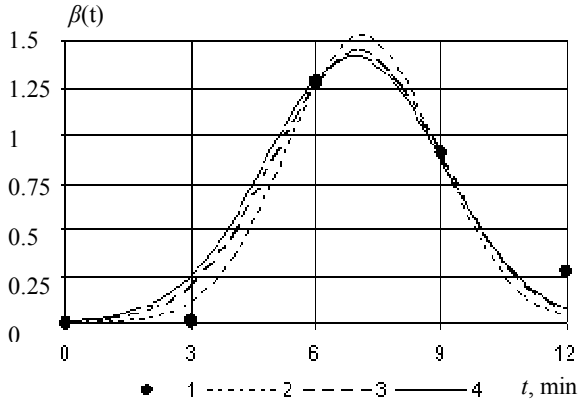

Fig. 4. Time function $\beta(t)$ calculated (*1*) from experimental data and at (*2*) $A = 7.50$, $B = 0.01$, $a = 6.90$, $b = 2.10$, (*3*) $A = 7.20$, $B = 0.01$, $a = 7.00$, $b = 2.00$, and (*4*) $A = 6.90$, $B = 0.01$, $a = 7.10$, $b = 1.80$.

Using the differential calculation methods [34], determining the derivatives $\beta'(t)$, $\beta''(t)$, and the functions $\beta(t)$, and setting them equal to 0, the greatest time t_0, before which the formation of a foam should be stopped (i.e., before the highest foam destruction rate is attained), was found. Hence, the point $t = a$ determines the maximum of the function $\beta(t)$, the inflection point of the function $\beta'(t)$, and the extremum of $\beta''(t)$, $t = a \pm b$ is the inflection points of $\beta(t)$ or the extremum of the function $\beta'(t)$, and $t = a \pm \sqrt{3}b$ is the inflection points of $\beta'(t)$ and the extrema of $\beta''(t)$.

Note that full symmetry is hardly probable in reality (Fig. 4). To estimate the deviation from the symmetry point, if necessary, it is also possible to use the third moment about the mean for the calculation of the asymmetry coefficient. The corresponding formula is derived by solving the differential equation, which is additional to set (1) and obtained by the same method as for the equations of the mathematical expectation and dispersion [29–32]. The found functional dependence $\beta(t)$ was substituted into the set of differential equations describing the mathematical expectation and dispersion to obtain

$$\begin{cases} \dfrac{d}{dt}M_i(t) + \beta(t)\cdot M_i(t) = \alpha \\ \dfrac{d}{dt}Q_i(t) + 2\beta(t)\cdot Q_i(t) = 2\alpha M_i(t) \\ Q_i(t) = D_i(t) + M_i^2(t) - M_i(t), \end{cases} \qquad (6)$$

with initial condition (2). Note that the derivation of differential equations immediately for the numerical characteristics is insensitive to the form of an equation, i.e., independent of whether the parameters α and β are constants or time functions, although this can not be ensured for the set of differential equations of system probabilities, thus accentuating the advantages of the derivation of differential equations immediately for the numerical characteristics [29–34]. However, the precise analytical solution of set (6) is of no interest due to its cumbersomeness, as it is presented in the

form of quadratures or a series, so the specialized Mathematica 5.2 software was used to solve the set by numerical methods. The obtained results are plotted in Fig. 5.

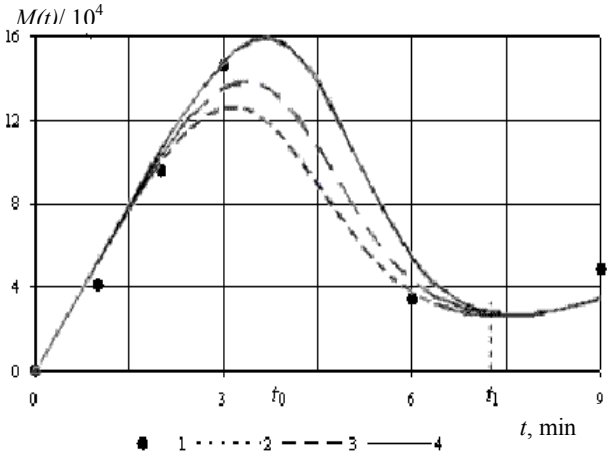

Fig. 5. Number of bubbles versus time t in the rotor-stator processing of a protein solution for (*1*) experimental data, (*2*) $A = 7.50$, $B = 0.01$, $a = 6.90$, $b = 2.10$, (*3*) $A = 7.20$, $B = 0.01$, $a = 7.00$, $b = 2.00$, and (*4*) $A = 6.90$, $B = 0.01$, $a = 7.10$, $b = 1.80$.

From Fig. 4 it can be seen that the empirical curves attain the highest value at a time moment t_0 within the interval [6, 8], i.e., it is unadvisable to perform the process of generation for longer than 8 min, as it appears to be just the time moment, in the neighborhood of which the greatest combined action of all the destruction factors takes place. This is confirmed by Fig. 5. The solution of set (1) with the variable parameter $\beta = \beta(t)$ in the form of Eq. (5) is plotted in Fig. 4. The obtained results for the different values of the parameters of the function $\beta(t)$ describe experimental data at a various precision, which decreases with increasing time interval, and each of them rather precisely describes the overall character of the process on the interval to 9 h. For this reason, after studying the effect of the behavior of the function $\beta(t)$ on the foam destruction dynamics, the foam generation process is considered as a whole and after destruction. From Fig. 5 it can be seen that the interval, on which the foaming process should be interrupted, is [3, t_0]. Similarly, the function *M(t)* attains the lowest value at a maximum point t_1 of the function $\beta(t)$, and it is obvious that this is inadmissible in the foam generation process.

From the comparison of the results shown in Figs. 4 and 5 it is obvious that the singular points of the function $\beta(t)$ are also singular for the function *M(t)*, so the parameter *a* is determined by the time moment, at which a foam is maximally destructed, and $t = a \pm b$ is the time moment, at which the destruction rate begins to intensively change, namely, *a-b* corresponds to an intensive increase in the destruction rate, *a+b* corresponds to its intensive decrease, $t = a \pm \sqrt{3}b$ are the beginning moments of stable acceleration in the foam destruction rate, i.e., intensive acceleration ($t = a - \sqrt{3}b$) or deceleration ($t = a + \sqrt{3}b$). In this case, the time interval $[a - \sqrt{3}b, a - b]$, to which the terminal time moment of the foaming process must belong, is unequivocally selected. As is evident, the value $t = a - \sqrt{3}b$ is ideal. For the considered function $\beta(t)$ (where $A = 7.50$, $B = 0.01$, $a = 6.90$, $b = 2.10$, $A = 7.20$, $B = 0.01$, $a = 7.00$, $b = 2.00$, and $A = 6.90$, $B = 0.01$, $a = 7.10$, $b = 1.80$, Fig. 4), such time intervals are [3.26, 4.80], [3.54, 5.00], and [3.98, 5.30], and the "ideal" terminal time moments are 3.26, 3.54, and 3.98 min, respectively (note that the error of the description of the protein foam destruction rate by the function $\beta(t)$ on the interval from 0 to 9 min was 15, 18, and 20%, respectively).

Similarly, the intervals [3.45, 4.91], [3.24, 4.76], and [2.36, 4.25] are obtained via the approximation with the function $\beta_\nu(t)$ at $\nu = 1750$, 2000, and 3000 rpm with an error of less than 18% on the interval from 0 to 9 min. The recommended terminal time moments are ≈ 3.5, 3.3, and 2.4 min, respectively.

Note that the difficulties arising in the derivation of the analytical dependence are resolved by finding a numerical solution, which unfortunately does not enables the study of process trends and the system approach to the study of the process, but allows the calculation of numerical results for the average parameters at $\beta = \beta(t)$. In this case, it is almost impossible to obtain any formulas convenient for the application in engineering practice, but, as shown by performed studies, it is quite realistic. The suggested hypothesis on the possibility of the application of Eqs. (3) with constant values of the parameter β averaged over each interval (in our case, these intervals were determined via the regular measurements of samples) was confirmed after the comparison of the experimental data approximation results with the theoretical values, which are the solutions of sets (1) and (6) with initial conditions (2), where $\beta(t) = \overline{\beta}$ and $\beta = \beta(t)$, respectively. In this case, it is evident that the shorter is a considered interval, the smaller is the difference between the averaged parameter β and it real value. Note that the application of differential calculation to the analysis of the function $M_i(t)$ allows (due to that $\beta(t) \neq 0$) the estimation of its maximum value attained at a certain point t_0 from the first equation of set (6) using the inequality

$$M_i(t_0) = \lim_{t \to t_0} \frac{\alpha}{\beta(t)} > 0 \cdot$$

At a constant value of the parameter α, the function $M(t)$ attains the highest value for such an argument, at which the function $\beta(t)$ takes the lowest value on the interval [0, 9). Let us approximate the experimental data on the number of foam bubbles per unit volume by the "pieces" of the function $M(t)$ with the constant parameter $\overline{\beta}$ [25, 28]:

$$\overline{\beta} = \frac{1}{T} \cdot \int_0^T \beta(t) dt ,$$

where T is a considered interval. The results plotted in Figs. 4 and 6 demonstrate the nearly identical description of experimental data for both theoretical dependences on the interval [0, 4). The approximation error does not exceed 4%, but an increase in the length of the considered interval appreciably rises it up to 30% and more. Hence, the formulas of set (1) at $\beta(t) = \overline{\beta}$ or simple formulas (3), where β is constant, can be used for the analysis of the foaming process on the interval [0, t_0).

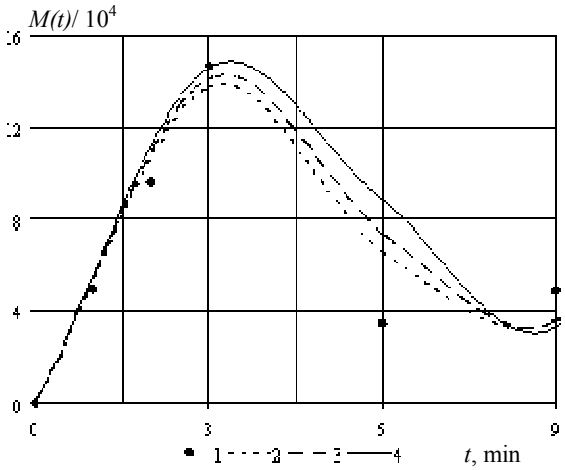

Fig. 6. Number of bubbles versus time t in the rotor-stator processing of a protein solution at $\beta(t) = \overline{\beta}$ for (*1*) experimental data, (*2*) $A = 7.50$, $B = 0.01$, $a = 6.90$, $b = 2.10$, (*3*) $A = 7.20$, $B = 0.01$, $a = 7.00$, $b = 2.00$, and (*4*) $A = 6.90$, $B = 0.01$, $a = 7.10$, $b = 1.80$.

The obtained data were further used as a basis to formulate the constraints of the time of action on a processed mass. Comparing the experimental data and the values of the function $M_i(t)$ expressed by the equation of set (1) with the variable parameter $\beta(t)$ in the form of Eq. (5) on the intervals from 0 to 4.8, 5.0, and 5.3 (Fig. 6), it has been ascertained that the error is from 8 to 2%, respectively, and does not exceed 3% on the interval [0, 4). This allows the error values to be considered as falling within statistical disperancy. The foaming process was studied for t_0 minutes. The interval from 0 to 4 was partitioned with unit steps, at each of which the average value of the parameter β was determined, and the functions $M_i(t)$ and $D_i(t)$ (Fig. 7), which are the solution of set (3), were considered. The preliminary analysis of the experimental data (Fig. 1) shows that the average values of the parameter $\beta(t) = \beta$ on this time interval nearly coincide and equal to 0.010 1/min at a rotor revolution speed of 3000 rpm, except the case corresponding to the generation of a protein concentrate foam. Knowing the capacity of the used equipment for the foaming of a protein solution (the parameter α) and using Eqs. (3) for the description of the process, the range $(M_i(t) - \sqrt{D_i(t)}; M_i(t) + \sqrt{D_i(t)})$ for the number of bubbles per unit volume was obtained (Fig. 7).

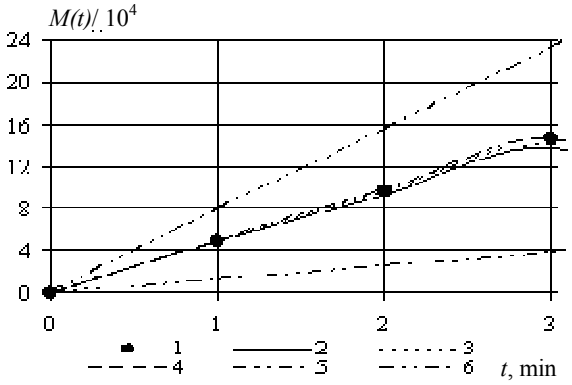

Fig. 7. Number of bubbles versus time t in the rotor-stator processing of a protein solution for (*1*) experimental data, (*2*) $M_0(t)$, $\alpha = 49500$ 1/min, $\beta = \beta(t)$, $A = 7.50$, $B = 0.01$, $a = 6.90$, $b = 2.10$, (3) $M_0(t)$, $\alpha = 49500$ 1/min, $\beta = \beta(t)$, $A = 7.20$, $B = 0.01$, $a = 7.00$, $b = 2.00$, (4) $M_0(t)$, $\alpha = 49500$ 1/min, $\beta = \beta(t)$, $A = 6.90$, $B = 0.01$, $a = 7.10$, $b = 1.80$, and (5,6) boundary $M_0(t)$, $\alpha = 13270$, 77800 1/min, $\beta = 0.010$ 1/min

Note that the experimental data on the number of bubbles per unit volume in the repeated series of experiments on the rotor-stator processing of a protein solution (skim milk protein concentrate with an initial mass fraction of solids of 9.2%) at 1750, 2000, and 2500 rpm (Fig. 7) will also fall into this interval with a near unitary probability. The case corresponding to 3000 rpm is of no interest, as excess hydrodynamic action leads to the considerable destruction of a foam due to the rotation of the device's working body alone.

Hence, the first cycle of the process of protein foam formation and destruction has systematically been studied. The constructed stochastic model has allowed the mathematical expectation and dispersion of the number of protein foam bubbler per unit volume to be determined at a random time moment of gas saturation within the first cycle. It has been shown that the applied numerical methods are in good agreement with the analytical methods, which give simple formulas convenient for engineering calculations. A method of determining the parameters of this model and the dependence of the parameter $\beta(t)$ has been developed. The proposed model has enabled the quantitative description of the foaming process both on average and by states. It has been established that the protein foaming time in a rotor-stator device at specified process parameters is advisable to be limited by the moment, at which the highest foam destruction rate is attained.

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REFERENCES

1. Fridrikhsberg, N.N., *Kurs kolloidnoi khimii* (Course in Colloid Chemistry) (Khimiya, St. Petersburg, 1995).

2. Gel'fman, M.I., Kovalevich, O.V., and Yustratov, V.P., *Kolloidnaya khimiya* (Colloid Chemistry) (Lan', St. Petersburg, 2003).
3. Kruglyakov, P.M. and Ekserova, D.R., *Pena i pennye plenki* (Foam and Foam Films) (Khimiya, Moscow, 1990).
4. Tikhomirov, V.K., *Peny. Teoriya i praktika ikh polucheniya i razrusheniya* (Foams. Theory and Practice of Their Formation and Destruction) (Khimiya, Moscow, 1983).
5. Kann, K.B., *Kapillyarnaya gidrodinamika pen* (Capillary Hydrodynamics of Foams) (Nauka, Sib. Otd., Novosibirsk, 1989).
6. Bikerman, J., *Foams* (Springer, Berlin, 1973).
7. Schramm, L.L., *Emulsions, Foams, and Suspensions: Fundamentals and Applications* (Wiley-VCH, Weinheim, 2005).
8. Merkin, A.P. and Traube, P.R., *Neprochnoe chudo* (Fragile Miracle) (Khimiya, Moscow, 1983).
9. Walstra, P., *Physical Chemistry of Foods* (Marcel Dekker, New York, 2003).
10. Dickinson, E., *Food Emulsions and Foams* (Royal Soc. Chem., London, 1987).
11. Balerin, C., Aymard, P, Ducept, F., Vaslin, S., and Cuvelier, G., Effect of formulation and processing factors on the properties of liquid food foams, *J. Food Eng.,* 2007. V.78. №3. P. 802–809.
12. Narchi, I., Vial, C., Labbafi, M., and Djelveh, G., Comparative study of the design of continuous aeration equipment for the production of food foams, *J. Food Eng.,* 2011. V.102. №2. P. 105–114.
13. Indrawati, L., Wang, Z., Narsimhan, G., and Gonzalez, J., Effect of processing parameters on foam formation using a continuous system with a mechanical whipper, *J. Food Eng.,* 2008. V.88. №1. P. 65–74.
14. Müller-Fischer, N., Suppiger, D., and Windhab, E.J., Impact of static pressure and volumetric energy input on the microstructure of food foam whipped in a rotor-stator device, *J. Food Eng.,* 2007. V.80. №1. P. 306–316.
15. Meshalkin, V.P. and Boyarinov, Yu.G., Semi-markovian models of the functioning of complex chemical engineering systems, *Theor. Found. Chem. Eng.,* 2010. V.44. №2. P. 186–191.
16. Hanselmann, W. and Windhab, E., Flow characteristics and modelling of foam generation in a continuous rotor/stator mixer, *J. Food Eng.,* 1998. V.38. №4. P. 393–405.
17. Ho, Q.T., Carmeliet, J., Datta, A.K., Defraeye, T., Delele, M.A., Herremans, E., Opara, L., Ramon, H., Tijskens, E., van der Sman, R., Liedekerke, P.V., Verboven, P., and Nicolaï, B.M., Multiscale modeling in food engineering, *J. Food Eng.,* 2013. V.114. №3. P. 279–291.
18. Vetoshkin, A.G. Hydromechanics of separation of gas–liquid systems with foam structure, *Theor. Found. Chem. Eng.,* 2004. V.38. №6. P. 569–574.
19. Liszka-Skoczylas, M., Ptaszek, A., and Żmudziński, D., The effect of hydrocolloids on producing stable foams based on the whey protein concentrate (WPC), *J. Food Eng.,* 2014. V.129. P. 1–11.
20. Oboroceanu, D., Wang, L., Magner, E., and Auty, M.A.E., Fibrillization of whey proteins improves foaming capacity and foam stability at low protein concentrations, *J. Food Eng.,* 2014. V. 121. P. 102–111.
21. Indrawati, L. and Narsimhan, G., Characterization of protein stabilized foam formed in a continuous shear mixing apparatus, *J. Food Eng.,* 2008, V. 88. №4. P. 456–465.
22. Britten, M. and Lavoie, L., Foaming properties of proteins as affected by concentration, *J. Food Sci.,* 1992. V. 57. №5. P. 1219–1241.
23. Damodaran, S., Protein stabilization of emulsions and foams, *J. Food Sci.,* 2005. V.70. №3. P. 54–66.
24. Germick, R.J., Rehill, A.S., and Narsimhan, G., Experimental investigation of static drainage of protein stabilized foams—Comparison with model, *J. Food Eng.,* 1994. V.23. №4. P. 555–578.
25. Saaty, T., *Elements of Queueing Theory with Application* (Dover Publications, New York, 1961).
26. Ventsel', E.S. and Ovcharov, L.A., *Teoriya sluchainykh protsessov i ee inzhenernye prilozheniya* (Theory of Stochastic Processes and Its Engineering Applications) (Vysshaya shkola, Moscow, 2000).
27. Kleinrock, L., *Queueing Systems. Volume I: Theory* (Wiley, New York, 1975).
28. Feller, W., *An Introduction to Probability Theory and Its Applications* (Wiley, New York, 1968).
29. Pavsky, V.A., Pavsky, K.V., and Khoroshevsky, V.G., Vychisleniye pokazatelei zhivuchesti raspredelennykh vychislitel'nykh sistem i osushchestvimosti resheniya zadachi (Calculating the characteristics of the robustness of distributed computational systems and the feasibility of problem solutions), *Iskusstvennyi intellekt* (Artificial Intelligence), 2006. №4. P. 28–34.
30. Khoroshevsky, B.G., Pavsky, V.A., and Pavsky, K.V., Raschet pokazatelei zhivuchesti raspredelennykh vychislitel'nykh sistem (Calculation of the robustness characteristics of distributed computational systems), *Vestnik Tomskogo Gosudarstvennogo Universiteta. Upravlenie, vychislitel'naya tekhnika i informatika* (Tomsk State University Journal of Control and Computer Science), 2011. №2. P. 81.
31. Khoroshevsky, V.G. and Pavsky, V.A., Calculating the efficiency indices of distributed computer system functioning, *Optoelectron. Instrum. Data Process.,* 2008. V. 44. №2. P. 95–104.
32. Yustratov, V.P., Pavskii, V.A., Krasnova, T.A., and Ivanova, S.A., Mathematical modeling of electrodialysis demineralization using a stochastic model, *Theor. Found. Chem. Eng.*, 2005. V.39. №3. P. 259–262.
33. Ivanova, S.A, *Stokhasticheskie modeli tekhnologicheskikh protsessov pererabotki dispersnykh sistem obezzhirennogo moloka* (Stochastic Models of the Processing of Dispersed Skim Milk Systems) (KemTIPP, Kemerovo, 2010).
34. Ivanova, S.A. and Prosekov, A.Yu., *Intensifikatsiya tekhnologii aerirovaniya molochnykh productov* (Intensification of Dairy Product Aeration Technologies) (KemTIPP, Kemerovo, 2011).

THEORETICAL AND PRACTICAL ASPECTS OF THE THERMOGRAPHIC METHOD FOR MILK COAGULATION RESEARCH

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Abstract: The precise and objective estimation of the beginning of gelation in milk is topical for both laboratory studies and industrial dairy production. In this work, the principles of the thermographic method of monitoring milk coagulation are formulated. This method has evolved from the well-known hot-wire method; it is based on the measurement of the temperature difference between two thermometers, one of which is heated. Unlike the hot-wire method, the thermographic method can even be used in processes that require significant changes in milk temperature, for example, during heat–acid milk coagulation. Two basic designs of thermographic systems, using as thermometers either differentially connected thermocouple junctions or two identical thermistors connected as two legs of a bridge circuit, are described. In both cases, the temperature difference between the heated and unheated thermometers at about 0.5 W of thermal power supply is about 3°C for incoagulated milk and 8–10°C after clot formation. The qualitative agreement of the results of rheological and thermographic methods has been demonstrated. A thermographic research technique for heat–acid or heat–calcium milk coagulations has been developed. Within the effective viscosity model, the numeric solution of the problem of temperature field simulation in the vicinity of the heated thermometer has been obtained. On the basis of the simulation results, the possibility of studying structure formation in milk during its coagulation has been analyzed using the thermographic data. Experimental results obtained during thermographic research of milk coagulation are presented.

Keywords: milk coagulation control, heat convection, thermographic method

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INTRODUCTION

It is common knowledge that the process of milk coagulation represents the transition of a micellar colloidal system of caseins to a gel-like state. This process underlies many dairy technologies, particularly, cheese making, largely predetermining cheese quality [1]. The latter circumstance makes the coagulation of milk proteins the subject of thorough research for scientists specializing in the properties of milk and dairy products.

The estimation of the beginning of gelation in milk is topical both for scientific research and for process monitoring in industry. Suffice it to note that the exact estimation of the beginning of coagulation directly in the cheese vat makes it possible, in principle, to adjust automatically the process schedule as the physic-chemical indicators of milk change (for example, the protein content) in order to economize on both milk-clotting preparations and cheese production times. Very important is also the correct and unambiguous determination of the duration of the main coagulation stages for research purposes, in particular, for building adequate models of the coagulation process.

Despite the very long history of dairy technology, the methods of objective monitoring of the milk coagulation process appeared relatively recently. We may say that the main method of determining the time of coagulation was visual observation using a cheesemaking knife or a cheesemaker's finger practically until the mid-20th century. Note that the first experience of objective observation over the rennet clotting of milk with a viscometer dates back to 1932 [2].

At present, there are a large number of various methods for objective monitoring of milk coagulation. They all may conventionally be divided into several groups characterized by the choice of milk parameters that undergo changes during coagulation [3–5]. Basically, these are certainly rheological and optical methods, based on observing changes in milk structure during its coagulation. In addition, the rheological methods record structural changes by the system's response to applied mechanical stresses or deformations, and the optical methods, by the change in the absorption or dissipation of visible light, as well as in the emission of the infrared band. The wide use of these two methods depends, primarily, on the possibility of their use directly online. Moreover, these methods ensure obtaining well-correlated data [6–8].

Another quite widespread method to monitor milk coagulation due to the possibility to use it online is the hot-wire method, developed in 1985 [9]. Its essence is that the temperature of an electric current–conducting wire placed in milk increases during coagulation because of a decrease in a convective heat sink resulting from the formation of a structure in milk. This method is indirect rheological in essence; therefore, the results obtained with it correlate well with data obtained both rheologically and optically [10]. Thanks to its simplicity and possibility to be used online, this method continues to be topical nowadays [11].

A disadvantage of the hot-wire method is the dependence of the sensor temperature not only on convective heat exchange in milk but also on milk temperature, which can change, for example, as a result of various production processes. Therefore, the purpose of this work is the development of a simple universal method for the objective monitoring of milk coagulation based on the hot-wire method but without the above disadvantage. In addition, the work has also analyzed a number of theoretical models that describe the operation of the presented method.

OBJECTS AND METHODS OF RESEARCH

The object of research was reconstituted fat-free milk. In order to obtain it, 90 g of instant fat-free milk powder (Milk Factory, Kemerovo, Russia) were dissolved in 910 mL of distilled water and stirred thoroughly. Into the resulting reconstructed milk, 4 cm^3 of a 10% medical solution of $CaCl_2$ (Shenlu Pharm, China) were added, after which it was aged for 12 hours at 6±2ºC.

For rennet clotting, microbially derived chymosin, Maxiren® (DSM, the Netherlands), was used. In order to prepare the solution, 0.1 g of enzyme powder was dissolved in 100 cm^3 of distilled water.

During the simulation of acid clotting, a 10% solution of lactic acid was slowly introduced into milk and stirred thoroughly. The solution was obtained by diluting a 40% nutritive solution of lactic acid (Univerkhim, Chelyabinsk, Russia).

Milk coagulation was carried out in a thermostatted cell of 200 mL.

Thermographic method

Milk coagulation was monitored by the change in its effective viscosity with the help of temperature-sensing viscosity sensors of in-house design, attached to a computer. The principle of their operation is in measuring the temperature difference between two thermometers stationed in milk at a short distance from each other, one of which being heated. This method is similar to the hot-wire method, but, unlike the latter, it is not sensitive to changes in the environmental temperature, because it links the rheological parameters of milk not with the absolute temperature of the heated thermometer but with temperature difference between the heated and unheated thermometers.

The amount of heat fed to the heated thermometer per time unit depends on the capacity of the electric heater. In our devices, it was 400–600 mW. Heat is removed from the thermometer through heat exchange and convective transfer. As a result of heat equilibrium established between heat release and its removal, the temperature difference between the two thermometers placed in milk takes on a certain value rather quickly. In our experiments, a typical temperature difference value for incoagulated milk was 3–4°C, depending on the heat-release capacity. As a result of milk coagulation, the increased effective viscosity hinders convection, causing the growth of temperature difference between the thermometers. Finally, the structure emerging in milk ceases the convective flow practically altogether, and the heat equilibrium changes. The temperature difference established after coagulation depended in our experiments mainly on the power of heat release and on the quality of the clot formed, being approximately 7–10°C.

We developed two principal circuits that implemented the above method. In the first case, two junction points of a differentially connected thermocouple, one of which was glued directly to the heating resistor, served as thermometers. In the second case, two thermistors connected as two legs of a bridge circuit were used as thermometers. In the latter case, the heating element was a thin wire wound directly on one of the cylindrical thermistors. In both cases, the thermometers are positioned in thin leakproof stainless-steel tubes filled with a dielectric heat-conducting paste at a distance of 2–3 cm from each other. Both ways of implementing our method yield identical results and differ only in the manner they transform the output signal for connection with the personal computer.

As was noted above, the described method is indirectly rheological, characterizing a change in the effective viscosity of milk during coagulation. In order to substantiate this statement, Fig. 1 shows the comparison of a typical rheogram of rennet clotting, obtained with a Brookfield rotary viscosimeter (a) [12], and a typical curve of temperature difference between the heated and unheated thermometers placed in milk under rennet clotting (b).

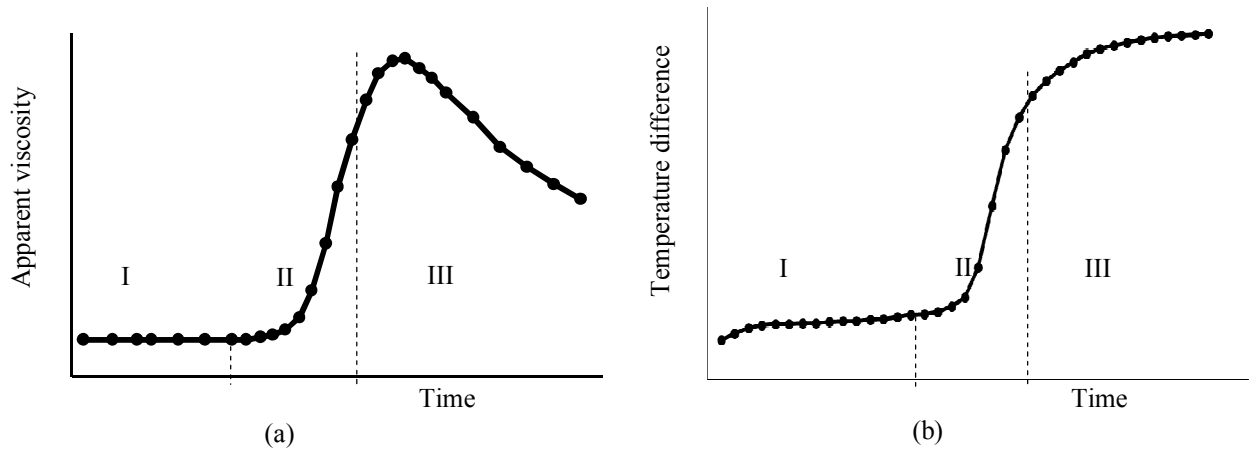


Fig. 1. Comparison of (a) a typical rheogram and (b) thermogram of rennet coagulation.

As is seen from the figure, informationally, the thermogram is practically equivalent to the rheogram. Both curves show distinctly the stages of latent (I) and explicit (II) coagulation. However, stages (III) in Figs. 1a and 1b differ noticeably. Stage III in Fig. 1a corresponds to the syneretic layering of the clot, which results in a decrease in its observable viscosity. As is known, a mechanical impact on the clot is necessary for intensive whey separation to begin, which, in the case of a rotary viscosimeter, is ensured by the moving elements of a measuring cell. There were no mechanically moving parts in our method, which led to a delay in the metastable equilibrium stage. In addition, a higher temperature of the heated thermometer contributed to the further strengthening of the clot around it and, consequently, to the further slow growth of the temperature difference.

On the other hand, the absence of moving parts makes it possible to use easily our sensor to monitor gelation directly in the cheese vat (online), and the simplicity of the design ensures a high reliability at a relatively low cost.

Hereinafter, the curves, obtained with the help of the thermal viscosity meter, that characterize the change in the effective viscosity of milk during coagulation, will be called thermograms (by analogy with rheograms), and the method of deriving them will be called the thermographic method.

Insensitivity of the thermographic method to milk temperature changes during coagulation allows us to use it in production processes accompanied by such changes. Heat–acid milk clotting, which underlies the production of well-known cheeses, is a good example of this. For instance, Quesco Blanco, which was brought from Spain, is quite popular in South and Central Americas. Paneer is well known in South Asia. Adygei cheese is popular on the territory of the CIS. Heat–acid milk coagulation is based of the settling of milk proteins under the action of an acid and high temperature. The main merit of this method of milk clot is a high degree of deproteinization of raw milk by settling whey proteins together with casein. In addition, compared with casein, whey proteins within the heat–acid clot have a more balanced amino acid composition, improving the biological value of products produced on the basis of heat–acid coagulation.

The direct use of the thermographic method to study the heat–acid coagulation process is complicated by the fact that the application of an acid to heated milk is accompanied by intensive stirring, which hinders structure formation. As a consequence, no strongly marked change in the temperature difference between the heated and unheated thermometers occurs. In order to resolve this problem, we developed the following technique of studying heat–acid milk coagulation. Acid agents are introduced preliminarily into milk, as well as additive substances that are able to affect heat–acid clotting in amounts that admittedly do not cause coagulation at room temperature. The prepared samples are then heated under control until gel appears, which is recorded thermographically. In our opinion, this method allows us to obtain objective data for the analysis of physicochemical specifics of destabilization of the milk protein system under high temperatures. The obtained data may be the basis for the standardization of conditions for the high-temperature coagulation of milk proteins in the production of heat–acid cheeses in the dairy industry.

A mathematical model of the thermographic method

According to Archimedes' law, in a liquid in a gravitational field, the local area of a less density generates a buoyancy force directed upward. This force leads to the emergence of convective flows in liquids whose density ρ depends on temperature T.

Fluid flow equations may be written on the basis of general physical principles: the equation of continuity, which states that a change in fluid density at a given point may only depend on its expansion or compression; the balance of impulses (Newton's second law), which is written as the result of equalizing inertial forces and viscous forces; and the balance of energy, which takes into account, along with other energy types, the diffusive and convective transfer of heat energy. Usually, the equations of motion of a viscous fluid in this form are called the Navier–Stokes equations [13]:

$$\begin{aligned}
&\frac{d}{dt}\rho(\mathbf{r},t)+\rho(\mathbf{r},t)\nabla\cdot\boldsymbol{\upsilon}(\mathbf{r},t)=0,\\
&\rho(\mathbf{r},t)\frac{d}{dt}\boldsymbol{\upsilon}(\mathbf{r},t)=-\nabla p(\mathbf{r},t)+\nabla\cdot\mathbf{T}(\mathbf{r},t)+\mathbf{f}(\mathbf{r},t), \qquad (1)\\
&\frac{d}{dt}w(\mathbf{r},t)=-p\nabla\cdot\boldsymbol{\upsilon}(\mathbf{r},t)+\Phi(\mathbf{r},t)-\nabla\cdot q(\mathbf{r},t)+e(\mathbf{r},t).
\end{aligned}$$

The values given in this system of equations are set in a point determined by radius vector $\mathbf{r}$ at time point t and have the following meaning:

ρ is a fluid density;

$\boldsymbol{\upsilon}$ is a fluid velocity vector liquid;

P is a pressure created by external forces;

$\mathbf{T}=\mu(\nabla\boldsymbol{\upsilon}+(\nabla\boldsymbol{\upsilon})^{\mathrm{T}})+\mu^{*}(\nabla\cdot\boldsymbol{\upsilon})$ is the viscous part of the stress tensor, where μ is the shear (usual) viscosity coefficient, and μ^{*} is an additional viscosity coefficient related to the fluid's volume deformation;

$\mathbf{f}$ is the density of volumetric forces within the fluid; in our case, $\mathbf{f}=\rho\mathbf{g}$, where $\mathbf{g}$ is the free fall acceleration;

w is the density of the fluid's internal (heat) energy; in our case, we may take that $dw=\rho cdT$, where c is the fluid's specific heat capacity;

$\Phi=\mathrm{tr}(\mathbf{T}(\nabla\boldsymbol{\upsilon})^{\mathrm{T}})$ is a dissipative function that describes the amount of mechanical energy that turns into heat in a unit time in the fluid's' unit volume;

$q=\kappa\nabla T$ is the heat flow vector, where κ is the coefficient of heat conductivity and T is temperature;

e is the density of the volume sources of heat; and

the full time derivative is determined by the expression

$$\frac{d}{dt}\equiv\frac{\partial}{\partial t}+\boldsymbol{\upsilon}\cdot\nabla.$$

Thus, we have at least three simultaneous equations that determine flow parameters: velocity, pressure, and temperature. In addition, we need some equations that link state parameters, in particular, equation $\rho=\rho(T)$.

We also have to know the coefficients of molecular transfer: viscosity μ for Newtonian fluid, coefficient of thermal conductivity κ, and some other coefficients for special flow cases.

The main difficulty in solving the above equations arises owing to a possible change in transfer parameters μ and κ, as well as density ρ. Since μ and κ mainly depend on temperature, they change substantially in processes with large temperature differences. In other cases, these parameters may often be assumed as constant. However, in order to obtain motion, we should always take into account changes in pressure.

Let us first note that, at low temperature differences and, consequently, at small flow rates, there is a stationary limit to the solving system (1), which corresponds to conditions $\frac{\partial \rho}{\partial t} = 0, \quad \frac{\partial \upsilon}{\partial t} = 0, \quad \frac{\partial T}{\partial t} = 0$.

In our case, the stationary condition should also take into account the possible change in viscosity during the milk clotting process. Indeed, if the characteristic time during which the milk viscosity changes noticeably exceeds considerably the time of setting up the stationary flow mode, then the process may be considered quasi-stationary.

Note also that the heat release by the volume sources is the main cause of the fluid temperature change. Then we obtain the following system of stationary equations to determine velocity and temperature distributions in the fluid:

$$\begin{aligned}
&(\boldsymbol{\upsilon}(\mathbf{r})\cdot\nabla)\rho(\mathbf{r}) + \rho(\mathbf{r})\nabla\cdot\boldsymbol{\upsilon}(\mathbf{r}) = 0,\\
&\rho(\mathbf{r})(\boldsymbol{\upsilon}(\mathbf{r})\cdot\nabla)\boldsymbol{\upsilon}(\mathbf{r}) = -\nabla p(\mathbf{r}) + \mu\nabla^2\boldsymbol{\upsilon}(\mathbf{r}) + \lambda\nabla\cdot\boldsymbol{\upsilon}(\mathbf{r}) + \rho\mathbf{g}, \qquad (2)\\
&\rho(\mathbf{r})C_P(\boldsymbol{\upsilon}(\mathbf{r})\cdot\nabla)T(\mathbf{r}) = -\kappa\nabla^2 T(\mathbf{r}) + e(\mathbf{r}).
\end{aligned}$$

The next approximation may be the assumption of the fluid's practical incompressibility. Let us assume that the fluid density is constant in all cases, except for taking into account the buoyancy force when we describe convection (the Boussinesq approximation). In this approximation, the difference of pressure and gravity forces may be represented as

$$\rho\mathbf{g} - \nabla p \approx \mathbf{B} + \nabla p^* = \mathbf{g}(\rho_T - \rho_0) + \nabla p^*,$$

where ρ_T is the fluid density at temperature T; ρ_0 is the fluid density at temperature T_0; and p^* is the nonhydrostatic part of the fluid pressure. Under T_0 we mean the fluid temperature far from the heat source. Using the condition of a small change in density, we may write down the buoyancy force through the temperature coefficient of the fluid volume expansion, β. Indeed, $\rho_T \approx \rho_0(1-\beta(T-T_0))$; therefore,

$$\mathbf{B} = -\mathbf{g}\rho_0\beta(T-T_0).$$

In addition, in system (2), we should set

$$\rho(\mathbf{r}) = \rho_0 = const.$$

In many flows induced by the buoyancy force, there is axial symmetry, since a surface or body near which a flow occurs is symmetrical in relation to the vertical axis. A flow close to axisymmetric should, obviously, also appear in the case of a convective flow formed by a small heat source. Such flows represent jets and rising plumes.

In the Boussinesq approximation, $\nabla\rho = 0$, $\nabla\cdot\boldsymbol{\upsilon} = 0$, and the quasi-stationary equations of system (2) with a point heat source, written in the cylindrical coordinate system, will have the following form:

$$\begin{aligned}
&\frac{1}{r}\frac{\partial(r\upsilon_r)}{\partial r} + \frac{\partial\upsilon_z}{\partial z} = 0\\
&\upsilon_z\frac{\partial\upsilon_z}{\partial z} + \upsilon_r\frac{\partial\upsilon_z}{\partial r} = \nu\left(\frac{\partial^2\upsilon_z}{\partial z^2} + \frac{\partial^2\upsilon_z}{\partial r^2} + \frac{1}{r}\frac{\partial\upsilon_z}{\partial r}\right) - \frac{\partial p_z^*}{\partial z} + g\beta(T-T_0)\\
&\upsilon_z\frac{\partial\upsilon_r}{\partial z} + \upsilon_r\frac{\partial\upsilon_r}{\partial r} = \nu\left(\frac{\partial^2\upsilon_r}{\partial z^2} + \frac{\partial^2\upsilon_r}{\partial r^2} + \frac{1}{r}\frac{\partial\upsilon_r}{\partial r} - \frac{\upsilon_r}{r^2}\right) - \frac{\partial p_r^*}{\partial r} \qquad (3)\\
&\upsilon_z\frac{\partial T}{\partial z} + \upsilon_r\frac{\partial T}{\partial r} = \lambda\left(\frac{\partial^2 T}{\partial z^2} + \frac{\partial^2 T}{\partial r^2} + \frac{1}{r}\frac{\partial T}{\partial r}\right) + W_h f,
\end{aligned}$$

where $\nu = \frac{\mu}{\rho_0}$ is the fluid's kinetic viscosity; $\lambda = \frac{\kappa}{\rho_0 c}$ is the coefficient temperature conductivity; W_h is the power fed to the point heat source; and $f(z,r)$ is the function of heat source distributions, which in the case of a point heat source has the following form: $f(z,r) = \frac{\delta(z)\delta(r)}{2\pi r}$. The velocity, temperature, and pressure fields are determined from the solution of equation system (6) with the corresponding boundary conditions. The problem was solved numerically by the finite element method in the COMSOL Femlab system.

RESULTS AND DISCUSSION

Results of numeric calculations

Figure 2 shows the numeric solution of system (3) for a small cylindrical heat source 3 mm long and 2 mm in diameter, in which a constant power of 0.5 W is given out. Milk is considered a fluid with the following parameters: the density, ρ_0 = 1030 kg/m^3; the coefficient of heat conductivity, κ = 0.55 W/(m·K); and the coefficient of volume expansion, $\beta = 10^{-4}$. The viscosity of this fluid can change in very wide limits, as shown in the figure. The absence of radial and axial flows on the region boundaries were selected as the boundary conditions: $r \subset [0, r_{\max}]$; $z \subset [-z_{\max}, +z_{\max}]$.

$$\left.\frac{\partial\upsilon_r}{\partial r}\right|_{r=r_{\max}} = 0; \quad \left.\frac{\partial\upsilon_r}{\partial z}\right|_{z=\pm z_{\max}} = 0; \quad \left.\frac{\partial\upsilon_z}{\partial r}\right|_{r=r_{\max}} = 0;$$

$$\left.\frac{\partial\upsilon_z}{\partial z}\right|_{z=\pm z_{\max}} = 0; \quad \left.\frac{\partial T}{\partial r}\right|_{r=r_{\max}} = 0; \quad \left.\frac{\partial T}{\partial z}\right|_{z=\pm z_{\max}} = 0.$$

In addition, the fluid flow on the boundaries is absent $\upsilon_z(r_{\max}) = 0$; $\upsilon_r(r_{\max}) = 0$; $\upsilon_z(\pm z_{\max}) = 0$; $\upsilon_r(\pm z_{\max}) = 0$, and the temperature is constant $T(r_{\max}) = T_0$; $T(\pm z_{\max}) = T_0$, where T_0 = 30°C. The dimensions of the region for numeric integration were chosen in the following way: $r_{\max}$ = 5 mm; $z_{\max}$ = 10 mm.

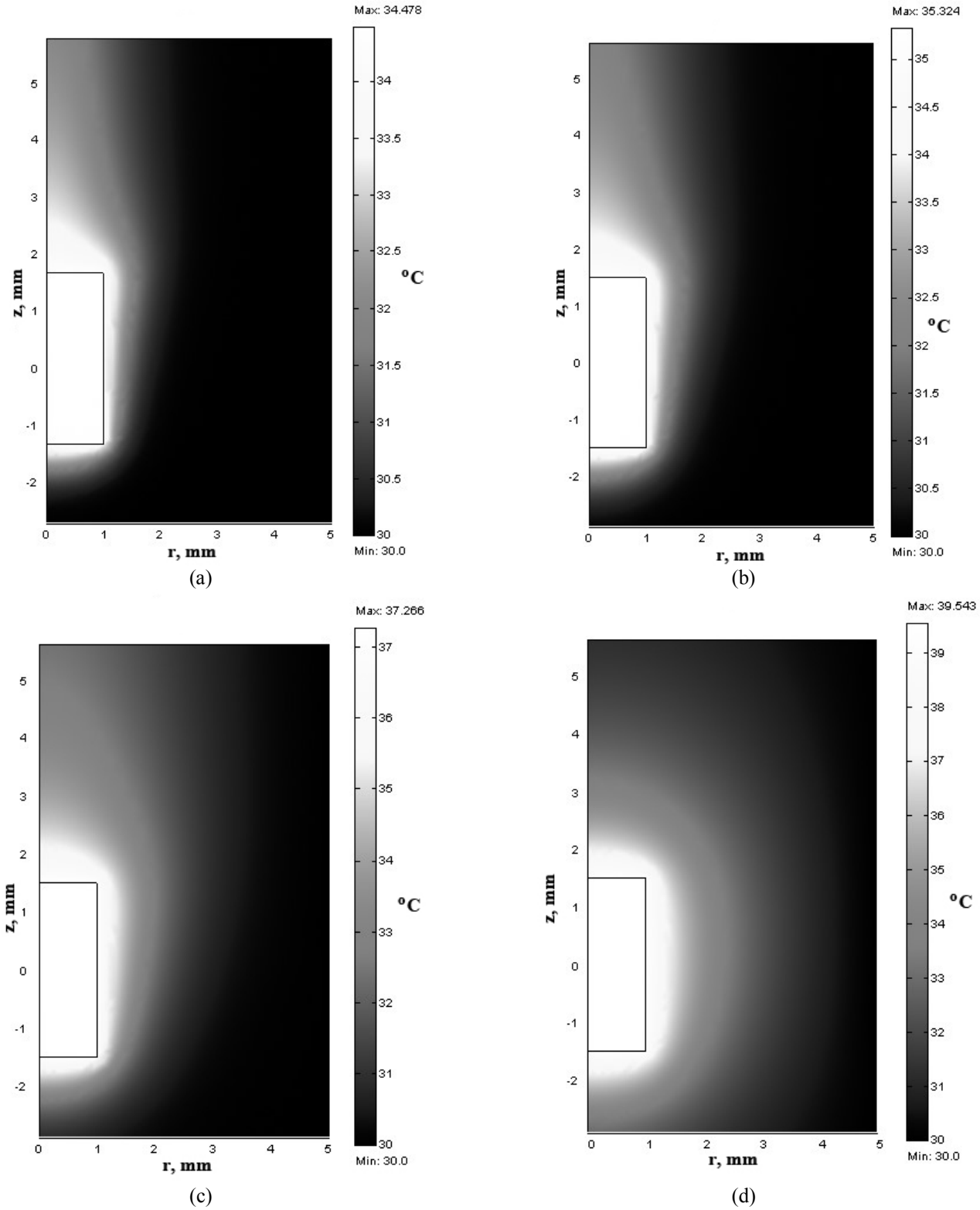


Fig. 2. Temperature distribution in milk with different apparent viscosities around the heated resistor. (a) $\mu = 1.5 \cdot 10^{-3}$ Pa·s, (b) $\mu = 1.0 \cdot 10^{-2}$ Pa·s, (c) $\mu = 1.0 \cdot 10^{-1}$ Pa·s, and (d) $\mu = 1.0$ Pa·s.

As is seen from the figure, the change in the temperature difference between the heated resistor and the main part of milk in an interval from $\Delta T \approx 4$°C to $\Delta T \approx 10$°C, which corresponds to the thermographic findings, occurs as the fluid viscosity changes by about 1000 times.

Obviously, in this case we are speaking about a certain effective viscosity of milk, which is an analog of the apparent viscosity, recorded with a rotary viscometer. Figure 3 shows the dependence of the thermographic temperature difference on the effective viscosity of milk, derived from the results of numeric simulation.

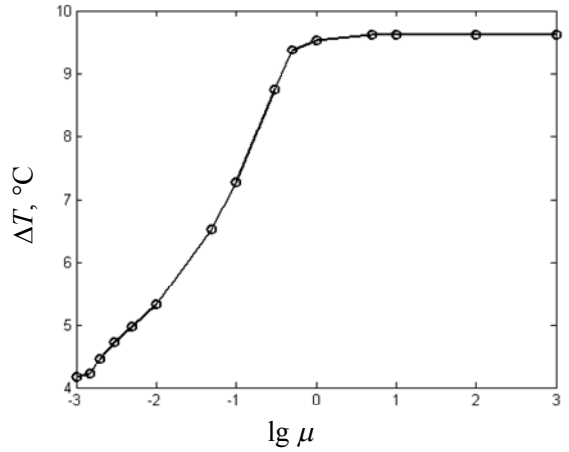


Fig. 3. Dependences of thermographic temperature difference on log of apparent milk viscosity as a result of numeric simulations.

The real dynamic viscosity of milk changes only by several times at the initial stage of flocculation, after which the main resistance to the convective flow comes from the hydrodynamic interaction of the fluid with the forming gel structure. Therefore, the analysis of the numeric estimation data may become the basis for the quantitative study of the forming structure of the milk clot. Roughly speaking, a change in effective viscosity by three orders of magnitude, according to Poiseuille's law for the flow of a viscous fluid through a capillary tube, should correspond to a decrease in the mean pore diameter in the clot by about 5–6 times.

Let us assess the relationship between the permeability of the porous structure of the milk gel and the thermographic temperature difference using Darcy's law:

$$U = \frac{k}{\mu}\operatorname{grad} p \,. \qquad (4)$$

where U is the convective flow's velocity and k is the coefficient of permeability of the medium.

After the formation of a structure in milk, we may ignore the hydrodynamic part of the pressure owing to the practical absence of flows. Then the fluid at depth z amounts to $p = -\rho g z$. Consequently:

$$\operatorname{grad} p = \frac{\partial p}{\partial z} = g\frac{\partial(\rho z)}{\partial z} = g\left(z\frac{\partial \rho}{\partial z} + \rho\right) = g\left(z\frac{\partial \rho}{\partial T}\frac{\partial T}{\partial z} + \rho\right). \qquad (5)$$

As was noted above, the milk density depends on the local temperature according to the law: $\rho = \rho_0(1 - \beta\Delta T)$, then:

$$\frac{\partial \rho}{\partial T} = -\rho_0\beta \,. \qquad (6)$$

Expression (5) with account for (6) will look as follows:

$$\frac{\partial p}{\partial z} = g\left(\rho - z\rho_0\beta\frac{\partial T}{\partial z}\right). \qquad (7)$$

The first summand in (7) represents the density of the pressure force that equalizes the gravity force in a stationary fluid, and the second represents a component of the density of forces that cause the convective transfer of the fluid owing to the temperature difference. It follows from expression (7) that the mean density of these forces is $\left\langle\frac{\partial p}{\partial z}\right\rangle = \rho_0 g\beta\Delta T$.

Taking into account all the transformations, expression (4) will take the form:

$$\langle U\rangle = \frac{k}{\mu}\left\langle\frac{\partial p}{\partial z}\right\rangle = \frac{k}{\mu}\rho_0 g\beta\Delta T \,. \qquad (8)$$

where $\langle U\rangle$ is the mean velocity of the flow.

Expression (8) determines the relationship between the coefficient of permeability and the thermographic temperature difference:

$$k = \frac{\langle U\rangle\mu}{\rho_0 g\beta\Delta T} \,. \qquad (9)$$

The analysis of velocity fields derived from model calculations at various viscosities allowed us to obtain a dependence of the mean flow velocity, $\langle U\rangle$, on the temperature difference (Fig. 4).

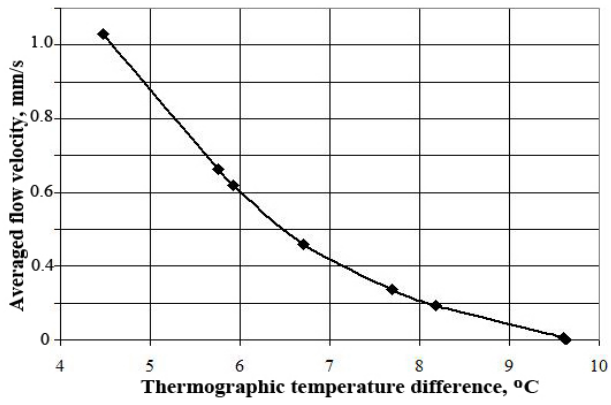


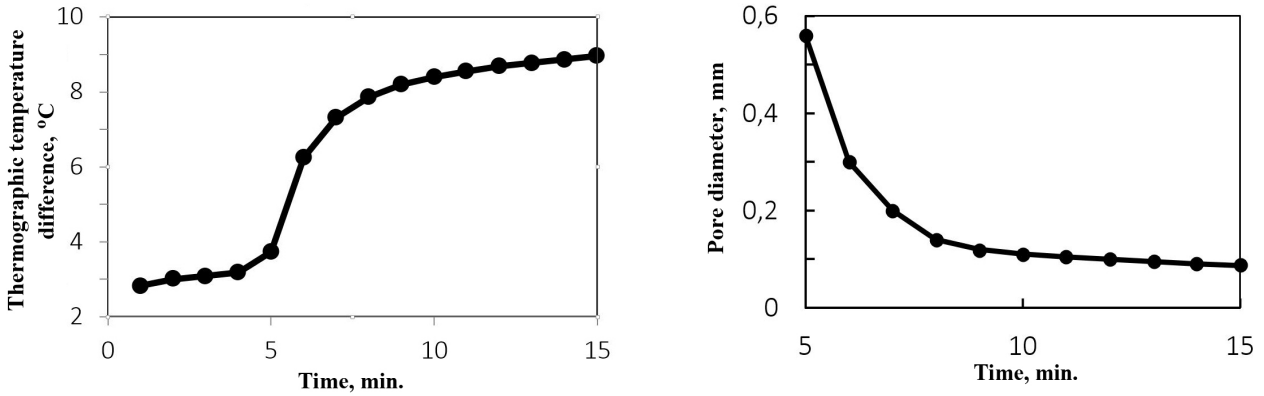
Fig. 4. Dependences of averaged flow velocity on thermographic temperature difference as a result of numeric simulations.

Using formula (9) and the diagram in Fig. 4, we can establish a relationship between the coefficient of permeability and the thermographic temperature difference for the chosen thermogram.

Experimental results

Fig. 5a shows a thermogram of the rennet clotting of milk, and Fig. 5b shows the result of its processing in accordance with the procedure described above. When plotting dependence 5b, we assumed that the coefficient of permeability was related to the mean size of pores formed during coagulation by the simplest dependence $k \approx \delta^2$, where δ is the pore diameter. As is seen from the figures, the mean pore size in the milk gel decreases from about 0.6 mm at the very beginning of clotting to about 0.1 mm after the actual completion of structure formation.

Figure 6 shows thermograms of the rennet clotting of reconstituted fat-free milk into which different amounts of calcium chloride were added. As was expected, the introduction of soluble calcium into milk has reduced significantly the duration of the inductive stage of coagulation. The experimental findings have served as the basis for the development of a phenomenological model of milk coagulation, which explains the role of calcium in this process [14].



(a) (b)

Fig. 5. A thermogram of reconstituted skim milk rennet coagulation (a) and (b) estimation of mean pore diameter in this process.

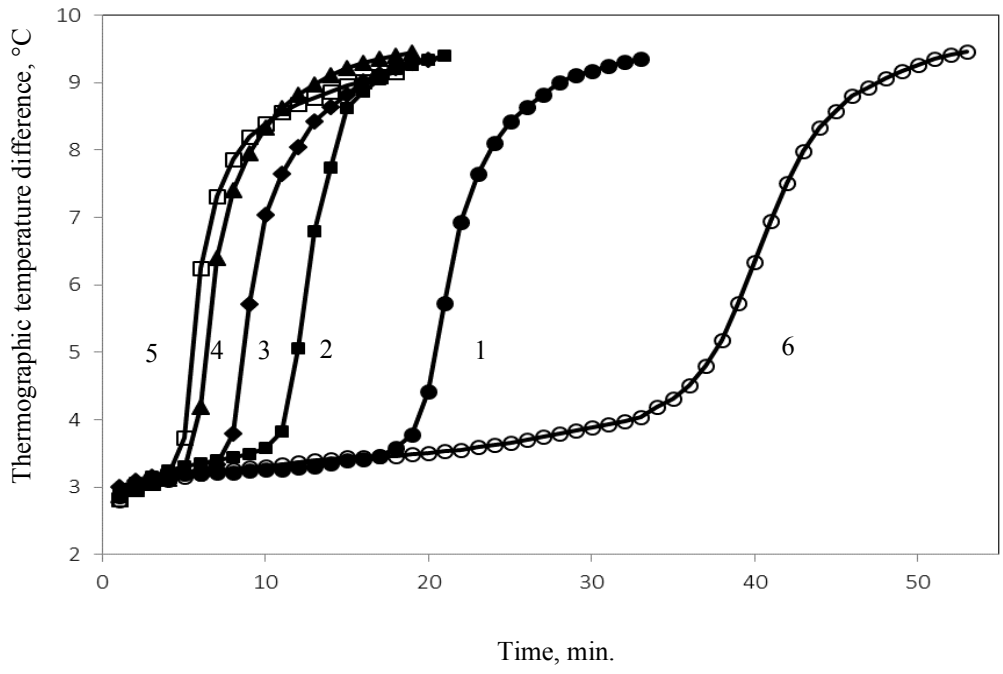


Fig. 6. Thermograms of reconstituted skim milk coagulation by chymosin (25 mg/dm^3) at 30°C with different addition of $CaCl_2$.
Curves: (1) addition of 0.4 g of $CaCl_2$ per 1 dm^3 of milk;
(2) addition of 0.8 g of $CaCl_2$ per 1 dm^3 of milk;
(3) addition of 1.2 g of $CaCl_2$ per 1 dm^3 of milk;
(4) addition of 1.6 g of $CaCl_2$ per 1 dm^3 of milk;
(5) addition of 2.0 g of $CaCl_2$ per 1 dm^3 of milk;
(6) no $CaCl_2$ added to milk.

The data in Fig. 7 exemplifies the application of the thermographic method to processes whose technology is associated with changes in milk temperature, demonstrating the dependence of clotting temperature on the initial milk acidity. Unlike standard thermograms, where the abscissa axis reflects the clotting time, in Fig. 2 the milk temperature is plotted along the abscissa axis. A sharp increase in the thermographic temperature difference corresponds to structure formation, as in a standard case.

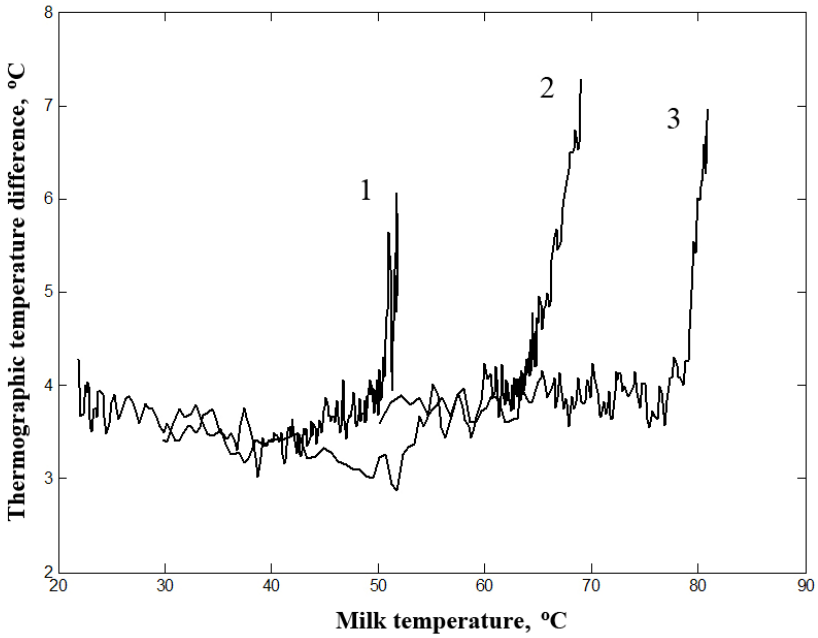


Fig. 7. Dependences of thermographic temperature difference on milk temperature for milk samples with different initial acidity: (1) pH = 5.6, (2) pH = 5.9, and (3) pH = 6.2.

The study of heat–acid heat-calcium milk coagulation by the thermographic method underlay the recently published physicochemical model of these processes, explaining their similarities and differences [15].

CONCLUSION

Thus, we have developed the thermographic method, which combines the simplicity of the hot-wire method and, in addition, allows milk coagulation monitoring even during the production processes related to changes in milk temperature. Hydrodynamic and heat processes that take place in the vicinity of the heated thermometer have been modeled, and the principal possibility to analyze the forming structure of the milk clot has been demonstrated on the basis of the thermographic method. Note that the thermographic sensors that we have developed can easily be combined with other sensors of milk technological parameters, providing thus the possibility of complex monitoring of the cheese production process, as well as the production of other dairy products at the milk coagulation stage both during laboratory research and directly during online production processes.

REFERENCES

1. Fox, P.F., Guinee, T.P., Cogan, T.M., and McSweeney, P.L.H., *Fundamentals of Cheese Science* (Aspen, 2000), 639 p.
2. Holter, H., Über die Labwirkung, *Biochemische Zeitschrift*, 1932. V. 255. P. 160.
3. O'Callaghan, D.J., O'Donnell, C.P., and Payne, F.A., A comparison of on-line techniques for determination of curd setting time using cheesemilks under different rates of coagulation, *Journ. Food Eng.*, 1999. V. 41(1). P. 43.
4. O'Callaghan, D.J., O'Donnell, C.P., and Payne, F.A., Review of systems for monitoring curd setting during cheesemaking. *Int. Journ. Dairy Technol.*, 2002. V. 55(2). P. 65.
5. Lucey, J.A., Formation and physical properties of milk protein gels, *Journ. Dairy Sci.*, 2002. V. 85, P. 281.
6. Klandar, A.H., Lagaude, A., and Chevalier-Lucia, D., Assessment of the rennet coagulation of skim milk: A comparison of methods, *Int. Dairy Journ.*, 2007. V. 17(10). P. 1151.
7. Sandra, S., Cooper, C., Alexander, M., and Corredig, M., Coagulation properties of ultrafiltered milk retentates measured using rheology and diffusing wave spectroscopy, *Food Res. Int.*, 2011. V. 44(4). P. 951.
8. Cipolat-Gotet, C., Cecchinato, A., De Marchi, M., Penasa, M., and Bittante, G., Comparison between mechanical and near-infrared methods for assessing coagulation properties of bovine milk, *Journ. Dairy Sci.*, 2012. V. 95(11). P 6806
9. Hori, T., Objective measurements of the process of curd formation during rennet treatment of milks by the hot wire method, *Journ. Food Sci.*, 1985, V. 50. P. 911.

10. O'Callaghan, D.J., Mulholland, E.P., Duffy, A.P., O'Donnell, C.P., and Payne, F.A., Evaluation of hot wire and optical sensors for on-line monitoring of curd firmness during milk coagulation, *Irish Journ. Agric. Food Res.*, 2001. V. 40(2). P. 227.
11. Sbodio, O.A. and Revelli, G.R., Milk's coagulation. Development of a device for online "monitoring" of the process. Progress made in Argentina. *Revista de Investigaciones Agropecuarias* (Agricultural Research Journal), 2012. V. 38(3) (published online: http://ria.inta.gov.ar/english/wp-content/uploads/2013/06/By-Sbodio-ingles-4.pdf)
12. Upreti, P., Mistry, V.V., and Acharya, M.R., Characterization of rennet coagulation of milk concentrated by vacuum condensing and ultrafiltration, *Dairy Sci. Technol.*, 2011. V. 91(3). P. 383.
13. Gebhart, B., Jaluria, Yo., Mahajan, R.L., and Sammakia, B., *Buoyancy-Induced Flows and Transport* (Hemisphere, New York, 1988), 971 p.
14. Osintsev, A.M., Gromov, E.S., and Braginsky, V.I., A phenomenological model of milk coagulation, *Foods Raw Mater.,* 2013. V. 1(1). P. 11.
15. Osintsev, A.M., Braginsky, V.I., Chebotarev, A.L., Osintseva, M.A., and Syrtseva, A.P., Investigation of heat-acid milk coagulation by the thermographic method. *Tekhn. tekhnol. pishchevykh proizvodstv* (Food Processing: Techniques and Technology), 2013. № 4. P. 69.

SYSTEMIC REGULARITIES IN THE STUDY AND DESIGN OF TECHNOLOGICAL COMPLEXES FOR THE PRODUCTION OF INSTANT BEVERAGES

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Abstract: This article is devoted to the state-of-the-art systemic approach to the analysis and synthesis of process flows for the production of instant polydisperse granular functional beverages. The distinctive feature of these studies is the methodological approach developed by Academician V.A. Panfilov, representing a quantitative description of the integrity level of a large production process in a technological complex, based on the results of its diagnostics and comprising sequential transition in studies from a system of technologies to a system of processes and form a system of processes to a system of apparatuses and machines. The definition of a technological system as an interrelated whole creates a certain logic and methodology of its qualitative and quantitative study and develops a system-centered opinion on production.

Keywords: process flow, granular, instant, disperse, system, analysis, synthesis

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INTRODUCTION

Extensive materials accumulated as a result of studies on the production of instant food products currently represent a totality of specific solutions (mainly, empirical) and do not give an insight into the shaping theoretical basics of instantization techniques, economic expediency, and energy consumption, which insistently require systematization and generalization. With all the diversity of technologies for the production of instant, quick-dispersing, and quick-swelling beverages, which are called in a word *instant* in foreign literature, there is neither a single classification nor a single approach to the formation of such production technologies and processes.

Technological flows in the production of dry granular beverages cannot be viewed either as a sum of known individual technologies, the dry concentrate technology, and the granulation and drying technology or as a sum of individual physicochemical phenomena and processes. Each influences both directly and indirectly the process of the formation of a polydisperse multicomponent system with properties of an instant product.

The definition of a technological system as an interrelated whole creates a certain logic and methodology of its qualitative and quantitative studies and develops a system-centered opinion on production. We may say that the technological system actively influences its components and transforms them.

In the real conditions of interaction between these two systems, technological and disperse, it is obvious that they should be considered as a complex, taking into account their integrated essence, optimizing production, and taking it to a totally different level. Without learning the essence of phenomena, it is impossible to create a new whole.

Building a model of fast-prepared beverages also determines the choice of process-flow equipment. In the theory of systems, the making of the most rational decisions and the optimization of system control in the broadest meaning of this term have led to the appearance within system analysis of a section on decision making in the conditions of the so-called unique choice [1, 2, 5, 7, 9].

The unique-choice situation is characterized by three necessary elements: a problem to be resolved, a designer of a technology or a process flow who makes decisions, and a few alternatives from which to choose.

THE PRINCIPLES OF SYSTEMATIZATION OF INSTANT-PRODUCT TECHNOLOGIES

Literary sources [3, 5, 8] give us a number of instant-product technologies. Let us consider the main principles of their systematization. As a rule, decisions in frequently recurring situations also recur and are transferred, proceeding from the similarity criterion, to similar problems. Obviously, these are complex, nonstandard, and unique in their own way situations that deserve special attention of process-flow designers. In addition, we should bear in mind a number of specific features of the principles of systematization of instant-product technologies.

Usually, we fail to assess fully every proposed alternative by one numerical criterion, for example, by porosity or by solubility. However, when making a multicriterion assessment of each alternative, we face two problems:

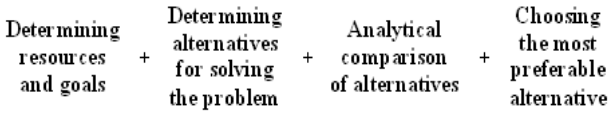
– whether we have taken into account all material indicators (completeness of the indicator list) and

– methodological difficulties when we simultaneously compare various criteria, for example, the native and

gustative properties of a product, as well as its porosity, solubility, wettability, and strength (the dimensionality rule).

The subjectivity of the quality assessments of alternatives is obvious even in assessments by one criterion, all the more so in multicriterion cases. Such difficulties make solving the problem of the optimal choice anything but simple. A way out of this situation was the creation of expert panels, for example, a commission for the assessment of the organoleptic properties of food products. The problem of choice becomes simpler when we have a large number of publications and patents systematized by certain technical indicators, which allow the developers of process flows to improve to the maximum the level of problem structuralization by making it more transparent.

An increased degree of problem structuralization is a basic problem of system analysis. Let us consider what methods are used to solve problems that are reduced to the comparison of alternatives. The general algorithm of actions when solving the problem of the unique choice according to [4, 9] represents the following scheme:



The first two stages largely depend on problem specifics. At the current level of system engineering, they should, already at the stage of establishing a scientific rationale, engineering-and-economic solutions, and the terms of reference, take into account the regularities of process-flow development, the need for production modernization and reprofiling, the possible change in the amount of raw materials, the personnel qualifications, etc. If we do not consider the above factors, this may lead to the inanity of implementing the project itself already in the near future. The role of exploratory design, both scientific–engineering and environmental, has increased greatly.

Moreover, the more complex the structure of a newly designed process flow, the more operations and relationships it has, and the more efforts are needed to organize its normal functioning. A newly created object (process flow) will act according to new laws [4, 6, 8, 9]. Therefore, a system of machines (processing line) should be built with regard to the regularities of the system of processes of a specific technology.

Food production is a complex of integral systems. When analyzing one of the integral systems (a process system), the subject of study becomes, primarily, its structure, the laws of combining parts into a single whole, and its integrative regularities. When analyzing a system complex, the subject matter is the relatedness of two or several objects–systems that form a polysystem complex.

Thus, the methodological cycle of creating a highly efficient process line should be as follows: *"from a process flow to a system of processes and from a system of processes to a system of machines*." The most science-intensive notion in this methodological cycle is the system of processes [6, 7].

The work of a scientist or an engineer on a project is reduced to two large stages: the systemic consideration of a problem and system design.

1. **Systemic consideration of a problem**.

– the preliminary formation of a problem,
– the systemic study of the problem,
– the definition of the designed object, and
– the definition of the facility's monitored parameters and limitations to its general characteristics.

2. **System design**.

– The modeling of the designed product,
– finding the best structure and optimizing the object's internal characteristics,
– checking the controllability of the object formation process,
– developing technical requirements on object-forming components, and
– determining the order of technical flow formation by production stages of the end product.

In this case, the problem may be formulated as "How to obtain a dry instant food product that preserves its native properties, has a good looseness and extended storage life, and, at the same time, represents a simple processing technique**."**

The problem cannot always be formulated simply at the start; all the more so, we do not have an answer about the preferable options of its solution at once. The right formulation of a problem is half the battle.

Complex studies include a general overview of similar processes of polydisperse systems in other industries, such as the metallurgical, chemical, agricultural, and food industries [8]. Only after performing all the above studies, it is possible to finally specify the problem, taking into account the possible development of structure formation in disperse systems. Then it is time to state the goals the implementation of which will help resolve the problem of the creation of instant beverages.

At present the main production industries, including the food industry, feel increasingly sharply the need for a systemic approach to the creation of state-of-the-art technological complexes. Most authors of articles, monographs, and textbooks who realize this need see its cause in the fact that, as state-of-the-art technological systems are created, their traditional consideration without using systemic insights already does not allow the adequate accounting for the range of emerging interactions, the nature of the integral properties of a system, the possible anomalous modes and functional side effects, etc. This article focuses on the fact that, **at the current level of complexity of technological systems, their functionality and development are more and more affected by general systemic regularities.** Therefore, the current stage needs transition from moderately elementary and structure-centered versions of the systemic approach, which focus on the study of relationships, interactions, and other structural characteristics, to more developed concepts of systemacity based on the identification of systemic regularities that characterize the structure, dynamics, and organization of a complex object in

their integrity. Let us consider the nature of general systemic regularities in their application to technological complexes, relying on theoretical–systemic ideas [1, 2, 7].

According to these ideas, a system is considered as an organized unity whose stability, functionality, and development are based on resolving topical contradictions (problems) in intended environmental conditions. This definition sets the coordinates for the constructive understanding of systemacity, in terms of which the main system-constituting principle is not structure, relation forms, interaction types. etc., but primarily the nature of topical contradictions (problems), the resolution of which allows a system to function and develop. In addition, structure types, methods of operation, forms of interaction with the environment, and other systemic characteristics depend on the logic of the resolution of topical problems, which are primary in systemic studies and predetermine all other systemic parameters.

The main law of complex systems, which expresses their essential specifics, is the law of focused action. The essence of this law is that, in order to resolve topical contradictions (problems), a system acts as a focusing lens: it concentrates the potential of its components, relationships, actions, and resources on the attainment of functional results that resolve these contradictions. The higher the focus of system parameters on the attainment of functional results, the higher its effect is and the better its topical problems are resolved. The good organization of a system differs from the bad one in a higher focus of the system parameters (goals, structures, operating methods, forms of control, etc.) on attaining functional results. Obviously, all other systemic phenomena and regularities should be considered through the prism of the above law, which reflects the basic mechanism of the systemic operation of complex objects.

When assessing or designing technological complexes, the law of focused action is, primarily, oriented at the sequential analysis of the degree of the focus of system parameters (from goals (objectives) to the functional properties of an object and from them to its structure, dynamics, organizational and control forms, and its interaction with the environment). Violation of the focus of the operations of a complex or even the detection of factors of dysfunctional focus can manifest themselves at the level of any of the above parameters. For example, at the structural level, during analysis it is advisable to look at the possible existence of latent dysfunctional structures, formed by subsystem interfaces, communication networks, auxiliary equipment, etc., in the system along with the explicit and purposefully designed structure. Ideally, this analysis should be aimed at achieving the functional unity of operations of the explicit (functional) and latent structures.

In the light of the law of focused action, traditional and seemingly well-known requirements of the systemic approach acquire a substantially new meaning. For example, the well-known requirement of the comprehensiveness of an approach to an object is transformed into the principle of **the combination of the comprehensiveness of studies with the focus of its results on the object's functional characteristics.** This transformation is necessary because an object may have many sides, aspects, and facets, many of which are not topical in terms of problems being resolved. In addition, considering many sides without focusing the analysis on functional characteristics leads not to a systemic but to a summative ("mosaic") picture of the object. Thus, only the combination of comprehensiveness and functionality in consideration yields the final picture that corresponds to the requirements of a systemic representation of an object.

The law of focused action is closely related to the law of functional complementarity, which is also a central provision of the general theory of systems. The essence of the **law of functional complementarity** is that an integral system, unlike a systemless conglomerate, is characterized by complementarity of the functional properties of its elements [2, 10]. In higher systems, the complementarity of properties of elements manifests itself in the fact that they mutually support one another during the process of functioning and contribute to the restoration of defective elements, extending the range of complementary properties, etc**. The law of functional complementarity reflects the structural mechanism of the focused operation of a system: the functional complementarity of the properties of elements is the necessary condition for their functionally focused actions.** If we take into account the law of functional complementarity, this will allow us to purposefully design elements of technological complexes, securing a division of processes, properties, and functional modes that will contribute to the complementarity of their properties and, consequently, to the integrity and functional efficiency of a complex. For example, an important aspect of achieving the functional complementarity of the elements of a complex is to ensure their relative equifunctionality. This requirement is associated with the so-called **"law of the least,"** established by A.A. Bogdanov in his *Tectology* [1]. According to this law, the stability (functionality) of a whole is limited by the stability (functionality) of its weakest link. It follows from this law that a significant condition of system optimality is the relative equifunctionality of its elements, the absence of both the "weak" links, which restrict the general functionality (productivity) of a complex, and the excessively "strong" links, whose potential cannot be used fully due to restrictions on behalf of other, functionally weaker, elements

An important regularity of complex systems is **the unequal influence of various elements on the general condition of a system, the results of its functioning, and the way of its development.** Methodologically, this regularity entails the **principle of isolating the main (leading) links and determining their system-integrating relationships and functions in a system.** Since the main functional processes and the main contradictions of a complex are concentrated in the leading links, it is advisable to begin configuration analysis with these links and their integral relationships, which creates the possibility of a more substantiated approach to the study of other, less

important, elements of the complex [2, 10]. The identification of the leading ("central" or "backbone") subsystems makes it possible to anticipate their possible functional effects on other subsystems in designing a complex, as well as to predict developmental options for a technological complex as a whole, since possible transformations of these subsystems predetermine the ways of transition to technological systems of qualitatively different types and levels. In characterizing the main links of a system, it is important to pay attention not only to the "leading," but also to the "mass," links, i.e., repeated uniform elements or processes. Even an insignificant improvement of such elements can noticeably upgrade the characteristics of a system as a whole by multiple accumulations of small effects.

A number of significant methodological consequences are predetermined by the law of hierarchy of complex systems. The **law of hierarchy** means that any object (phenomenon) under study has both superior, suprasystemic, and subordinate, subsystemic, metalevels, which are materially related to this phenomenon and which largely determine its nature and quality. For example, if a process flow is the object under study, then the ambient suprasystem for this object will be a workshop or a factory and the subsystems will be complexes, aggregates, machines, apparatuses, tools, mechanisms, implements, and parts. **Methodologically, the hierarchical multidimensionality, typical of systemic objects, and coherence require studies not only at the level of these objects but also at the level where they are affected by both the ambient metasystems and the microcharacteristics of their subsystems** [2, 9]. The consideration of an object with respect to influences exerted on it by its suprasystemic levels and the properties of its subsystems leads to the synthesis of split-level pictures into a multidimensional and volumetric representation, much more manifold and much deeper than the one that we have when we consider this object only at its own level. As we design a specific technological system, the principle of hierarchy makes us consider this system not only with a view to its specifics and objectives but also in the light of the history of designing technological systems in a given industry in general or even in the light of the experience accumulated by the technologically most advanced industries. For example, we know the practices of modernizing several civil industries, where the solutions to numerous technical problems, chronic and formidable for those particular industries, were successfully found by transferring technological experiences accumulated by the military-industrial complex.

The consideration of a technological system from the subsystem level implies taking into account the possible influence of subsystems on the nature of the system's functioning in general, as subsystems that operate in various technological modes; use various raw materials, technological ingredients, and materials; and function under various design options of the subsystems. Such consideration may reveal effects and phenomena predetermined by the nature of the system itself, which often fall out of the sight of designers if they consider and design a technological system within its narrow niche.

The consideration of a technological system within its own scale from a position of the system approach also acquires multidimensionality. The **system approach implies comparison of a system under consideration with similar systems of the same order: competing systems, alternative systems, and systems of the same or congenial type.** Such comparison allows us to take into account the experience of various design approaches, creates opportunities for their fruitful synthesis, and reveals criteria and forms of design thinking beyond the grasp of the "object-oriented" approach. Thus, if we take the principle of hierarchy into account, this allows us to transfer from unidimensional, object-oriented, thinking to multidimensional, system-oriented, thinking and to reach an incomparably deeper level of insight into the nature and essential basics of an object, letting us select the most efficient and reliable solutions.

The dialectical understanding of a complex system implies its consideration as a controversial integrity. Such a vision is not just a possible aspect of thinking; it has a real objective nature, characterized by the **law of contradictory integrity**. The essence of this law is that any complex system has both the system-integrating factors, which ensure the consistency, integrity, and functionality of a given system, and the opposing, system-destroying and disintegrating, factors. The second group of factors very rarely becomes the object of analysis during the design of technological systems, being often initiated as a result of emergencies and engineering disasters. An especially important aspect of analysis of system-destroying factors is associated with their possible transfer from the mode of disconnected operation to the mode of coherent and systemic operation. This particular evolution of system-destroying factors often leads to breakdowns, destructions, and shutdowns of technological complexes or to a sharp drop in the quality of their functioning. Thus, the **consideration of a technological complex as the opposition of system-integrating and system-destroying factors that operate within it and the revealing of their correlation, possible forms, and prospects for synergy and the coherent effect of the destroying factors, are important principles of the system approach,** which is very topical during the creation of state-of-the-art equipment and technologies.

Another relevant consequence of the law of contradictory integrity is the **need to reveal the critical boundaries of change in an object's functional parameters within integrity.** The search for such critical boundaries is currently a major problem for many specific sciences of complex systems. The study of critical boundaries and modes that change functional parameters is also very topical for the creation of state-of-the-art complex technological systems in terms of their reliability, protection, fail safety, and high-quality operation.

Thus, even a very brief and schematic overview of systemic regularities in the light of their possible uses

during the study and design of state-of-the-art technological systems shows that the consideration of these regularities is becoming a necessary condition for the creation of new-generation technological systems. The development of engineering and technology has reached a line where the creation of qualitatively new technological systems without using systemic theory and methodology is becoming increasingly ineffective and hazardous. This means that theoretical and systemic knowledge is becoming a component that is no less important for current engineering education than basic technical knowledge.

REFERENCES

1. Bogdanov, A.A., *Vseobshchaya organizatsionnaya nauka (tektologiya)* (General Organizational Science (Tectology)), 3rd ed., Part 3 (Ekonomika. Moscow, 1989), 270 p.
2. Vinograi, E.G., *Obshchaya teoriya organizatsii i sistemno-organizatsionnyi podkhod* (General Theory of Organization and the Systemic–Organizational Approach) (Izd. TGU, Tomsk, 1989), 236 p.
3. Dernei, I., *Proizvodstvo bystrorastvorimykh produktov* (Production of Instant Products) (Legkaya i Pishchevaya Prom–st', Moscow. 1983), 184 p.
4. Kafarov, V.V., Dorokhov, I.N., and Arutyunov, S.Yu., *Sistemnyi analiz protsessov khimicheskoi tekhnologii* (System Analysis of Chemical Processes) (Nauka, Moscow, 1985), 440 p.
5. Lipatov, N.N., Jr., Predposylki komp'yuternogo proektirovaniya produktov i ratsionov pitaniya s zadavaemoi pishchevoi tsennost'yu (Preconditions for computer design of food products and rations with preset nutrition values), *Khranenie Pererabotka Sel'khozsyr'ya (*Storage Process. Agric. Raw Mater.), 1995. № 3. P. 4.
6. Panfilov, V.A., *Tekhnologicheskie linii pishchevykh proizvotstv. (Teoriya tekhnologicheskogo potoka)* (Processing Lines for Food Production. (Theory of Process Flows)) (Kolos, Moscow, 1993), 288 p.
7. Peregudov, F.I. and Tarasenko, F.P., *Vvedenie v sistemnyi analiz* (Introduction to System Analysis) (Vyssh. Shkola, Moscow, 1989), 367 p.
8. Popov, A.M., Romanenko, R.Yu., Miller, E.S., Donya, D.V., and Popov, A.A., Osnovnye napravleniya formirovaniya struktur v dispersnykh sustemakh (Major Trends in Structure Formation in Disperse Systems), *Tekhnika Tekhnologiya Pishchevykh Proizvodstv* (Food Processing: Techniques and Technology), 2013. № 4 (31). P. 118.
9. Popov, A.M. and Panfilov, V.A., Sistemnye zakonomernosti slozhnykh ob''ektov i printsipy ikh ispol'zovaniya pri proektirovanii tekhniko-tekhnologicheskikh kompleksov (Systemic regularities of complex objects and the principles of their use in the design of technological complexes), *Khranenie Pererabotka Sel'khozsyr'ya (*Storage Process. Agric. Raw Mater.), 2005. № 10. P. 58.
10. Khomyakov, D.M. and Khomyakov, P.M., *Osnovy sistemnogo analiza* (Fundamentals of System Analysis) (MGU, Moscow, 1996), 108 p.

BIOSENSORS: DESIGN, CLASSIFICATION, AND APPLICATIONS IN THE FOOD INDUSTRY

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Abstract: Biosensors are promising analytical tools applicable in clinical diagnostics, food industry, environmental monitoring, and other areas in which rapid and reliable analyses are needed. This review covers the basic types of biosensors and their designs and general operating principles. A classification of biosensors according to the type of transducer they involve and according to the nature of the biological entity used as the recognition element is presented. Methods of immobilization of biological components, namely, adsorption, microencapsulation, inclusion, cross-linking, and covalent binding are briefly characterized. The main areas of application of biosensors in the food industry—food safety and quality assessment, process monitoring, and others—are considered.

Keywords: biosensor, transducer, immobilization, foods

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INTRODUCTION

The issue of food safety and quality is becoming increasingly challenging. This is due to the fact that hazardous foods that can contain toxic substances, including those of anthropogenic origin, appear on the market. Environmental pollution caused by sewage from industrial and agricultural enterprises, which contains residual pesticides, fertilizers, growth stimulants, and other harmful substances, leads to accumulation of toxicants in fish, meat, and dairy foods. In addition, in the 1980s some countries began extensive studies in the design of genetically modified organisms (GMOs) intended for the production of foods, animal feeds, and pharmaceutical and veterinary preparations. This highlights the need for extensive supervision aimed at revealing the strains that were created in violation of safety principles and at checking whether a product obtained using a GMO is identical to its natural prototype.

In view of the aforesaid, increasing attention is being focused on development of sufficiently sensitive and selective express analysis methods. Prominent examples of analytical systems combining all of the above merits are biosensors [1–4].

GENERAL OPERATING PRINCIPLES AND DESIGN OF BIOSENSORS

Biosensors are analytical devices that employ sensitive biological materials to "recognize" certain molecules and provide information on their presence and amount as a signal convenient for recording and processing [4–10].

Any biosensor consists of the following three basic components: recognition element, which is a biose-lective membrane involving various biological structures; physical transducer; electronic system for signal amplification and recording and for user-friendly data representation [4, 7–17]. Figure 1 presents a general scheme of a biosensor.

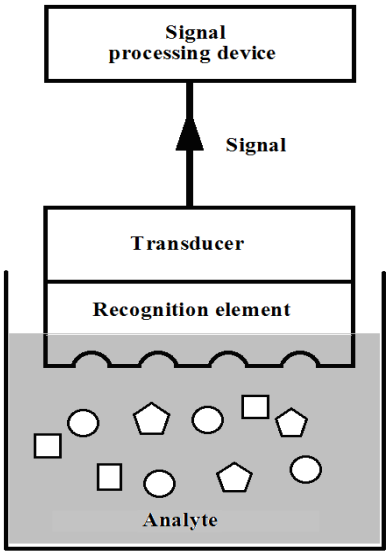

Fig. 1. General scheme of a biosensor.

A recognition element is the basic component of any sensor. It is due to its recognition element that a sensor can selectively respond to one or several analytes among a large number of other substances. All types of biological structures—enzymes, antibodies, receptors, nucleic acids, and even living cells—are used as a recognition element in biosensors.

A transducer converts the changes caused by the reaction between the selective biological layer and the analyte into an electric or optical signal. This signal is then measured using a light-sensitive and/or electronic device.

CLASSIFICATION OF BIOSENSORS

Biosensors are classified according to transducer type or according to the nature of their biological component. The following four basic transducer types can be distinguished [4, 7–10].

Electrochemical Transducers

– Potentiometric transducers. The analytical signal in this case is the potential drop between the working electrode and the reference electrode or between two reference electrodes separated by a semipermeable membrane (at a zero current through the electrochemical cell). An ion-selective electrode (ISE) usually serves as the transducer. The most widespread potentiometric biosensors employ pH electrodes.

– Voltammetric transducers. Here, the electroactive species oxidation or reduction current is measured. The latter is induced by producing the preset potential drop between the electrodes. In most cases, a constant potential vs. the reference electrode is applied to the working electrode (or a bundle of electrodes). The observed current is proportional either to the volume concentration of electroactive species or to the rate of their disappearance or formation in the biocatalytic layer [4].

– Conductometric transducers. These transducers measure the electrical conductivity of the solution in the course of a biochemical reaction. They are less commonly used in biosensors, particularly when the recognition element is an enzyme. However, they should not be discounted in detection of affine interactions [4].

– Impedimetric transducers. These devices measure the impedance of an electrochemical cell and the variation of this impedance with ac frequency [9].

– Transducers based on field-effect transistors. The systems involving ion-sensitive silicon field-effect transistors are, in essence, conventional potentiometric systems, with the only difference that the input transistor of the electronic circuit of the high-resistance voltmeter is transposed into the solution being analyzed. This considerably enhances the resolving power of the transducer and thereby raises the sensitivity of the biosensor. The biosensitive layer is usually placed directly on the surface of an ion-sensitive membrane as part of the gate of the field-effect transistor [4]. Biologically modified, ion-selective, field-effect transistors provide means to directly determine small peptides and proteins as their characteristic charge [18].

A serious drawback of all potentiometric systems based on the above operating principles is their sensitivity to the buffer capacity of the solution, which markedly restricts their application.

Optical Transducers

Optical transducers may be based on absorption, fluorescence, luminescence, internal reflection, surface plasmon resonance, or light scattering spectroscopy. For example, an immunosensor based on localized surface plasmon resonance on gold nanoparticles has been developed for determining casein in milk [19].

Piezoelectric Devices

Piezoelectric sensors employ crystals that undergo elastic deformation under the action of an electric potential. An alternating potential at a certain frequency generates a standing wave in the crystal. Analyte adsorption on the surface of the crystal, which is covered with a biological recognition element, alters the resonance frequency, and this is an indication of binding taking place. Piezoelectric immunosensors are considered to be among the most sensitive sensors developed to date, for they are capable of detecting antigens in the picogram range (http://www.tms.org/pubs/journals/JOM/0010/Kumar/Kumar-0010.html).

Thermometric Transducers

Measuring the amount of heat with a sensitive thermistor provides means to determine the analyte concentration. Thermal biosensors are quite uncommon.

Any type of biochemical element can be combined with different transducers to obtain a wide variety of biosensors. Up to 80% of the biosensors are electrochemical ones; depending on the nature of their biological component, they are also referred to as enzyme electrodes, immunosensors, and DNA sensors [3, 5, 8, 15].

The recognition elements in biosensors are biological entities that can recognize a single substrate among a multitude of others. This requirement is met by four entities: enzymes, antibodies, nucleic acids, and receptors [8].

Enzymatic Sensors

These include pure enzyme preparations or biological preparations (tissue or microbial culture homogenates) showing a certain biological activity. The simplest enzymatic biosensor design is used when the substrate or the product of the enzymatic reaction is electrochemically active, capable of being rapidly and reversibly oxidized or reduced on an electrode upon the application of an appropriate potential. According to their functions, enzymatic sensors are subdivided into substrate and inhibitor ones. Substrate biosensors are intended for determination of specific substrates of enzymatic reactions. Examples are glucose determination using a glucose oxidase–based enzymatic sensor and urea determination using a urease sensor [20, 21]. Inhibitor sensors are intended for determination of substances reducing the activity of an enzyme. An example is the determination of organophosphorus pesticides inhibiting acetylcholine hydrolysis catalyzed by acetylcholinesterase [6, 79].

The most common enzymatic biosensors are glucose and urea biosensors [8].

Immunosensors

Immunoglobulins, which are protective proteins secreted by the immune system of an organism in response to the ingress of alien biological compounds (antigens), are employed in this case as the biochemical receptor. Immunoglobulins, also known as antibodies, form strong complexes with antigens. Immunosensors are used to detect the participants of immunochemical

interaction, namely, the antibodies and antigen. The presence of antibodies in blood is a diagnostic indication of infection or a toxic action of certain substances.

Antigens can be determined not only in biological liquids, but also in other media, including the natural environment. Provided that there are specific antibodies, immunosensors can detect practically any compound, showing a high specificity and selectivity.

DNA Sensors

The biochemical components of DNA sensors are nucleic acids (DNA). Most frequently, they are not natural components isolated from a living organism, but their fragments called DNA probes or DNA primers. They are selected so that they reflect the specificity of the DNA structure as a whole. DNA probes are synthesized by DNA amplification via a polymerase chain reaction. They can be additionally modified so as to enhance their stability or facilitate their introduction into a biosensor. Oligonucleotide sequences having no natural analogue, selected according to their capability to interact with certain biomolecules, are also used in DNA sensors. These synthetic nucleic receptors received the name of aptamers [23, 24]. Since present-day science is unable to predict the aptamer structure required for each particular ligand, one has to synthesize all possible oligonucleotides for obtaining an aptamer (imposing a reasonable limit of, e.g., 40–100 nucleotides) and then select those which bind most strongly to the target.

Another purpose of DNA sensors is to reveal proteins and nonmacromolecular compounds specifically interacting with certain DNA fragments. These objects include regulatory proteins, tumor markers damaging DNA, and many anti-cancer drugs. Aptamers are nearly as specific as antibodies and exceed them in stability. The aptamer-based DNA sensors are called aptasensors. DNA sensors are used to determine the nucleotide sequence in a target DNA molecule that is complementary to the probe. This provides means to reliably diagnose pathogenic microorganisms and viruses and to solve problems of fine genetic diagnostics [3, 15, 25]. Examples of the latter application are affiliation, detection of genetic disorders, and detection of products made from genetically modified organisms.

Microbial Biosensors

In most common microbial biosensors, the biological component is separated from the recording device. This is due to the fact that the response of microorganisms to variations in the chemical composition of the medium is rather sluggish compared to the response of enzymes or antibodies, because the former is mediated by matter transfer across a biomembrane. For this reason, it is necessary to create a higher concentration of living cells than is allowed by the geometry of the transducer. A microbial biosensor may be a columnar or membrane reactor or a suspension of microorganisms in a solution with an immersed sensor [8, 14]. The microorganisms employed in these sensors can execute various functions. They can convert the analyte using enzymes they secrete into the culture medium during their metabolism or enzymes remaining in their living cells. These sensors are similar to enzymatic sensors, with the only difference that a group of enzymes, not necessarily a single one, may be involved in the conversion of the substrate [14, 16]. The action of microorganisms is based on the fact that they change their respiratory activity as they assimilate organic substances. These microbial biosensors are called respiratory biosensors. They are used in the determination of the total amount of oxidizable organics in, e.g., wastewater [14, 26–28]. Respiratory microbial biosensors are also usable in the determination of antimicrobial agents suppressing microbial respiration.

Microbial oxidation reactions are low-selective, because, as distinct from individual enzymes, unicellular organisms can decompose various organic substances at similar rates. Genetic engineering makes it possible to design microorganisms producing certain enzymes whose activity can be measured in the same way as in the case of enzymatic sensors. This is how the stability of enzymes can be enhanced and their concentration can be increased in the case of low-stability proteins. The best known examples of these sensors include toxin determination systems based on the inhibition of luciferase, a microbial enzyme that generates luminescence during the oxidation of some substrates [14, 29–31].

Another area of application of microbial sensors is investigation of the effect of substances on a cell as a model of a multicellular organism. These biosensors are also employed in toxicological studies to estimate the median lethal concentration of toxicants and in the optimization of individual doses of antibiotics and the amounts of antimicrobial and antifungal additives for paints and finishing materials. Finally, microbial biosensors are used to estimate the condition of natural microorganism communities, for example, in monitoring the performance of biological wastewater treatment systems.

Biosensors Based on Supramolecular Structures of a Cell

These biosensors occupy an intermediate place between enzyme and DNA sensors, since they involve intracellular entities that have a fairly complex hierarchical structure. These entities include lipid membranes with built-in receptors, cell organelles (mitochondria and chloroplasts), polyenzyme complexes, etc. These biosensors have not found wide application yet, because their biological components, when isolated from their natural medium, are insufficiently stable to maintain the operating parameters of a sensor for a long time. They are used in the investigation of biochemical processes, for example, for verifying the mechanism of the toxic action of pollutants and for determining the pathway via which an action potential or another biochemical signal is transmitted from a cell. These biosensors include phytotoxicity sensors involving components of the photosynthetic apparatus of plant cells.

IMMOBILIZATION OF BIOLOGICAL COMPONENTS

For a biosensor to operate reliably, its biomaterial should be bound to the transducer surface. This operation is referred to as immobilization of a biological component.

Immobilization means bringing a biomaterial (enzymes, antibodies, nucleic acids, oligonucleotides) into insoluble form by incorporating it in an inert support or by chemically of physically binding it to the transducer surface.

There are five basic methods of immobilizing biomaterials [8–10].

1. Adsorption. This is the simplest method that does not need any substantial pretreatment of sensor components or use of special-purpose chemicals. Alumina, activated carbon, clay, cellulose, collodion, silica gel, glass, hydroxylapatite, and many other substances are known to adsorb enzymes without affecting their native conformation.

Both physical adsorption and chemical adsorption (chemisorption) are usable in this case. In physical adsorption, the biomaterial is held on the surface by Coulomb, van der Waals, or ionic interactions or hydrogen bonding. In the case of immobilization of cellular structures, the adsorptive binding of cells to the surface of a pretreated polymer can be so strong that the removal of the cells from the polymer surface causes their lysis.

The result of adsorptive immobilization is largely determined by the properties of the transducer surface, including its charge, the presence of polar groups, its redox potential, and its energetic uniformity. Adsorption does not afford a high concentration of a biological component. In order to increase the amount of biological component adsorbed, the transducer is pretreated so as to generate charged or polar groups enhancing biomaterial adsorption. This is done by using various methods of oxidation and surface modification with polymers or functionalizing reagents. For example, the oxidation of gold and carbon electrodes increases their protein, nucleic acid, and microorganism adsorption capacity.

A considerably stronger binding between the biomaterial and the support is provided by chemisorption yielding covalent bonds (see below). The recognition elements prepared by adsorption are very sensitive to pH, temperature, ionic strength, and substrate concentration variations.

Adsorption is mainly used at the research stage, when even a weak binding between the biological material and the transducer is sufficient and the sensor is not intended for long-term operation.

2. Microencapsulation. This is one of the widespread methods of making electrochemical sensors. A biomaterial is placed near the transducer (electrode) so that it is separated from the rest of the solution by a semipermeable membrane allowing analyte molecules and the products of the catalytic reaction to pass through. For this purpose a direct or inverse emulsion is initially prepared from a polymer solution in an organic solvent and an aqueous solution of the biological component. The emulsion is dried to obtain a membrane whose polymeric matrix incorporates microcapsules of water containing biomolecules and nonmacromolecular electrolyte ions. This immobilization method leaves intact the hydrophilic environment of the biopolymer at all immobilization stages, making it possible to achieve a sufficiently high residual activity of the enzyme. The immobilized enzyme is actually free but is localized in a certain part of the measurement cell.

Several types of membranes are used in microencapsulation. Along with cellulose acetate (dialysis) membranes, which are impermeable to protein molecules and slow down transport of many nonmacromolecular compounds, membranes made from polycarbonate (Nucleopore), the natural protein collagen, and from polytetrafluoroethylene (Teflon) are employed, the latter being permeable only to some gases [8]. Negatively charged membranes used in glucose sensors are made from the Nafion polymer (http://www.biosensoracademy.com/rus/readarticle.php).

This immobilization method is readily applicable to various sensor models. It ensures a reproducible performance of the enzyme, protecting it against contamination and degradation. On the whole, microencapsulated enzymes are resistant to variations of the pH, temperature, ionic strength, and chemical composition of the medium. Nevertheless, some molecules and species, such as small gas and electrolyte molecules, pass through the membrane.

3. Inclusion. Biomaterial inclusion into a forming polymer matrix is actually a universal method applicable to various types of recognition elements. The polymer can be deposited from an organic solvent by diluting the solution with water or from a microemulsion by drying it on the sensor surface. The polymer can also be obtained by gelation from a gelatin, agar, polyacrylamide, or alginate solution or by polycondensation of some organic esters or chloroanhydrides [32]. The latter technique is called sol–gel immobilization (Fig. 2).

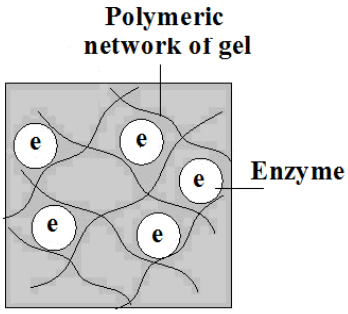

Fig. 2. Enzyme inclusion in gel pores.

A matrix consisting of a synthetic polymer is prepared in the presence of a biomaterial. A cross-linking agent is usually added in order to unite separate polymer strands into a three-dimensional network. The biologically active molecules find themselves entrapped in the polymer bulk. An obvious advantage of this method is its universality. Its drawback is that the network impedes diffusion and hampers analyte permeation. In addition, if the molecules included into

the network are not chemically bonded to it, they can be washed away.

Proteins and nucleic acids are immobilized in polymers of *o*- and *m*-phenylenediamine, *p*-aminophenol, thionine, other phenothiazine and phenoxazine dyes, and derivatives of pyrrole, thiophene, and aniline.

The inclusion of biomolecules in polyionic complexes results from complexation in the layer-by-layer deposition of polyelectrolytes from solution. The native environment of the enzyme is retained in synthetic lipid membranes, specifically, Langmuir–Blodgett (LB) films that are similar in composition and properties to natural biomembranes. These membranes are used as a model in the investigation of membrane processes, as well as in protein and nucleic acid immobilization [33]. LB films as such have a low mechanical strength; for this reason, they are deposited onto the surface of an inert hydrophobic polymer (polyvinyl chloride, Teflon).

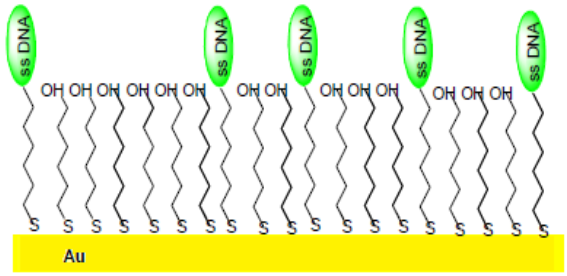

Fig. 3. Self- assembled monolayer of C_nH_{2n+1} alkanethiol on the surface of the gold electrode of a DNA sensor.

Self-assembled monolayers (SAMs) differ from LB films in that they are more strongly bound to the support [34, 35]. Monolayer formation begins with the interaction between the polar moiety (head) of separate molecules and the support surface (Fig. 3). The subsequent ordering of the molecules in the monolayer plane, or layer self-assembling, is due to the van der Waals interaction between the hydrophobic moieties (tails) of the molecules. Various materials, including silicon, metals, and oxides, can serve as the support [36]. SAMs are usable as a matrix for inclusion of biopolymers and hydrophobic nonmacromolecular compounds.

Photopolymerized layers are prepared by depositing a homogeneous mixture of monomers and a biological component onto a support and by exposing the mixture to UV radiation. This procedure exerts a weaker denaturing effect on the biomaterial than the chemical initiation of the same reaction.

4. Cross-linking. In this method, the biomaterial is chemically bound to a solid support or to a gel using so-called bifunctional reagents, for example, glutaraldehyde [13, 37, 38]. An example of such cross-linking is the action of glutaraldehyde, which forms Schiff bases with amino, hydroxyl, and thiol groups of proteins and nucleic acids (Fig. 4).

Various types of polymers (gelatin, agar, cyclodextrins, polyvinyl chloride, polyacrylamide, and many other polymers and gels) were investigated in detail as matrices for a biomaterial, but none of them was found to be perfect [39]. As in the case of encapsulation, substrate diffusion through the resulting material may be rather slow. Biologically active compounds in these materials can gradually degrade. Another disadvantage of the method is that the resulting materials have poor mechanical characteristics. At the same time, this method can be of use in enhancing the stability of adsorbed biomaterials.

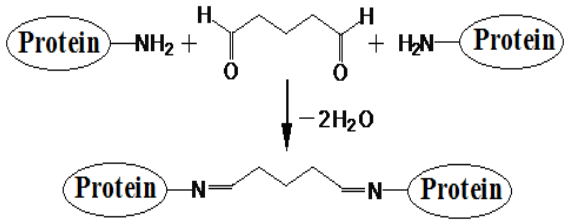

Fig. 4. Interaction between glutaraldehyde and an amino group of a protein.

5. Covalent bonding. Covalent bonding is likely the most widespread immobilization method. As follows from its name, it means the formation of a covalent bond between a biomaterial and a support. The choice of chemicals to be used in this immobilization method depends on the molecules to be bonded and on the support material. Covalent bonding is usually carried out in three steps: the first step is purification of the support and functionalization of its surface with the necessary groups, the second step is biomaterial deposition, and the third one is removal of weakly bound molecules with a pure solvent. Obviously, the sequence of chemical reactions should be chosen so that the bonds formed at the early stages persist at the later stages. The following support materials are used to produce sensors: metals, (usually gold, silver, or platinum), glass, carbon, polysaccharides (cellulose and its derivatives), nylon, poly(methyl methacrylate), and materials having free $–NH_2$, –SH, or –COOH groups or imidazol groups.

Usually proteins are covalently bonded through nucleophilic functional groups in their side amino acid chains that have no effect on their enzymatic activity. Covalent bonds form mainly at moderate temperatures, low ionic strengths, and physiologic pH values. In order to protect the active site of the enzyme during the reaction, the latter is conducted in the presence of a substrate.

The covalent immobilization of DNA and oligonucleotides is carried out by cross-linking them with chitosan to obtain a multitude of amide bonds [37]. Methods of covalent bonding of DNA to aminodextrins and silanized supports were also developed [40].

A widespread method is modification of terminal nucleotide residues. The introduction of thiol groups into these nucleotide residues provides means to obtain, via chemisorption on gold, regular layers of oligonucleotides that are mostly oriented orthogonally to the surface.

The main advantage of covalent bonding is that, on the one hand, it ensures strong biomaterial–support

binding and prevents biomaterial loss and, on the other hand, it provides means to produce sensors with a long service life [8].

In some cases, a biomaterial can be bound to the transducer by several methods. It is always necessary to investigate the efficiency of binding by each method; in particular, it is necessary to compare the activity of an enzyme in solution and the activity of the same enzyme in the immobilized state. A binding method and a matrix (support) should be chosen before completing the fabrication of the sensor.

APPLICATIONS IN THE FOOD INDUSTRY

The food industry needs express analysis methods for checking the quality and safety of foods, for process monitoring, for increasing the product yield, for energy input optimization, and for raising the process automation level. Determination of chemical and biological contaminants in foods is of paramount importance for ensuring healthy nutrition for people. The biosensors employed in the food industry are primarily intended for determination of contaminants, also covering a few important food components, such as sugars, alcohols, amino acids, phenolic compounds, lactic acid, malic acid, ascorbic acid, and acetic acid [8, 41, 42]. It is, therefore, necessary to invest in development of food quality biosensors, since they proved to be a viable alternative to conventional analytical methods, such as chromatography [41, 43]. However, very few biosensors are playing a significant role in quality control in the food industry. Considerable effort should be put into development of inexpensive and sufficiently reliable biosensors capable of operating under real conditions [44].

As was mentioned above, the sensors used in the food industry are mostly intended for food safety analysis (detection of contaminants, allergens, toxins, pathogenic microorganisms, detergents, etc.), for determination of the composition of foods and raw materials, and for fermentation process control (Table 1) [45].

Table 1. Main applications of biosensors in the food industry

Food safety		
Xenobiotics • Additives • Drugs • Pesticides and fertilizers • Other contaminants: dioxins, PCB's, PAH's, heavy metals, and biotoxins	Bacterial toxins • Mycotoxins • Marine toxins	Pathogens • Viruses • Bacteria • Protozoa
Food quality		
Food composition: • Sugars • Amino acids • Alcohols • Organic acids • Cholesterol	Shelf life: • Polyphenols and fatty acids (rancidity) • Sugars and organic acids (maturation) • Biogenic amines (freshness index) • Aliina (garlic and onions)	
Technological processes		
• Sugars (fermentation and pasteurization) • Amino acids (fermentation) • Lactic acid (cheese making) • Alcohols (fermentation)		
Other applications		
• GMO • Reproductive cycle of animals		

Food security is among the most important elements of the national security of any country. Food security can be conventionally divided into the following three components: ability to domestically manufacture a sufficient amount of food, protectability of the food industry against external and internal impacts, and ability of the government to control the quality of the foods sold to the population. In view of this, food and raw material quality control is of primary importance. For this purpose, it is necessary to have express, accurate, informative, and reliable analytical methods meeting present-day requirements. The amounts and types of food additives used in food production are regulated by the legislation of each particular country. Detection and quantification of food additives are essential for preventing manufacturers from abusive use of these components and for revealing substances that can cause allergy in certain groups of people. Table 2 presents examples of biosensors employed in the determination of various toxicants in foods and raw materials [14, 38, 41].

The safety regulations imposed on horticulture and animal husbandry products, including grain, milk, and meat, are becoming more stringent every year, and, accordingly, microtoxin control in foods is becoming more exacting. For example, a method for determining microtoxin traces has been developed for milk quality control. This method employs a bioluminescent biosensor based on a strain of the genetically modified yeast S*accharomyces cerevisiae* [46].

Piezoelectric quartz crystal immunosensors proved to be convenient tools for biochemical and clinical examinations of patients, for food and drug certifi-cation, and for environmental monitoring. They are also used in the kinetic study of biochemical interactions and in the characterization of immunoreagent cross-coupling [21, 38, 47–50]. The practice of employing piezoelectric immunosensors demonstrated that these analytical devices have a high potential for determining residual amounts of pollutants (Table 2) [38].

Table 2. Biosensors for toxicant detection in foods

Analyte	Biological component/matrix	Transducer	Detection limit, linear-response concentration range	Reference
Estrogenic mycotoxin	*Saccharomyces cerevisiae*	Luminescent	1–258 nM	[46]
Acetochlor	Hapten–protein conjugate/4-aminothiophenol or succinimidyl propionate	Piezoelectric quartz crystal	20 ng/mL	[51]
	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.02 ng/mL	[52]
Alachlor	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.02 ng/mL	[53]
Butachlor	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.002 ng/mL	[54]
Bisphenol A	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	0.5 ng/mL	[55]
Sulfamethoxazole	Hapten–protein conjugate/siloxane	Piezoelectric quartz crystal	1–50 ng/mL	[57, 58]

A large group of piezoelectric quartz crystal biosensors is intended for detection of pesticides: acetochlor in surface and potable water [51] and in milk and apple juice [52], alachlor in sausages [53], and butachlor in rice [54]. The detection limit is 0.02 ng/mL for alachlor and acetochlor and 0.002 ng/mL for butachlor, so these sensors can be used in the determination of herbicides at their TLV levels and below in surface, potable, and ground water.

At present, manufacturers of foods and plastic packaging materials widely use detergents, emulsifiers, and pigments, thus polluting the foods and environment with endocrine disruptors (bisphenol A, nonylphenol, linear alkylbenzenesulfonates, esters of phthalic acid, etc.). Dergunova et al. [55, 56] have developed piezoelectric quartz crystal immunosensors for the detection of trace nonylphenol and bisphenol A concentrations in aqueous solutions in the flow injection mode. These sensors provide means to detect nonylphenol and bisphenol A at a concentration of 0.8 and 0.5 ng/mL, respectively, in foods stored in plastic packages.

Use of antimicrobial drugs (sulfanilamides) in the medical treatment of cattle and poultry causes accumulation of these compounds in agricultural products. A sensor was suggested for the detection of residual amounts of sulfa drugs (0.15 mg/mL) in foods (milk, chicken meat, eggs) [57, 58].

A considerable number of publications have been devoted to heavy-metal biosensors. Voltammetric biosensors have been developed for detecting lead [59–61] and copper [62, 63]; fluorescent biosensors, for detecting zinc and copper [64, 65]; bioluminescent [66] and amperometric biosensors, for detecting mercury [67].

Biosensors for food quality control and food production monitoring are characterized in Table 3.

Biosensors are fairly widely employed in solving scientific and practical problems in alcohol manufacturing [14, 68]. The quality of ethanol production processes at all stages is characterized by dynamics of the time variation of the concentrations of dissolved starch, sugars, ethanol, and methanol, which are parameters determinable with biosensors. Ethanol determination in fermentation process control can be carried out using various types of biosensors. Enzymatic sensors for estimating the ethanol concentration may be based on alcohol dehydrogenase or alcohol oxidase immobilized on an appropriate transducer. An amperometric biosensor for ethanol determination in the vapor phase, based on alcohol dehydrogenase and nicotinamide adenine dinucleotide (NAD+) as a cofactor was presented by Park et al. [69]. Ethanol detection in the vapor phase was possible in the 20–800 ppm range. An ethanol biosensor based on alcohol oxidase and a Clark oxygen electrode was described by Morozova et al. [70]. The measurement range of this electrode is from 0.05 to 10 mM. An amperometric biosensor involving *Candida tropicalis* cells immobilized in gelatin using glutaraldehyde allows ethanol to be determined in the 0.5–7.5 mM range [71]. Here, ethanol determination is based on measuring the difference between the respiratory activities of the cells in the presence and absence of ethanol. Valach et al. [72] designed a new microbial amperometric biosensor for flow injection determination of ethanol. Hammerle et al. [73] developed an amperometric biosensor based on alcohol oxidase, involving the methylotrophic yeast *Pichia pastoris* as the catalyst for ethanol conversion into hydrogen peroxide. This biosensor can qualitatively determine the total volatile alcohol content of apple juice by analyzing the gas phase over the sample, requiring no preliminary absorption or concentration. A biosensor based on *Methylobacterium organophilium* immobilized on a thin membrane and an oxygen electrode was also developed for ethanol quantification. The linear response range of this biosensor is 0.050–7.5 mmol/L [74].

Table 3. Food quality control biosensors

Analyte	Biological component/matrix	Transducer	Detection limit, linear-response concentration range	Reference
Ethanol	Alcohol dehydrogenase and (NAD+)	Amperometric	20–800 ppm	[69]
	Alcohol oxidase		0.05–10 mM	[70]
	Candida tropicalis/gelatin, glutaraldehyde	Amperometric	0.5–7.5 mM	[71]
	Gluconobacter oxydans	Amperometric	10 μM – 1.5 mM	[72]
	Methylobacterium organophilium	Oxygen electrode	0.050–7.5 mM	[74]
	Alcohol oxidases	Amperometric	0.7–12.3 mM	[77]
Volatile alcohols	*Pichia pastoris*	Amperometric	0.10–30 mM	[73]
Sugars	Glucose oxidases	Amperometric	0.5–2.5 mM	[77]
D-Glucose and D-xylose	XDH-bacteria	Voltammetric	0.25–6 mM (−0.5 V) 0.24–4 mM (+0.55 V)	[76]
Clotting activity of rennet	Casein micelles/gold electrode	Impedimetric	–	[82]
Lactate	Lactate oxidase	Amperometric	$5 \cdot 10^{-7}$–$5 \cdot 10^{-4}$ M	[83]
Casein	Anti-casein antibody/gold-capped nanoparticle substrate	LSPR	0.1– 10 mg/mL	[19]
Oxalate	Oxalate oxidase/ chitosan	Potentiometric	–	[41]
	Oxalate oxidase/ gold nanoparticles	Amperometric	1–800 μM	[41]
Amygdalin	β-Glucoxidases	Potentiometric	–	[41]
	Peroxidase	Potentiometric	–	[41]
Caffeine	*Pseudomonas alcaligenes*	Amperometric	0.1–1 mg/mL	[85]

Starch can be determined using both enzyme and microbial biosensors. The analytical procedure in this case typically includes starch hydrolysis to glucose by amylolytic enzymes (α-amylase, glucoamylase) followed by glucose determination with an amperometric sensor based on glucose oxidase or microbial cells. For estimating the total utilizable sugar content of wort being brewed, the microbial biosensor may be preferable, because the wide substrate specificity of the microorganisms makes it possible to obtain an integral estimate of the total sugar content [75]. There is a rich assortment of amperometric biosensors for glucose determination [8].

Food manufacturers extensively use sweeteners, such as D-glucose and D-xylose. The co-immobilization of glucose oxidase and xylose dehydrogenase on an electrode modified with nanocomposite films afforded a voltammetric biosensor for the simultaneous determination of D-glucose and D-xylose [76]

Researchers of Tula State University developed various biosensors for determining the ethanol, glucose, and starch contents of brewing semiproducts [77–79] and for determining the biochemical oxygen demand of alcohol production waste [80, 81]. For example, Alferov et al. [77] developed an amperometric biosensor for ethanol, glucose, and starch quantification in brewing semiproducts. They demonstrated that the biosensor involving glucose oxidase allows the glucose concentration to be measured in the 0.5–2.5 mM range and the biosensor based on alcohol oxidase allows ethanol determination in the 0.7–12.3 mM range.

Milk protein coagulation is among the basic processes in cheese making. In this process, milk is curdled using rennet, which eventually destabilizes casein micelles. For the first time, this process was monitored by electrochemical impedance spectroscopy using a faradic impedimetric biosensor, and the curdling activity of rennet was estimated using the hexacyanoferrate(II)/(III) redox couple [82].

Lactic acid is among the most important substances to be analyzed, since it is a product of the metabolism of practically all living organisms and a native or artificial component of many foods. Lactate oxidase immobilization in a conductive polymer film on the surface of planar electrodes modified with Prussian blue made it possible to develop a lactate biosensor characterized by a high sensitivity (190 ± 14 mA/(M cm^2)), a linear response range of $5 \cdot 10^{-7}$–$5 \cdot 10^{-4}$ M, and a high operating stability. This sensor was demonstrated to be applicable to food (kvass) quality control [83].

Owing to their balanced amino acid composition and high digestibility, casein and caseinates are widely used as filler materials in the production of sausages, bread, tinned stew, ice-cream, sauces, confectioner's frosting, etc.; at the same time, casein is among the

most potent allergens. Ha Minh Hiep et al. [19] developed a localized plasmon resonance based immunosensor for casein determination in milk. This immunosensor is easy to manufacture and maintain and is highly sensitive, having a lower casein detection limit of 10 ng/mL.

A quick method involving an amperometric glucose biosensor was suggested for testing chilled meat for freshness [84].

Quality of coffee is determined by its caffeine content. Babu et al. [85] developed an amperometric caffeine biosensor by immobilization of *Pseudomonas Alcaligenes* MTCC 5264 on a cellophane membrane.

A challenging present-day problem is that of detecting GMOs. DNA- or oligonucleotide-based sensors capable of detecting complementary segments of DNA or RNA molecules upon hybridization can be successfully used for this purpose [38]. This was demonstrated by studies on food quality assessment [86, 87]. The data of these studies indicate that these sensors provide means to selectively and specifically detect GMOs via hybridization between the gene fragment (single-stranded DNA molecule) that is immobilized on the sensor surface and is responsible for mutations and the DNA isolated from the material being examined [38].

The concentration of antioxidants is estimated as their effect on the DNA sensor signal measured in the presence of a DNA-damaging factor (Fenton's reagent, copper(II) phenanthroline complexes, ionizing radiation, etc.). In testing an antioxidant mixture of unknown composition, such as plants, foods, and tea extracts, the antioxidant content can be expressed in units of standard antioxidant (e.g., quercetin) concentration. For example, natural flavonoids were determined as their effect on the cleavage of thermally denatured DNA by active oxygen species generated in the Cu(II)–H_2O_2–ascorbic acid system [88, 89].

The analytical potential of the above biosensors is not limited to the examples presented here. Many systems have been developed and tested in recent years, and some of them have found wide application in environmental and analytical monitoring, medicine, biotechnologies, and food quality control. However, although there have been numerous publications dealing with biosensors for food analysis, only a few types of biosensors are now on sale [41]. Unfortunately, most biosensors have been tested only in distilled water or a buffer solution, and only since very recently biosensors have increasingly been tested on real objects. In the food industry, biosensors can be of use in solving a number of specific problems: food aging, estimating the age of vine and distilled beverages, disclosing food falsifications, and the GMO problem. Obviously promising is the wide introduction of relatively cheap, portable biosensors into analytical practice, since they would make it possible to markedly shorten the analysis time, enhance the quality of analysis, and detect and quantify biological compounds in environmental objects, foods, and biological fluids.

REFERENCES

1. Babkina, S.S., Ulakhovich, N.A., Zyavkina, Yu.I., Moiseeva, I.N., Filyushina, E.E., Nikitin, E.V., and Zagumennov, V.A., *Khimiya i khimicheskaya tekhnologiya (Chemistry and chemical engineering),* 2005, vol. 48, no. 1, pp. 22–25.
2. Liu, J., Brown, A.K., Meng, X., Cropek, D.M., Istok, J.D., Watson, D.B., Lu, Y., and Liu, J., *Proceedings of the National Academy of Sciences of the United States of America,* 2007, vol. 104, no. 7, pp. 2056–2061.
3. Evtyugin, G.A., Budnikov, G.K., and Porfir'eva, A.V., *Rossiiskii khimicheskii zhurnal (Russian chemical journal),* 2008, vol. 52, no. 2, pp. 66–79.
4. Karyakin, A.A., Ulasova, E.A., Vagin, M.Yu., and Karyakina, E.E., *Sensor (Sensor),* 2002, no. 1, pp. 16–24.
5. Korotkaya, E.V., *Biosensory na osnove kollodievykh plenok: Monografiya* (Biosensors Based on Collodion Films: A Monograph), Kemerovo: Kemerov. Tekhnol. Inst. Pishchevoi Prom–sti., 2011.
6. Evtyugin, G.A., Budnikov, G.K., and Nikol'skaya, E.B., *Russian Chemical Reviews,* 1999, vol. 68, no. 12, pp. 1041–1064.
7. Budnikov, G.K., *Sorosovskii obrazovatel'nyi zhurnal (Soros educational journal),* 1996, no. 12, pp. 26–32.
8. Eggibs, B., *Chemical Sensors and Biosensors,* New York: Wiley, 2002.
9. *Biosensors: Fundamentals and Applications,* Turner, A., Karube, I., and Wilson, G., Eds., Oxford: Oxford Univ. Press, 1987.
10. Rastislav Monošíka, Miroslav Streďanskýb, and Ernest Šturdík, *Acta Chimica Slovaca,* 2012, vol. 5, no. 1, pp. 109–120. doi 10.2478/v10188-012-0017-z
11. Cavalcanti, A., Shirinzadeh, B., Zhang, M., and Kretly, L.C., *Sensors,* 2008, vol. 8, pp. 2932–2958.
12. Lojou, É. and Bianko, P., *Journal of Electroceramics,* 2006, vol. 16, no..1, pp. 79–91.
13. Kitova, A.E., *Extended Abstract of Cand. Sci. (Biol.) Dissertation,* Saratov: Institute of Biochemistry and Physiology of Plants and Microorganisms, 2009.
14. Chunhui Dai and Seokheun Choi, *Open Journal of Applied Biosensor,* 2013, vol. 2, pp. 83–93.
15. Mohanty, S.P. and Kougianos, E., *IEEE Potentials,* 2006, vol. 25, no. 2, pp. 35–40. doi 10.1109/MP.2006.1649009
16. Lei, Y., Chen, W., and Mulchandani, A., *Analytica Chimica Acta*, 2006, vol. 568, nos. 1–2, pp. 200–210. doi 10.1016/j.aca.2005.11.065
17. D'Souza, S.F., *Biosensor and Bioelectronics,* 2001, vol. 16, no. 6, pp. 337–353. doi 10.1016/S0956-5663(01)00125-7.
18. Lud, S.Q., Nikolaides, M.G., Haase, I., Fischer, M., and Bausch, A.R., *ChemPhysChem,* 2006, vol. 7, no. 2, pp. 379–384.
19. Ha Minh Hiep, Tatsuro Endo, Kagan Kerman, Miyuki Chikae, Do-Kyun Kim, Shohei Yamamura, Yuzuru Takamura, and Eiichi Tamiya, *Science and Technology of Advanced Materials,* 2007, vol. 8, pp. 331–338.
20. Kitova, A.E. Alferov, V.A., and Ponamoreva, O.N., in *Mikrobnye biokatalizatory i perspektivy razvitiya fermentnykh tekhnologii v pererabatyvayushchikh otraslyakh APK* (Microbial Biocatalysts and Outlooks for the Development of Enzyme Technologies in Processing Sectors of Agribusiness), Moscow: Pishchepromizdat, 2004, pp. 255–262.

21. Reshetilov, A.N., Kitova, A.E. Alferov, V.A., and Ponamoreva, O.N., *Biotekhnologiya-2003: Tezisy dokladov seminara-prezentatsii innovatsionnykh nauchno-tekhnicheskikh proektov* (Biotechnology 2003: Workshop and Presentation of Innovative Research and Development Projects), Pushchino, Moscow oblast, 2003, pp. 64–65.
22. Shevtsova, O.A., *Zbirnik naukovikh prats' Kharkivs'kogo universitetu povitryanikh sil (Transactions of the Kharkiv university of air forces),* 2010, issue 1 (23), pp. 201–205.
23. Spiridonova, V.A., *Biochemistry* (Moscow) S*upplement Series A: Membrane and Cell Biology,* 2010, vol. 4, no. 2, pp. 138–149.
24. Juan Zhang, Lihua Wang, Dun Pan, Shiping Song, Freddy Y.C. Boey, Hua Zhang, and Chunhai Fan, *Small,* 2008, vol. 4, no. 8, pp. 1196–1200.
25. Lui, C., Cady, N.C., and Batt, C.A., *Sensors,* 2009, vol. 9, pp. 3713–3744.
26. Reshetilov, A.N., Ponamoreva, O.N., Arlyapov, V.A., Alferov, V.A., Rogova, G.V., Blokhin, I.V., and Chepkova, I.F., *Voda: khimiya i ekologiya (Water: chemistry and ecology),* 2008, no. 3, pp. 23–30.
27. Perov, S.N., Kalach, A.V., and Meshcheryakova, O.L., *Sorbtsionnye i khromatograficheskie protsessy (Sorption and chromatographic processes),* 2008, vol. 8, no. 6, pp. 1008–1012.
28. Reshetilov, A.N., *Applied Biochemistry and Microbiology,* 2005, vol. 41, no. 5, pp. 442–449.
29. Deryabin, D.G. and Aleshina, E.S., *Applied Biochemistry and Microbiology,* 2008, vol. 44, no. 3, pp. 292–296.
30. Deryabin, D.G. and Aleshina, E.S., *Gigiena i sanitariya (Hygiene and sanitation),* 2008, no. 4, pp. 83–86.
31. Lavrinenko, I.A. and Lavrinenko, V.A., *Vestnik Novosibirskogo gosudarstvennogo universiteta, seriya: Biologiya, klinicheskaya meditsina (Bulletin of Novosibirsk State University, series: Biology, clinical medicine),* 2007, vol. 5, no. 1, pp. 118–126.
32. Zubov, V.P., Ivanov, A.E., Zhigis, L.S., Rapoport, E.M., Markvicheva, E.A., Lukin, Yu.V., and Zaitsev, S.Yu., *Bioorganicheskaya khimiya (Bioorganic chemistry),* 1999, vol. 25, no. 11, pp. 868–880.
33. Koval'chuk, M.V., Klechkovskaya, V.V., and Feigin, L.A., *Priroda (Nature),* 2003, no. 12, pp. 1–12.
34. Madou, M., *Fundamentals of Microfabrication: The Science of Miniaturization,* Boca Raton, Fla.: CRC, 2002, pp. 62–63.
35. Kaifer, A. and Gómez-Kaifer, M., *Supramolecular Electrochemistry,* Weinheim: Wiley–VCH, 1999, pp. 191–193.
36. Schramm, L., *Dictionary of Nanotechnology, Colloid and Interface Science,* Weinheim: Wiley–VCH, 2008.
37. Gol'dfarb, O.E., *Extended Abstract of Cand. Sci. (Chem.) Dissertation,* Kazan: Kazan State University, 2005.
38. Ermolaeva, T.N., Kalmykova, E.N., and Shashkanova, O.Yu., *Rossiiskii khimicheskii zhurnal (Russian chemical journal),* 2008, vol. 52, no. 2, pp. 17–29.
39. *Analytical Chemistry: A Modern Approach to Analytical Science,* Kellner, R., Mermet, J.-M., Otto, M., Valcarcel, M., and Widmer, H., Eds., Weinheim: Wiley–VCH, 2004.
40. Shlyapnikov, Yu.M., *Extended Abstract of Cand. Sci (Chem.) Dissertation,* Moscow: Moscow State University, 2010.
41. http://www.intechopen.com/books/state-of-the-art-in-biosensors-environmental-and-medical-applications/biosensors-for-contaminants-monitoring-in-food-and-environment-for-human-and-environmental-health
42. Vaefolomeev, S.D., *Sorosovskii obrazovatel'nyi zhurnal (Soros educational journal),* 1997, no. 1, pp. 45–49.
43. Luong, J.H.T., Groom, C.A., and Male, K.B., *Biosensors and Bioelectronics,* 1991, vol. 6, pp. 547–554.
44. Kopa, Yu. and Momynaliev, K., *Nanoindustriya (Nanoindustry),* 2007, no. 1, pp. 28–33.
45. Rumayor V.G, Iglesias, E.G, Galán, O.R, and Cabezas, L.G., *Aplicaciones de biosensores en la industria agroalimentaria,* Madrid: Comunidad de Madrid y la Universidad Complutense de Madrid, 2005.
46. Valimaa, A., Kivisto, A.T., Leskinen, P.I., and Karp, M.T., *Journal of Microbiological Methods,* 2010, vol. 80, no. 1, pp. 44–48.
47. Kalmykova, E.N., Melikhova, E.V., Dergunova, E.S., Eremin, S.A., and Ermolaeva, T.N., *Sorbtsionnye i khromatograficheskie protsessy (Sorption and chromatographic processes),* 2004, vol. 4, no. 5, pp. 597–605.
48. Pohanka, M., Pavlis, O., and Skladal P., *Sensors,* 2007, vol. 7, pp. 341–353.
49. Kalmykova, E.N. and Ermolaeva, T.N., *Izvestiya vysshikh uchebnykh zavedenii, seriya: Khimiya i khimicheskaya tekhnologiya (Bulletin of higher education institutions, series: Chemistry and chemical engineering),* 2005, vol. 48, no. 12, pp. 76–80.
50. Ermolaeva, T.N. and Kalmykova, E.N., *P'ezokvartsevye sensory: Analiticheskie vozmozhnosti i perspektivy* (Piezoelectric Quartz Crystal Sensors: Analytical Potential and Prospects), Lipetsk: Lipetsk. Gos. Tekh. Univ., 2007.
51. Lebedev, M.Yu., Eremin, S.A., Skladal, P., *Analytical Letters,* 2004, vol. 36, pp. 2443–2457.
52. Nartova, Yu.V., Eremin, S.A., and Ermolaeva, T.N., *Sorbtsionnye i khromatograficheskie protsessy (Sorption and chromatographic processes),* 2006, vol. 6, no. 5, pp. 764–772.
53. Nartova, Yu.V., Eremin, S.A., and Ermolaeva, T.N., *Rossiiskaya shkola-konferentsiya molodykh uchenykh "Ekotoksikologiya: Sovremennye bioanaliticheskie sistemy, metody i tekhnologii"* ("Ecotoxicology: Advanced Bionalytical Systems, Methods, and Technologies," Russian Young Scientists' School and Conference), Pushchino, Moscow oblast, 2006, pp. 68–70.
54. Nartova, Yu.V., *Mezhdunarodnaya konferentsiya molodykh uchenykh po fundamental'nym naukam "Lomonosov-2007", Khimiya* ("Lomonosov 2007," International Young Scientists' Conference on Fundamental Sciences, Section: Chemistry), Moscow, 2007, vol. 1, p. 39.
55. Dergunova, E.S. and Ermolaeva, T.N., *Vserossiiskaya studencheskaya nauchno-tekhnicheskaya shkola-konferentsiaya "Imzhenernye nauki — zashchite okruzhayushchei sredy"* ("Engineering Science for Environmental Protection," Russian National Students' Science & Technology School and Conference), Tula, 2006, pp. 136–140.

56. Ermolaeva, T.N., Dergunova, E.S., Kalmykova, E.N., and Eremin, S.A., *Journal of Analytical Chemistry,* 2006, vol. 61, no. 6, pp. 609–613.
57. Kalmykova, E.N., Melikhova, E.V., Eremin, S.A., and Ermolaeva, T.N., *Antibiotiki i khimioterapiya (Antibiotics and chemotherapy),* 2004, vol. 49, no. 1, pp. 8–13.
58. Melikhova, E.V., Kalmykova, E.N., Eremin, S.A., and Ermolaeva, T.N., *Journal of Analytical Chemistry,* 2006, vol. 61, no. 7, pp. 687–693.
59. Yüce, M., Nazir, H., and Dönmez, G., *Biosensors and Bioelectronics,* 2010, vol. 26, no. 2, pp. 321–326.
60. Yüce, M., Nazir, H., and Dönmez, G., *Bioresource Technology,* 2010, vol. 101, no. 19, pp. 7551–7555.
61. Yüce, M., Nazir, H., and Dönmez, G., *New Biotechnology,* 2011, vol. 28, no. 4, pp. 356–361.
62. Alpat, S., Aplat, S.K., Cadirci, B.H., Yasa, I., and Telefoncu, A., *Sensor and Actuators B: Chemical,* 2008, vol. 134, no. 1, pp. 175–181.
63. Yüce, M., Nazir, H., and Dönmez, G., *Bioelectrochemistry,* 2010, vol. 79, no. 1, pp. 66–70.
64. Ravikumar, S., Ganesh, I., Yoo, I., and Hong, S., *Process Biochemistry,* 2012, vol. 47, no. 5, pp. 758–765.
65. Liu, P., Huang, Q., and Chen, W., *Environmental Pollution,* 2012, vol. 164, pp. 66–72.
66. Barrocas, P.R.G., Landing, W.M., and Hudson, R.J.M., *Journal of Environmental Sciences,* 2012, vol. 22, no. 8, pp. 1137–1143.
67. Singh, J. and Mittal, S.K., *Sensors and Actuators B: Chemical,* 2012, vol. 165, no. 1, pp. 48–52.
68. Reshetilov, A.N. and Kitova, A.E. http://cbio.ru/page/43/id/4791/.
69. Park, Je-K., Yee, H.-J., Lee, K. S., Lee, W.-Y., Shin, M.-C., Kim, T.-H., and Kim, S.-R., *Analytica Chimica Acta,* 1999, vol. 390, pp. 83–91.
70. Morozova, N.O., Ashin, V.V., Trotsenko, Yu.A., and Reshetilov, A.N., *Applied Biochemistry and Microbiology,* 1999, vol. 35, no. 5, pp. 537–541.
71. Akyilmaz, E. and Dinckaya, E., *Biosensors and Bioelectronics,* 2005, vol. 20, no. 7, pp. 1263–1269.
72. Valach, M., Katrlik, J., Sturdik, E., and Gemeiner, P., *Sensors and Actuators B: Chemical,* 2009, vol. 138, no. 2, pp. 581–586.
73. Hammerle, M., Hilgert, K., Marcus, A.H., and Ralf, M., *Sensors and Actuators B: Chemical,* 2011, vol. 158, no. 1, pp. 313–318.
74. Wen, G.M., Shuang, S.M., Dong, C., and Choi, M.F., *Chinese Chemical Letters,* 2012, vol. 23, no. 4, pp. 481–483.
75. Reshetilov, A.N., Kitova, A.E., Alferov, V.A., Ponamoreva, O.N., Kuzmichev, A. V., and Ezhkov, A.A., *Biotekhnologiya-2003: Tezisy dokladov seminara-prezentatsii innovatsionnykh nauchno-tekhnicheskikh proektov* (Biotechnology 2003: Workshop and Presentation of Innovative Research and Development Projects), Pushchino, Moscow oblast, 2003, pp. 64–65.
76. Li, L., Liang, B., Li, F., Shi, J., Mascini, M., Lang, Q., and Liu, A., *Biosensors and Bioelectronics,* 2013, vol. 42, no. 12, pp. 156–162.
77. Alferov, V.A., Arlyapov, V.A., and Kamanin, S.S., *Izvestiya Tul'skogo gosudarstvennogo universiteta, seriya: Estestvennye nauki (Bulletin of Tula State University, series: Natural sciences),* 2010, no. 2, pp. 247–255.
78. Il'nitskii, M.Yu. and Zaitsev, M.G., *III Obshcherossiiskaya studenscheskaya elektronnaya nauchnaya konferentsiya "Studenscheskii nauchnyi forum"* ("Students' Scientific Forum," III Russian National Students' e-Conference), 2011. http://www.rae.ru/forum2011/133/
79. Zaitsev, M.G., *Extended Abstract of Cand. Sci. (Chem.) Dissertation,* Moscow: Moscow State University of Fine Chemical Technologies, 2013.
80. Khitrova, Yu.S. and Indzhigiya, E.Yu., *III Obshcherossiiskaya studenscheskaya elektronnaya nauchnaya konferentsiya "Studenscheskii nauchnyi forum"* ("Students' Scientific Forum," III Russian National Students' e-Conference), 2011. http://www.rae.ru/forum2011/133/
81. Sibirtsev, I.A., Kamanin, S.S., and Arlyapov, V.A., *III Obshcherossiiskaya studenscheskaya elektronnaya nauchnaya konferentsiya "Studenscheskii nauchnyi forum"* ("Students' Scientific Forum," III Russian National Students' e-Conference), 2011. http://www.rae.ru/forum2011/133/
82. Panagopoulou Maria, A., Stergiou Dimitrios, V., and Roussis Ioannis, G., *Analytical Chemistry,* 2010, vol. 82, pp. 8629– 8636
83. Korneyeva, L.Kh., Borisova, A.V., Yashina, E.I., Karyakina, E.E., Voronin, O.G., Kosnier, S.. and Karyakin, A.A., *Moscow University Chemistry Bulletin,* 2010, vol. 51, no. 1, pp. 49–56.
84. Kolesnikov, P.S., Extended Abstract of Cand. Sci. (Vet.) Dissertation, Moscow: Russian Academy of Agricultural Sciences, 2005.
85. Babu, V.R.S., Patra, S., Karanth, N.G., Kumar, M.A., and Thakur, M.S., *Analytica Chimica Acta,* 2007, vol. 582, no. 2, pp. 329–334.
86. Minunni, M., Tombelli, S., Mariotti, E., and Mascini, M., *Fresenius Journal of Analytical Chemistry,* 2001, vol. 369, no. 7/8, pp. 589–593.
87. Minunni, M., Tombelli, S., Pratesi, S., and Mascini, M., *Analytical Letters,* 2001, vol. 34, no. 6, pp. 825–840.
88. Korbut, O., Bučková, M., Labuda, J., and Gründler, P., *Sensors,* 2003, vol. 3, pp. 1–10.
89. Labuda, J., Buckova, M., Heilerova, L., Silhar, S., and Stepanek, I., *Analytical and Bioanalytical Chemistry,* 2003, vol. 376, pp. 168–173.

INFORMATION FOR AUTHORS

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