ADVANTAGES OF PORCINE BLOOD PLASMA AS A COMPONENT OF FUNCTIONAL DRINKS

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(Received March 26, 2014; Accepted in revised form April 14, 2014)

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

UDC [637.66:636.4]: 66.094.941
DOI 10.12737/5456

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.

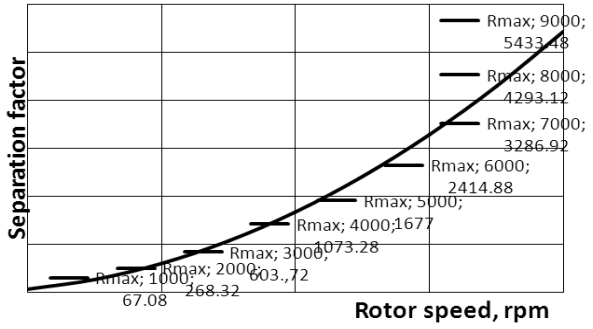

Fig. 1. Separation factor values for different rotation speeds (centrifuge CM - 50).

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.

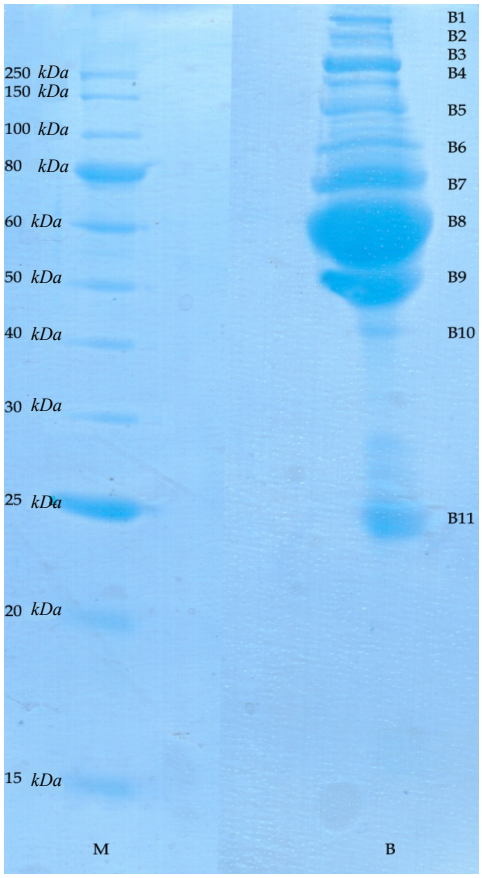

Fig. 2. Polyacrylamide gel electrophoresis (12% separating gel, 4% stacking gel): M - protein markers; B –blood plasma.

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).

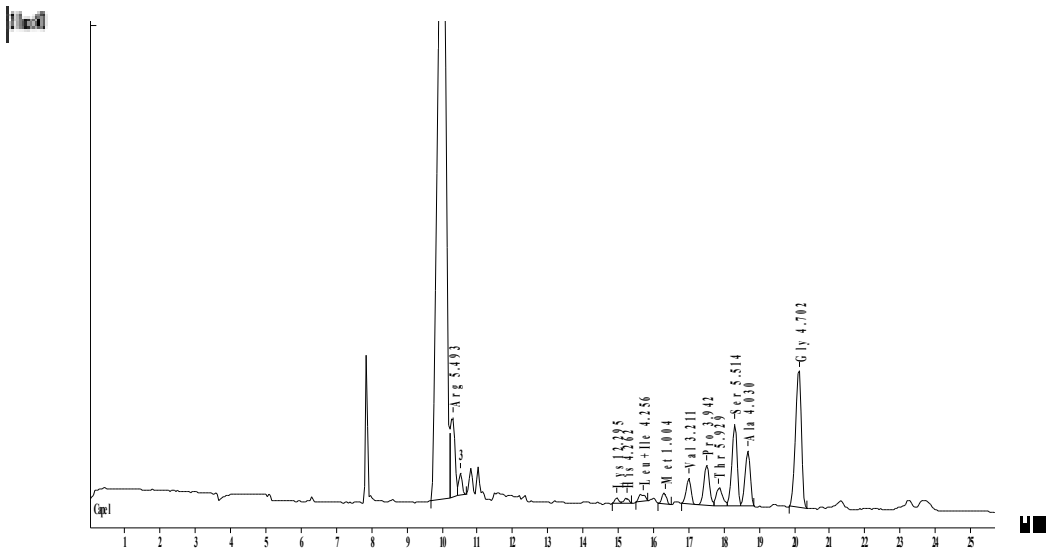

Fig. 3. Amino acid composition of proteins of pig blood plasma.

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.

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