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## Biologically Active Extracts From Plant Based On Blood Plasma.

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#### **ARSTRACT**

Development of functional beverages with extracts from raw materials of plant origin, containing many biologically active components remains the most promising direction in the creation of healthy foods. Special interest is production the protein-containing drinks, while along with traditional milk drinks products based on animal blood plasma deserves special attention. This paper contains the results of studying of possibility to use hydrolyzed blood plasma as an extractant in preparation a base for producing functional beverages. The investigations were carried out into laboratories of the department of technology of production and processing of agricultural products of the Stavropol State Agrarian University. As objects of research were used: peppermint, carcade, lentils, chickpea, hydrolyzed blood plasma and extracts obtained on its basis. Hydrolyzed blood plasma was obtained by pretreatment with collagenase enzyme: protein concentration was 4.0-4.5%, enzyme concentration was 0.37% (with an activity of 900 uPA/g), the hydrolysis temperature - 37-42 °C, the pH - 6.6-7.1, hydrolysis duration 2.5 hours, hydrolysis degree of plasma proteins was about 82%. Data were analyzed for significance using Statistical Analysis System software version 9.1. By calculation was established that the plant extracts have a high biological value, which confirms the good balance of the amino acid composition. Studying of storage ability revealed the absence of pathogenic microflora into the dry product with a shelf life of up to 12 weeks by temperature not higher than 4 °C. The indicator KMAFAnM remained within  $2.4 \times 10^3$  CFU per 1 g of the product. Thus, using the hydrolyzed blood plasma as an extractant of plant raw materials can be recommended as the basis for producing functional beverages with high biological value.

**Keywords:** blood plasma, functional drinks, plant extracts, extraction.

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#### INTRODUCTION

Drinks are excellent objects for the enrichment of biologically active components, acquiring the ability to provide an effective preventive and health effects on the human body.

The development of functional beverages with the use of extracts from raw materials of plant origin, containing many biologically active components, remains the most promising direction in the creation of healthy foods. Inclusion in the composition of drinks of plant extracts with antioxidant properties has a tonic effect on the body, increases the adaptability of the nervous system and the body's resistance to adverse external factors. Technological methods of processing plant materials allows to obtain extracts and concentrated bases containing protein components, products of hydrolysis of non-starch polysaccharides, and bioactive substances.

Of particular interest is the production of protein-containing drinks, while along with traditional milk drinks, drinks based on blood plasma of slaughter animals deserve special attention. This secondary raw materials of animal origin, characterized by the full value and high digestibility of the proteins included in its composition, finds its application in the production of food, medical, feed and technical products. Blood plasma drinks do not contain any indigestible or hardly digestible nutrients, have a low calorie content and can be used for disorders of protein metabolism, digestive functions, recommended for postoperative patients, children, the elderly [2, 3, 5].

In addition, deep processing of secondary protein raw materials from the meat industry will not only find an additional source of high-quality animal protein, but also reduce the discharge of slaughtered blood into sewer systems, thereby reducing the environmental burden on the environment [6].

The aim of the work is to study the feasibility of using blood plasma of slaughter animals for the production of plant extracts as a basis for the production of functional beverages.

### **MATERIAL AND METHODS**

The studies were conducted in the conditions of the laboratories of the department of agricultural production and processing technology of the Stavropol State Agrarian University.

As objects of research, peppermint, carcade, lentils, chickpea, hydrolyzed blood plasma and extracts obtained on its basis were used.

Hydrolyzed blood plasma of farm animals was obtained by pretreatment with collagenase enzyme preparation in accordance with the developed recommendations: the protein concentration in the system was 4.0-4.5%, the concentration of the enzyme preparation in the system was 0.37% (with an activity of 900 uPA / g), the temperature of the hydrolysis is 37-42 °C, the pH of the system is 6.6-7.1, the duration of hydrolysis is 2.5 hours, the degree of hydrolysis of plasma proteins is about 82%. This treatment allowed to increase the temperature of extraction, providing a more complete extraction of extractive substances and increase the digestibility of the extract - the basis for the production of beverages [3].

Plant material was used in the amount of 2.5 g per 100 ml of hydrolyzed animal blood plasma.

In the course of research, the main indicators were determined by the following methods: protein content - by the Kjeldahl method; fat - soxhlet; carbohydrates - by Bertrand method; mineral composition spectrophotometrically according to GOST R 55484-13; vitamin composition - on the LCMS-10EV liquid chromatograph according to the instructions; amino acid composition - on the AAA-400 amino acid analyzer by standard methods; amino acid fast and biological value - by calculation method according to N.N. Lipatov [1].

The studies were conducted in triplicate, with the subsequent processing of the results obtained by standard statistical methods in the program Microsoft Office Excel 2007.



## **RESULTS AND DISCUSSION**

It is known that the amount of extracted substances is directly related to the degree of grinding of the material and temperature regimes. In this regard, it was decided to crush the caracada and peppermint to a particle size of 1-3 mm with a characteristic lamellar form. The specified grinding degree provides the minimum amount of dust particles and is the best for extraction. The temperature range of extraction was in the range of 60-70 °C, since the temperature of the hydrolyzed plasma shifted to higher values led to a clouding of the system and a flocculent precipitate due to protein denaturation. The effectiveness of the factors of the experiment was evaluated by the quantitative change in the mass fraction of dry substances in the extraction system.

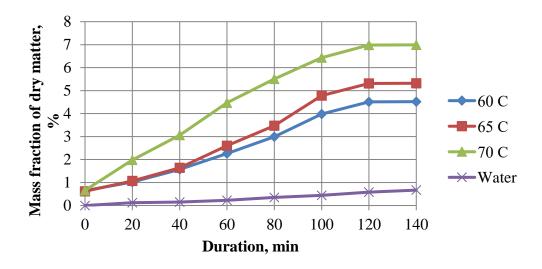


Figure 1: Dynamics of extraction of dry substances of peppermint hydrolyzed blood plasma in the temperature range of 60 - 70 ° C

The graph in Figure 1 shows that the mass fraction of dry substances in the extract is directly dependent on the time of extraction, and the highest value is achieved at a shutter speed of 120 minutes.

As a control experiment, extraction was performed in parallel with water at a temperature of 70 ° C. As a result, it was found that under identical conditions, biomodified plasma is a significantly better extractant.

The minimum concentration of extractive solids in hydrolyzed plasma at 60 ° C is 4.6%, which is about 10 times higher than the result of the water system (0.5%). An increase in the temperature of the "hydrolyzed blood plasma - peppermint" system to 65 ° C led to an increase in the dry matter extracted from the raw materials to 5.3%, which is 11 times higher than this figure when using water. A further increase in the temperature of extraction to 70 ° C was characterized by an increase in the dry matter content to 7.0%, which is 14 times higher than the similar indicator of the "water - peppermint" system.

Thus, the highest content of extractive substances in the "hydrolyzed blood plasma-peppermint" system is observed when kept for 120 minutes at 70 °C. At the same time, the use of water as an extractant could not provide a solids content above 0.6%, even with an increase in exposure to 140 minutes.

Further, by analogy, studies were carried out on the extraction conditions of carcade solids (Figure 2).

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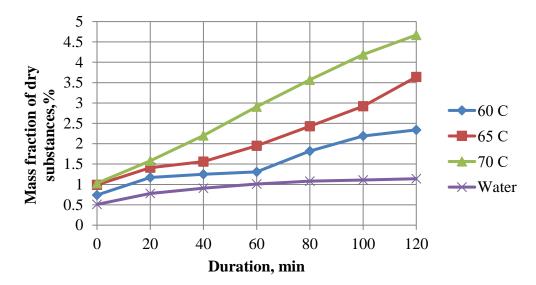


Figure 2: Dynamics of extraction of dry substances by carcade of hydrolyzed blood plasma in the temperature range of 60 - 70 ° C

The experimental data presented in Figure 2 confirms the validity of the established dependence of the efficiency of extraction of solids on the exposure time for both the water system and hydrolyzed plasma. The peculiarities of the chemical composition and properties of the carcade as a raw material led to a slightly better efficiency of aqueous extraction in comparison with mint, and somewhat less in experiments with hydrolyzed plasma.

The minimum proportion of extractives for hydrolyzed blood plasma at  $60\,^{\circ}$  C is 2.4%, which is 3 times higher than when using water. At the same time, as in the previous experiment, the best results of the extraction of hydrolyzed blood plasma were achieved at a temperature of 70  $^{\circ}$  C and a holding time of 120 minutes, at which the mass fraction of dry substances was 4.6%.

Blood plasma hydrolyzate consists of more than 90% of water, but the presence in it of a significant amount of protein components, which are surface-active substances, significantly alter its properties as a solvent. Presumably, the present surfactants activate the extractability of dry substances, increasing their transition to the liquid phase of the system, and, at the same time, affect the permeability of the solid phase. Probably, this can explain the significantly higher extraction ability of the blood plasma hydrolyzate of slaughter animals in comparison with water.

Further studies were related to the study of the efficiency of hydrolyzed blood plasma extraction of the components of plant protein raw materials - chickpea and lentils. In order to avoid denaturation of proteins of raw materials, the temperature factor was studied in the range of 45-55 ° C. Extrapolating the pattern of previous experiments and on the basis of preliminary studies, the maximum permissible extraction temperature of 55 ° C was chosen for the study. The interval of the system exposure factor ranged from 20 to 120 minutes. Studies have shown that the highest degree of extraction of dry substances is achieved at an extraction time of 100-120 minutes (Figure 3).



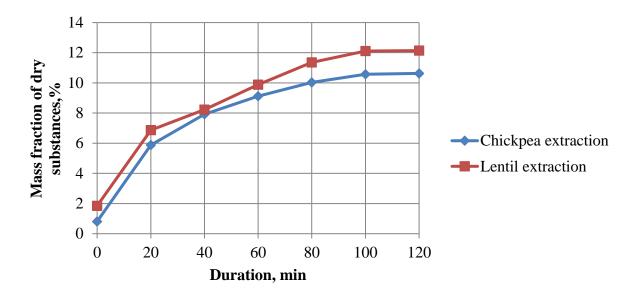


Figure 3: Dynamics of extraction of dry substances of chickpea and lentils by hydrolyzed blood plasma of farm animals at 55 ° C

The results of the experiment made it possible to establish that at 55 ° C and a holding time of at least 100 minutes, the mass fraction of extractable solids was 10.4% for chickpea and 12.0% for lentils. This exceeds the same indicator at a temperature of 45 ° C by 12 and 5%, respectively.

The use of such an unconventional extractant as hydrolyzed blood plasma certainly requires the study of the organoleptic indicators obtained for the production of beverage extracts (table 1).

Table 1: Results of the evaluation of organoleptic characteristics of extracts based on hydrolyzed blood plasma of slaughter animals

Indicators	Peppermint extract	Carcade extract	Chickpea extract	Lentil extract		
Taste	Cooling, refreshing	Sweet and sour	Sweet			
Appearance	There is an insignificant amount of sediment or suspension characteristic of the product.					
Aroma	Mint	Weak floral	Light bean flavor, characteristic of the ra materials used			

Evaluation of the organoleptic characteristics showed that the extracts obtained can be used to prepare drinks, however, the taste characteristics will require some adjustment due to the addition of additional flavor ingredients.

The obtained extracts for reasons of improving their usability and storage capacity were further dried on a spray dryer. The solubility of the obtained dry product is not less than 97%.

The obtained dry extracts were subjected to a thorough qualitative and quantitative study of the composition. It is difficult to overestimate the role of vitamins in the course of metabolic processes in the human body, ensuring normal metabolism and maintaining homeostasis. Avitaminosis inevitably leads to violations of all processes and functions of the body, undermining human health. In this regard, we have investigated not only the chemical composition, but also the content of vitamins and minerals in the extracts (table 2).

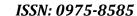




Table 2: Results of the study of the composition of dry plant extracts based on blood plasma hydrolyzate

Indicators	Lentil	Chickpea	Karkade	Mint				
Protein,%	20,01	19,72	7,01	6,83				
Lipids,%	0,50	1,02	-	-				
Carbohydrates,%	18,23	15,03	11,25	15,05				
Minerals and vitamins, mg%								
Sodium (Na)	23,82	25,81	18,23	32,05				
Potassium (K)	504,34	752,06	7,31	3,14				
Calcium (Ca)	56,83	80,40	25,11	40,67				
Phosphorus (P)	212,84	276,30	94,48	72,18				
Iron (Fe)	11,34	1,89	1,12	1,25				
Magnesium (Mg)	72,65	86,37	34,00	29,60				
Retinol (A)	0,004	0,007	0,013	0,042				
Thiamine (B1)	0,053	0,062	0,053	0,361				
Riboflavin (B2)	0,041	0,057	0,289	0,236				
Pyridoxine (B6)	0,008	0,012	0,009	0,005				
Cyanocobalamin (B12)	0,011	0,023	0,023	0,013				
Ascorbic acid (C)	2,781	2,067	23,346	7,832				
Tocopherol (E)	6,257	7,812	5,786	4,739				
Calciferol (D)	0,008	-	-	-				
Niacin (PP)	0,058	0,065	0,052	0,065				

The research results showed that the dry product contains significant amounts of proteins, vitamins E and C (which have a pronounced antioxidant effect and are deficient in the diets of most people) and mineral components, in particular potassium and phosphorus, and are recommended for the production of functional beverages.

The human diet should contain essential amino acids not only in sufficient quantities, but also their ratios in total protein and the timing of intake are important. It is these conditions that ensure the normal course of protein synthesis processes.

The most deficient in human nutrition are amino acids such as tryptophan, methionine and lysine. In this regard, the quality of the protein in the diet is most often assessed precisely by saturation with these essential amino acids.

Amino acid analysis of the obtained plant extracts (table 3) led to the conclusion that they have a high biological value due to the high content of essential amino acids, including the most scarce ones.



Table 3: Results of the analysis of the amino acid composition of the obtained dry extracts of peppermint (1), carcade (2), chickpea (3), lentils (4)

Amino acids	Amino acid content in FAO /	The amino acid content in the product, g / 100 g			Amino acid score, %				
Amino acids	WHO reference protein	1	2	3	4	1	2	3	4
Isoleucine	0,40	0,23	0,31	0,40	0,85	57,5	77,5	100,0	212,0
Valin	0,50	0,27	0,29	0,98	1,04	54,0	58,0	196,0	198,0
Methionine + cystine	0,35	018	0,19	0,63	0,59	51,4	54,3	178,0	168,0
Leucine	0,70	0,77	0,87	2,5	2,54	110,0	124,3	357,0	363,0
Lysine	0,55	0,60	0,65	2,00	2,02	109,0	118,2	364,0	367,0
Threonine	0,40	0,35	0,46	1,16	1,17	87,5	115,0	290,0	203,0
Tryptophan	0,60	0,11	0,14	2,11	2,37	110,0	98,3	352,0	395,0
Phenylalanine + tyrosine	0,10	0,68	0,59	0,36	0,34	113,0	140,0	360,0	340,0
Biological value,%	-	-	-	-	-	96,48	95,61	82,7	87,5

By calculation, it was established that the plant extracts under study have a high biological value, which confirms the good balance of amino acid composition.

The study of storage ability revealed the absence of pathogenic microflora in a dry product with a shelf life of up to 12 weeks at a temperature not higher than 4  $^{\circ}$  C. The indicator KMAFAnM remained within  $2.4 \times 10^3$  CFU per 1 g of the product.

The obtained plant extracts based on hydrolyzed blood plasma have a high biological value and can be recommended for use as a basis for the production of functional beverages.

#### CONCLUSION

Studies have shown that the use of hydrolyzed blood plasma of slaughter animals as an extractant of plant materials allows to obtain biologically valuable extracts characterized by a balanced amino acid composition, rich in vitamins and minerals, as well as acceptable organoleptic characteristics and persistence. Thus, they can be recommended as the basis for the production of functional beverages with high biological value.

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