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TECHNOLOGY DEVELOPMENT OF WHIPPED DRINK BASED ON BIOMODIFIED BLOOD PLASMA

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ABSTRACT

This paper is devoted to developing a non-alcoholic cocktail technology based on the biomodified blood plasma of farm animals. There are results of the most effective enzyme preparation and optimization of its conditions for enzymatic hydrolysis of blood plasma. There was studying the extraction dynamics of dry substances of plant raw materials (peppermint, hibiscus, chickpeas, and lentils) using hydrolyzed blood plasma as an extractant. There were developed the recipe and technology to make a whipped cocktail and studied quality indicators of the finished product.

Disciplinary: Food & Beverage Technology and Production.

1 INTRODUCTION

In the category of functional products, a significant role is played by drinks, which are able not only to satisfy the body's needs for fluid but also serve as a source of deficient food components, playing the role of a tool for the prevention of alimentary-dependent diseases [2, 5, 6]. The development of functional drinks using extracts from plant raw materials containing many biologically active components remains the most promising direction in creating healthy food products [3, 4]. The inclusion of herbal extracts with antioxidant properties in beverages has a tonic effect on the body, increases the adaptability of the nervous system and the body's resistance to adverse external factors [10, 16]. Technological methods of processing plant raw materials make it possible to obtain extracts and concentrated bases containing protein components, hydrolysis products of non-starch polysaccharides, and bioactive substances [7, 8, 9, 12].

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Of particular interest is the production of protein-containing beverages, while, along with traditional dairy beverages, beverages based on the blood plasma of slaughter animals deserve special attention. One of the most valuable sources of complete animal protein is the blood plasma of slaughtered animals [13, 15].

Also, deep processing of secondary protein raw materials in the meat industry will not only allow finding an additional source of high-quality animal protein but also reduce the discharge of slaughter blood into the sewer systems, thereby reducing the environmental burden on the environment.

At the same time, one of the limitations of using blood plasma is its short shelf life. Using the heat treatment to increase the blood plasma shelf life does not give the desired bactericidal effect, because heating the product over 52-55°C is not allowed due to protein denaturation [11]. One way to solve this problem is a directed change in the structure of blood plasma proteins, which would not undergo destruction during heat treatment in the temperature range of 60-65°C. It is possible by hydrolysis to obtain amino acids and peptides that have more excellent thermal stability due to the shorter molecular length.

This study develops a technology for a whipped dessert drink based on hydrolyzed blood plasma enriched with biologically active substances of plant origin.

2 RESEARCH GOAL, METHODS, AND MATERIALS

Following the set goal, the research objects were:

- blood plasma isolated by centrifugation in a laboratory centrifuge at 8000 RPM.
- enzyme preparations: protosubtilin G10x - total proteolytic activity - 101 units/g; lysoamidase - total proteolytic activity is 37.3 U/g; megaterin G10x - total proteolytic activity is 133 units/g; protepsin - with proteolytic activity - 50, 100 and 150 U/g;
- collagenase preparation from the hepatopancreas of crab with collagenolytic activity up to 750 U/mg, according to Mandl.

Vegetable raw materials (mint, hibiscus, chickpeas, lentils) had used in an amount of 2.5 g per 100 ml of prepared hydrolyzed blood plasma of farm animals. Technical processing of the samples to obtain an extract of hibiscus was at the temperature of 70°C for 40 min because further heating leads to an undesirable color change and deterioration of organoleptic properties. To get an extract of peppermint needed two hours, since complete extraction of extractives was at this duration without deterioration in the quality indicators of the product. The technological processing of chickpeas and lentils was in accordance with the known data. Extraction was in the temperature range 60-70°C.

To get the whole raw materials indicators, semi-finished products, and finished products used, the methods, materials, and technical equipment of standard, unique, and modified research methods (Table 1). The experimental results had mathematical processing using standard statistical methods.

3 RESULTS AND DISCUSSION

One of the ways of directed changes in the structure of proteins is the splitting of proteins into smaller fragments - peptides, amino acids, which, due to their shorter molecule length, are not able to denature even under the influence of relatively high temperatures.

Table 1: Research methods.

The studied indicators	The name and essence of the method, the regulatory document
Moisture content	The thermogravimetric method by the requirements of GOST 9793-74, GOST 33319-2015 using the FD-610 moisture meter; drying on a Chizhova moisture meter following GOST R 54607.4-2015 [1]
Mass fraction of protein	The Kjeldahl method, according to GOST 25011-81
Mass fraction of fat	Soxhlet method according to GOST 23042-86, by the refractometric method and on the SER 148 device according to the instructions
Mass fraction of ash components	gravimetric method after combustion of organic substances in a muffle furnace at a temperature of 500-700 ° C for 5-6 hours to constant weight according to GOST 31727-2012
Mineral composition (magnesium, calcium, sodium, potassium)	Spectrophotometric method on an atomic adsorption spectrophotometer S-115M1 by the instructions for the device, as well as following GOST R 55484-13
Phosphorus content	the spectrophotometric method according to GOST 32009-2013
Iron content	the colorimetric method following GOST 26928-86 and atomic absorption spectrophotometer AAS-703 (Perkin-Elmer, USA), by the instructions for the device
Active acidity (pH)	potentiometric method using pH meters pH-150, S220, pH-420 according to the instructions for the devices
Amino acid composition	by ion-exchange chromatography on an automatic amino acid analyzer brand AAA-T333 (Czech Republic) with separation of amino acids on an analytical column filled with Ostion LGFA cation exchange resin with step elution with three sodium citrate buffer solutions with different pH values (3.50; 4.25; 9.50).
Vitamin composition	on a liquid chromatograph LCMS-10EV according to the instructions
Foam ratio	was as the ratio of its volume to the volume of solution used to foam form; foam stability by the time of the destruction of the foam column
Solubility index	was determined according to GOST 30305.4–95 by the amount of the resulting wet sediment after centrifugation.
Evaluation of the balance of essential amino acids	By calculation methods (according to N.N. Lipatov Jr.) [1]
Organoleptic evaluation	The method of setting a tasting score according to GOST 9959-91 and GOST 9959-2015
Determination of microbiological indicators	According to standard methods: KMAFAnM, according to GOST 10444.15-94; bacteria of the Escherichia coli group according to GOST 31747-2012; pathogenic microorganisms (including salmonella and proteus) according to GOST 31659-2012; sulfite-reducing clostridia according to GOST 29185-2014; bacteria Staphylococcus aureus by GOST 31746-2012.

For a targeted effect on blood plasma proteins (BPP), we used proteolytic enzymes of various origins. For the successful hydrolysis of proteins, it is necessary to establish the pH optimum for enzyme preparations (Figure 1).

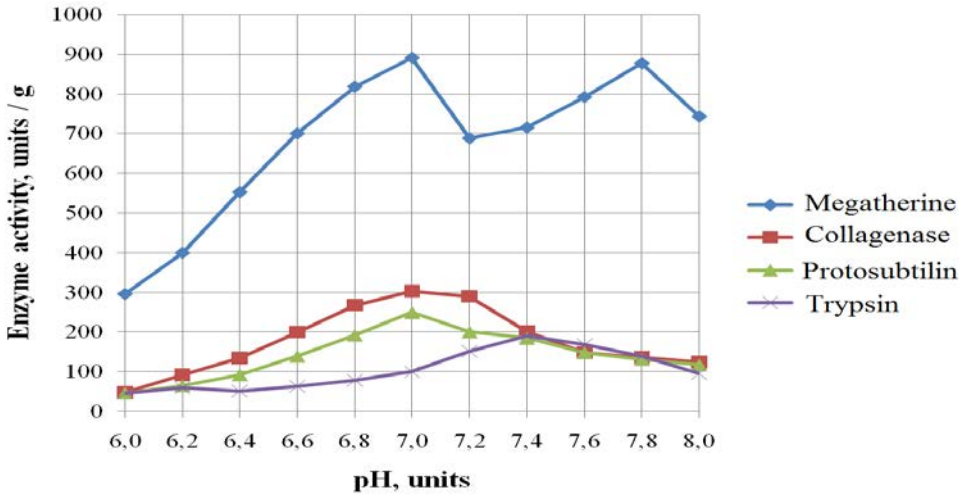


Figure 1: Dependence on the proteolytic activity of enzyme preparations on the active acidity of the medium.

Established that the enzymes exhibit maximum proteolytic activity at pH 6.9-7.1 (t = 37°C), i.e., they are neutral proteases. An exception is a trypsin, which has an optimum effect at pH 8 (t = 50°C), as well as megatheria, which is characterized by two maximum values of activity (pH 6.7-7.3; pH 7.7-8.1 at t = 37°C), which associated with the heterogeneity of the proteolytic complex of this preparation.

When assessing the hydrolytic capacity of enzyme preparations during the destruction of BPP, established that the best indicators are achieved when using collagenase (Table 1). Figure 2 shows the optimal concentration of drug in solution at a ratio of 6.8×10^{-3} g of enzyme per 1 g of the substrate.

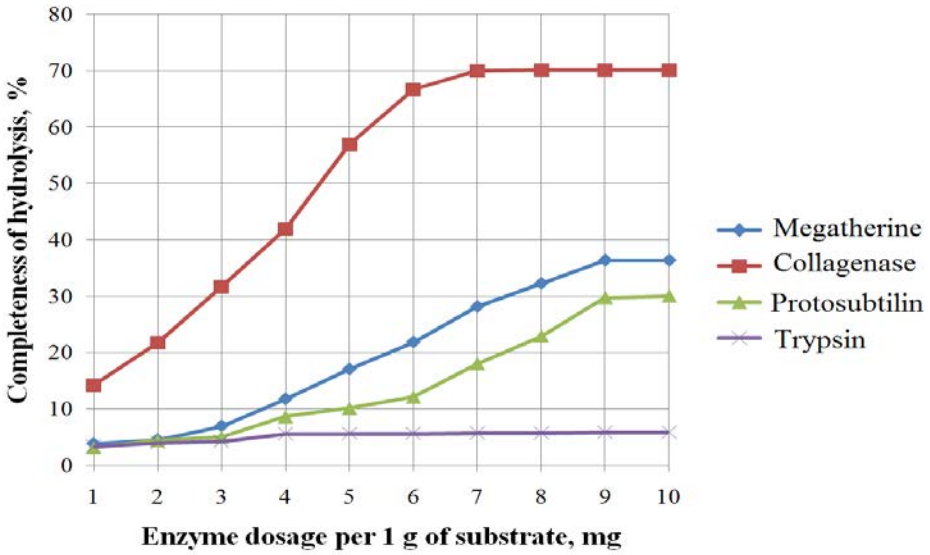


Figure 2: Dynamics of BPP hydrolysis by various enzymes.

Search for the value of the optimal duration of hydrolysis showed that during 2.8 - 3.2 hours during the hydrolysis of blood plasma by collagenase, an intensive accumulation of hydrolysis products occurs. With the further course of the process, the increase in the mass fraction of hydrolyzed protein is small (Figure 3).

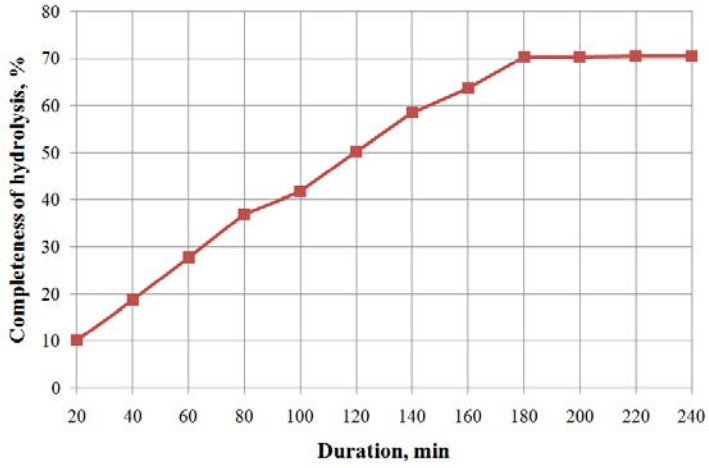
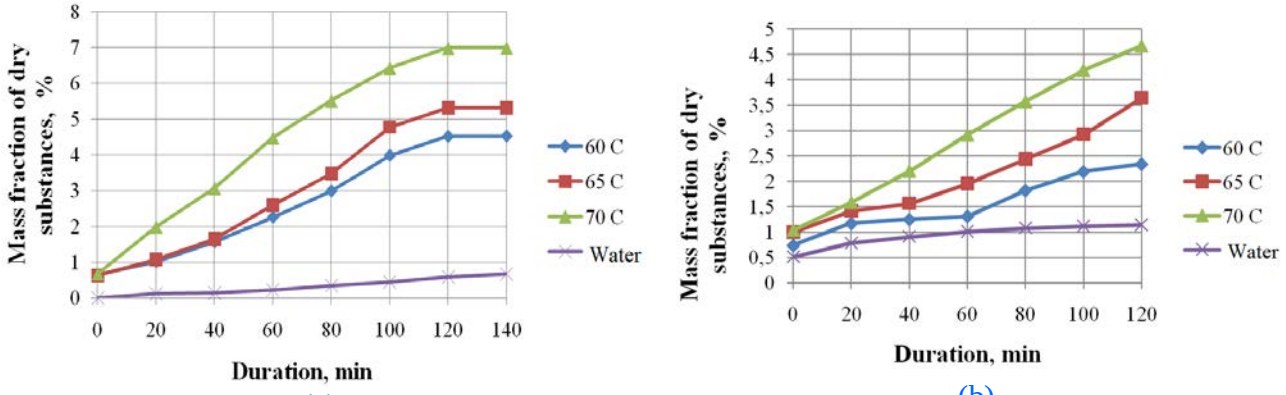


Figure 3: Dependence of the degree of enzymatic hydrolysis of BPP by collagenase on the duration of the reaction.

Thus, these studies made it possible to clarify the optimal conditions for the hydrolysis of blood plasma by the collagenase enzyme (Table 1).

4 OBTAINING BIOLOGICALLY VALUABLE EXTRACTS FROM PLANT MATERIALS

Plant extracts in drinks increase the tone of the body, the adaptive capabilities of the nervous system, the body's resistance to adverse environmental factors, and have antioxidant properties. For the experiment, we used blood plasma pretreated with an enzyme preparation with collagenase following the recommendations developed above. The extraction temperature was 70°C. The criterion for assessing the effect of factors was the mass fraction of dry substances in the extract.



(a) (b)

Figure 4: Influence of the duration of extraction on the mass fraction of dry substances in (a) peppermint extract and (b) hibiscus.

As shown in Figure 4, the degree of extraction of dry substances at a constant temperature both in hydrolyzed plasma and in water is directly proportional to the extraction time. It reaches a maximum at a duration of 120 minutes. In parallel, under the same conditions, extraction with water was carried out as a control. From the results of the experiment, it is evident that, under identical conditions, hydrolyzed blood plasma is the best extractant.

Although the main component of blood plasma is water, due to a significant amount of surfactants in the blood plasma of farm animals, its properties as a solvent change markedly. Probably, the substances present in the blood plasma, on the one hand, significantly activate the extractability of importance. That is, they increase their transfer to the blood plasma, and, on the other hand, they probably affect the permeability of the solid phase.

Probably, this explains the higher extracting ability of hydrolyzed blood plasma of farm animals in comparison with water. For sufficiently complete extraction of the solute, the extraction did several times. It is more efficient to extract the substance several times with small portions of the extractant than once with the same amount of the extractant. This principle is the basis for multiple extractions. The higher the frequency of extraction, the higher its efficiency.

Based on the experimental data obtained for the preparation of original protein extracts using the selected plant raw materials, the following extraction modes and conditions are holding time 2 hours, extraction temperature 70°C with three times infusion of plant materials.

The extraction of chickpeas and lentils was carried out in the temperature range 55-65°C because it excludes the process of denaturation of vegetable proteins. The variation of extraction process duration was from 20-120 minutes. The research way established that the highest percentage of dry

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matter recovery is achieved with an exposure of 100-120 minutes (Figure 5).

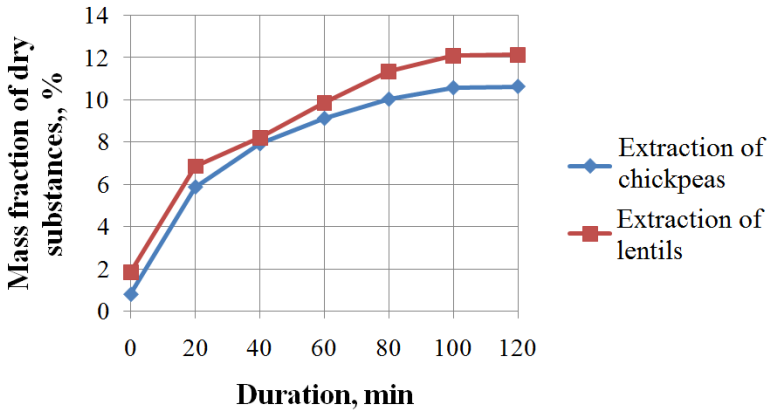

Figure 5: Influence of the extraction time on the dry matter content in chickpea and lentil extracts by hydrolyzed blood plasma

Analysis of the extraction results at different temperature conditions showed higher efficiency at 55°C. The mass fraction of dry substances in the extract of chickpea 12.5%, lentil 11%, which exceeds the level of the content of extracted essences at temperatures of 45°C, respectively, by 12% and 5%. Based on the experimental data obtained for the preparation of original combined extracts using the selected raw material, the following extraction modes and conditions should: holding time 100 min at 55°C.

5 DEVELOPMENT OF WHIPPED COCKTAIL RECIPE AND TECHNOLOGY

For the convenience of further use and increased shelf life, the obtained hydrolyzate dried on a spray dryer. That made it possible to prevent protein denaturation and ensure high solubility of the final product in water. We decided to use the dry hydrolyzate to develop a dry base for making cocktails. It contains 57% dry hydrolyzate, 39% fructose, and 4% ascorbic acid.

Dry extracts of lentil and hibiscus were selected for further research to develop the cocktail recipe. Foaming does not occur in chickpea extract because lipids are strong defoamers, and their content is higher in chickpea extract than in lentil and hibiscus extracts. Therefore, chickpea extract can be recommended for use as a protein fortifier in beverages that do not require whipping. The cocktail recipe is in Table 2.

Table 2: Cocktail recipe composition

Raw material component	Control	Experiment
Skimmed milk powder	10,0	-
Dry protein base (based on lentil extract)	-	10,0
Milk	45,0	45,0
Juice	45,0	45,0
Output	100,0	100,0

The technological scheme for producing a whipped drink is within the framework of traditional preparation technology. Still, it differs in introducing a dry base into it on the hydrolyzed blood plasma of farm animals.

The production process of a cocktail from a dry protein base is recommended organizing according to the following technological scheme, shown in Figure 8.

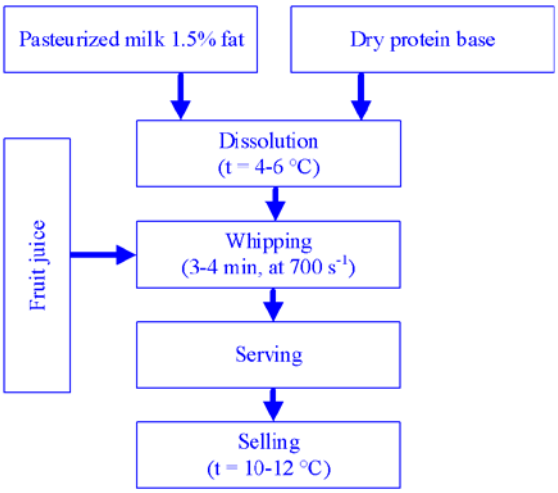

Figure 8: Technological scheme for a whipped drink (cocktail)

Table 3: Physicochemical and organoleptic characteristics of the developed drink

Indicators	Whipped drink (cocktail)	
	Control	Experiment
Mass fraction of dry substances, % not less	21.0	21.0
Protein, %	7.06	6.08
Carbohydrates, %	12.5	12.0
Acidity, °T	25.0	24.0
Ascorbic acid content, mg/100g, not less	29.58	195.65
Vitamin B_1 content, mg/100g, not less	0.34	0.78
Vitamin B_2 content, mg/100g, not less	0.23	0.44
Vitamin Ca content, mg/100g, not less	54.1	61.18
Vitamin Mg content, mg/100g, not less	3.26	7.11
Mass fraction of vitamin P, mg/100g, not less	11.6	34.4
Mass fraction of vitamin Fe, mg/100g, not less	0.011	0.025
Taste and smell	Pleasant, sweet and sour, fruity, without foreign tastes and smell	Pleasant, sweet and sour, fruity, without foreign tastes and smell
Colour	Color characteristic of the juice used	Color characteristic of the juice used
Structure and consistency	Foamy	Foamy

Because of plant components, products based on extracts enriched with vitamins, minerals (table 3), it also noted an increase in the content of essential amino acids (Table 4). The experimental sample is not inferior to the control one in terms of protein content. At the same time, it contains a significantly higher amount of vitamins and minerals. To assess the biological value of the dessert product was studying its amino acid compositions.

Table 4: The biological value of the protein of the developed drink

Amino acids, g / 100 g protein	Whipped drink (cocktail)	
	Control	Control
Valine	0.41	0.52
Isoleucine	0.55	0.6
Leucine	0.82	0.93
Lysine	0.97	0.93
Methionine + cystine	0.25	0.43
Threonine	0.62	0.73
Phenylalanine + Tyrosine	0.63	0.78
Tryptophan	0.54	0.88

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The amino acid composition showed that the content of essential amino acids in the protein of the test sample is higher, which indicates its higher biological value. Figure 9 shows the finished product of whipped drink based on biomodified blood plasma.

Figure 9: The finished product of whipped drink based on biomodified blood plasma.

6 CONCLUSION

The methods of biomodification of blood plasma by enzymatic hydrolysis have been studied. Established that using collagenase enzyme preparation promotes the most effective hydrolysis of plasma proteins under the following process conditions: temperature 36-38°C, active acidity 6.7-7.1 units, the concentration of the enzyme preparation 0.36%, duration 2.8-3.2 hours. The optimal conditions for extractives from plant materials have been determined: for hibiscus and mint, temperature 70°C, duration 120 min, for chickpeas and lentils, temperature 60-65°C, for 100 min.

A composition of a dry protein supplement for whipped drinks has been developed, including, in addition to the dry extract, fructose, and ascorbic acid. There proposed a recipe and a technological scheme for producing a new whipped drink using a dry protein base. The study of quality indicators showed that this product has high nutritional, biological value, and organoleptic characteristics.

7 AVAILABILITY OF DATA AND MATERIAL

Information can be made available by contacting the corresponding author.

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