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Technological Features of Obtaining an Antianemic Product with the Maximum Heme Iron Content

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Abstract

The stages of the technological process for obtaining an antianemic product from the pork and cattle blood were selected. The efficiency of the technological process was proved by the physicochemical parameters' measuring method. Microbiological indicators were studied. The antianemic product is a mixture of heme iron and aminopeptide complexes. The formulation of the finished product is selected, which is presented in 12 versions. The antianemic product is presented in dry form and as a concentrate (viscous liquid). Studies were conducted on the finished product shelf life selection.

***Keywords*:** Erythrocyte mass, pork blood, cattle blood, heme iron, fractional composition, physicochemical parameters, shelf life, technology.

1. INTRODUCTION

Nowadays the development of the meat-processing industry has positive dynamics and is characterized by the introduction of innovative technologies. However, such trend as processing the blood of slaughter animals has certain difficulties, the overcoming of which will allow obtaining various products. In this case, one of the most common products is the one for the prevention of iron deficiency of the human, which can be described as an "antianemic product." Thus, this line of research on the slaughter blood processing is very relevant and in high demand [1].

It should be taken into account that the erythrocytes are the iron-containing base in the blood, since they contain the hemoglobin protein, comprising the bivalent iron [2]. The use of this form of iron is a prerequisite for the creation of an effective antianemic product, also on the basis of some microbes [2].

The main drawback of common drugs for iron deficiency prevention is the risk of getting an excess of allergenic components represented by leukocytes and modifications thereof that are present in the blood of agricultural animals [3, 4].

The previous works of the authors presented the results of the technology development for a product containing an easily digestible form of iron from the blood of slaughter animals. The papers described the technology of blood stabilization with the subsequent fractionation for the maximum allocation of red blood cells from the total mass of blood. The regimes for conducting hydrolysis for the maximum cleavage of the protein components of erythrocytes were described [5, 6]. All the results obtained were relevant under condition of development in the laboratory.

The scope of the work was to conduct research in the conditions of the current production in order to identify technological features of obtaining antianemic products with the maximum content of heme iron from the blood of farm animals (cattle and pork blood). Based on the results achieved earlier and the goal of the work, the following research objectives were formulated:

1. Selection of technological stages and technological regimes for obtaining an antianemic product.
2. Selection of antianemic products formulation taking into account the features of technological regimes and stages.
3. Study of the effectiveness of the finished antianemic product. For this, it is necessary to study the physicochemical, microbiological and toxicological parameters.
4. Conduction of the studies on the finished product shelf life selection.

The subsequent sections of the article reflect the results, the discussion, and the conclusion of the research on which an inference can be made that the formulated objectives are achievable.

2. METHODS

The following were used as the objects of research:

- Whole cattle and pork blood (the choice in their favor was made due to their greatest distribution among farm animals and also taking into account a sufficiently large volume of blood in these animals);
- Acetic acid, edible, as per GOST 6968-76;
- Citric acid, edible, as per GOST 908-2004;
- 5.5 aqueous sodium citrate, edible as per GOST 31227-2004;
- Drinking water as per GOST 2874-82;
- Ancillary feedstock and materials that meet the

requirements of the existing documentation or obtained by import (sodium hydroxide and potassium hydroxide, hydrochloric acid, citric acid, mineral salts) and allowed for use in the food industry.

The mass fraction of dry substances and the mass fraction of moisture were determined as per GOST 33319-2015.

The average molecular weight of the aminopeptide complexes was determined by the Lamley method. The mass fraction of iron was identified as per GOST 26928-86.

The content of mesophilic aerobic and facultative-anaerobic microorganisms was determined as per GOST 10444.15-94, coliforms - as per GOST R 52816-2007, yeast and mold fungi - as per GOST 10444.12-88. Salmonella content was defined as per GOST R 50480-93 by inoculation on the Kaufmann culture medium, followed by inoculation on the Endo's medium. The sulfite-reducing clostridia were determined as per GOST 29185-91.

3. RESULTS

In carrying out research on the selection of technological stages and technological regimes for obtaining an antienamic product, the emphasis was placed on the technological features of the actual production conditions. Schematic representation of the technological stages with the indication of the modes is shown in Figure 1. The technology is a chain of interrelated stages, each of which has its own technological regimes.

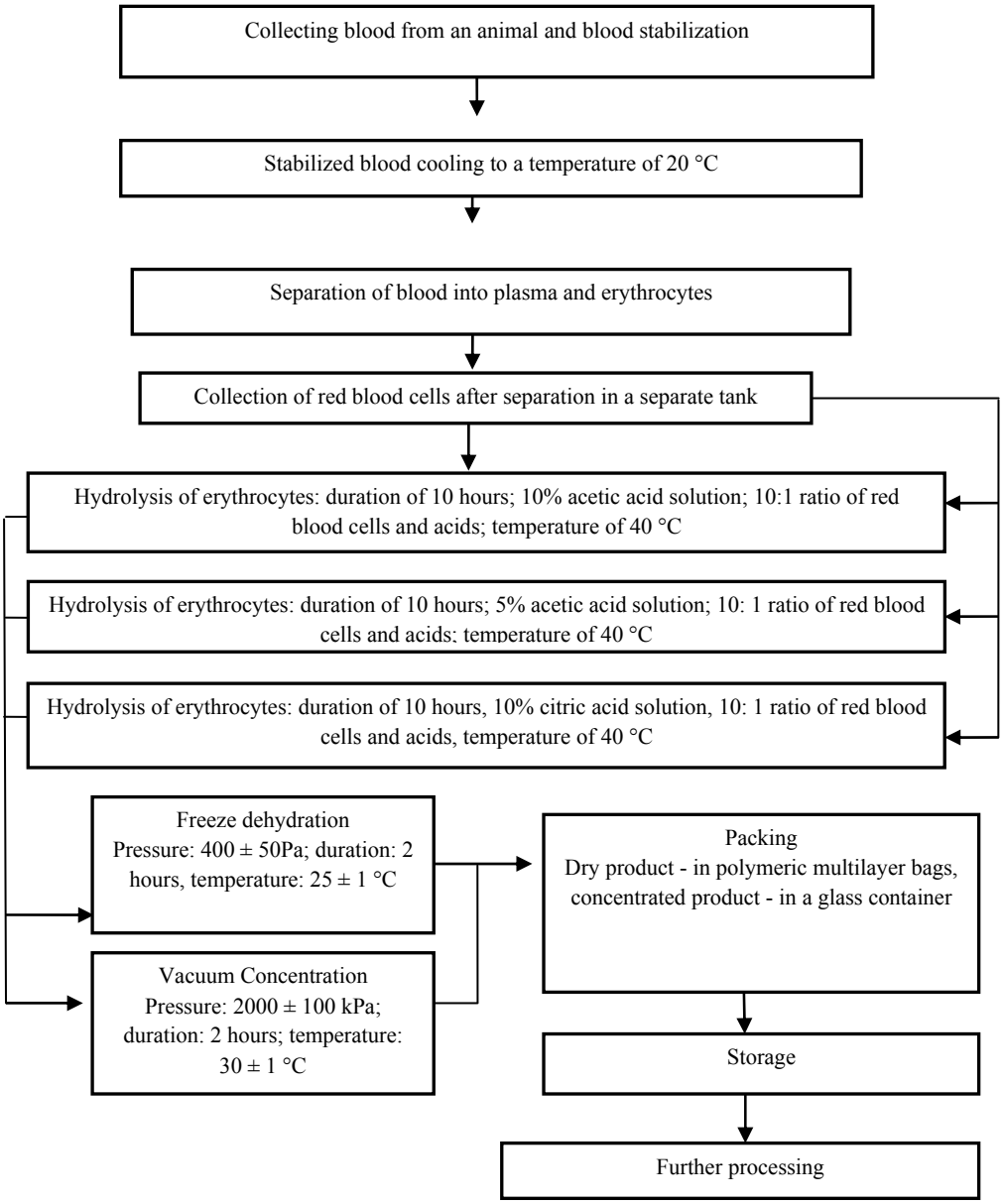

Figure 1. Process scheme for the production of an antianemic product from the cattle and pork blood

Table 1. Antianemic product formulations, kg

Components	Varieties					
	P KRS L 10%	P KRS U 5%	P KRS U 10%	S L 10%	S U 5%	S U 10%
blood	10.5	10.5	10.5	10.0	10.0	10.0
Sodium citrate	0.046	0.046	0.046	0.040	0.040	0.040
Citric acid for stabilizer	0.0016	0.0016	0.0016	0.0014	0.0014	0.0014
Citric acid for hydrolysis	0.12	-	-	0.10	-	-
Acetic acid	-	0.58	0.12	-	0.50	0.10
Water	2.15	2.15	2.15	2.05	2.05	2.05

The formulation components were selected during the selection of technological regimes. Table 1 describes six formulation options. Raw materials in the form of cattle and pork blood were taken into account in the selection of formulation components. The formulation options also depended on the hydrolysis method, namely, which acid was used for hydrolysis. Given that two types of product were used for each of the six options, namely dry and concentrated ones, twelve options of antianemic product were developed.

After the technology development and the selection of the finished product formulation, the physicochemical indices of the finished antianemic product were investigated, while the properties of the dry product and the concentrated one were separately studied. The results of the research are presented in Tables 2 and 3. To understand the degree of hydrolysis, the average molecular weight of aminopeptide complexes was investigated, and the mass fraction of iron, dry matter and the mass fraction of moisture were also studied.

Further, microbiological indices were studied in the finished antianemic product. Table 4 presents the results of these studies for the dry product, and Table 5 - for the concentrated product.

In the research of microbiological indicators, the emphasis was placed on the study of the presence of those microorganisms that are most characteristic for the product being developed. The obtained results were checked against the standards approved by Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor).

Further studies were carried out on the dynamics of the shelf life of the finished product. The results are presented in Table 8.

When investigating the shelf life, the dynamics of the development of microbiological indices during storage served as a landmark.

Table 2. Physicochemical parameters of dry antianemic product

Index	Value					
	Product obtained from cattle blood			Product obtained from pork blood		
	Citric acid, 10%	Acetic acid, 5%	Acetic acid, 10%	Citric acid, 10%	Acetic acid, 5%	Acetic acid, 10%
Mass fraction of moisture,%, below	2.5	2.5	1.5	1.5	1.5	1.5
Mass fraction of solids,%, above	97.5	97.5	98.5	98.5	98.5	98.5
Mass fraction of iron, %	5.5	6.8	6.9	7.0	7.6	8.0
Average molecular weight of aminopeptide complexes, kDa	22.6	22.4	23.7	22.0	22.4	25.5

Table 3. Physicochemical parameters of the concentrated antianemic product

Index	Value					
	Product obtained from cattle blood			Product obtained from pork blood		
	Citric acid, 10%	Acetic acid, 5%	Citric acid, 10%	Acetic acid, 5%	Citric acid, 10%	Acetic acid, 5%
Mass fraction of moisture, %, below	23.5	22.5	22.0	20.0	20.0	20.0
Mass fraction of solids, %, above	76.5	77.5	78.0	80.0	80.0	80.0
Mass fraction of iron, %	4.0	4.2	5.0	4.4	5.5	5.8
Average molecular weight of aminopeptide complexes, kDa	22.6	22.4	23.7	22.0	22.4	25.5

Table 4. Microbiological indices of dry antianemic product

Index name	Standard	Actual
QMA&OAMO CFU/g, below	$5*10^5$	$3.0*10^5$
Bacteria of the E. coli group (coliforms) in 0.1 g	not allowed	not detected
Pathogenic microorganisms, incl. bacteria of the Salmonella genus in 25 g	not allowed	not detected
Sulfite-reducing clostridia in 1.0 g	not allowed	not detected
Molds, CFU/g, below	not allowed	not detected

Table 5. Microbiological indices of a concentrated antianemic product

Index name	Standard	Actual
QMA&OAMO CFU/g, below	$5*10^5$	$2.5*10^5$
Bacteria of the E. coli group (coliforms) in 0.1 g	not allowed	not detected
Pathogenic microorganisms, incl. bacteria of the Salmonella genus in 25 g	not allowed	not detected
Sulfite-reducing clostridia in 1.0 g	not allowed	not detected
Molds, CFU/g, below	not allowed	not detected

Table 8. Dynamics of microbiological indices of a dry and concentrated antianemic product

Duration of storage, days	Microbiological indicators							
	Coliforms		Pathogenic microorganisms		Sulfite-reducing clostridia		Molds, CFU/g	
	dry	concentrate	dry	concentrate	dry	concentrate	dry	concentrate
0 (background)	No	No	No	No	No	No	No	No
10	No	No	No	No	No	No	No	No
20	No	No	No	No	No	No	No	No
30	No	No	No	No	No	No	5	No
40	No	No	No	No	No	No	12	No
50	No	No	No	No	No	No	15	4
60	No	No	No	No	No	No	60	10

4. DISCUSSION

Blood collection was carried out in a contactless way, i.e. preventing contact with atmospheric oxygen. This allows avoiding the activation of the blood coagulation enzymatic process. This process is carried out using hollow knives, through which blood enters the tanks for stabilization with the help of the pumping system. In these tanks, by means of special blades, the blood is mixed with stabilizer solution, which represents a mixture of solutions of 4% sodium citrate and 0.07 % citric acid; the weight fraction of the citric acid solution of the total stabilizer weight is not more than 0.035%. The stabilized blood enters the coolers, where it is cooled to the temperature of 20 °C. After that, chilled blood is necessarily checked for its quality indicators as per the current documentation.

Blood separation is carried out on a special separator, which allows monitoring the "separation factor" indicator (it should not exceed 2,000 units). The duration of the process is 5 minutes for both pork and cattle blood. In this case, the rotation speed of the separator drum will depend on the internal radius of the separation plates, so the rotation speed must be selected according to the calibration curve taking into account the size of the separation factor. After the separator, the red blood cells separated from the total blood mass enter the section through the piping system, where hydrolysis will be carried out.

Hydrolysis of erythrocytes is carried out using one of three options, the choice of which depends on the requirements to the quality indicators of the finished product.

The first hydrolysis option is carried out with 5% acetic acid, the erythrocytes and acid ratio is 10:1, process temperature is 40 °C, duration is 10 hours. The second hydrolysis option is carried out with 10% citric acid, the erythrocytes and acid ratio is 10:1, process temperature equals to 40 °C, duration is 10 hours. The third hydrolysis option is carried out with 10% acetic acid, the ratio of erythrocytes and acid ratio is 10:1, process temperature is 40 °C, duration is 10 hours.

Further, the process of freeze dehydration and vacuum concentration begins. Drying is carried out on the drying unit, in which the method of sublimation dewatering is implemented. The process is carried out at a drying chamber with pressure of 400 ± 50 Pa for the production of dry antianemic product, and 2000 ± 100 Pa - for the production of concentrated (liquid) one. The process temperature for obtaining dry antianemic product is 25 ± 1 °C and 30 ± 1 °C for the preparation of a concentrated one. In both cases the process duration is 2 hours.

Then the final stage - packing of the finished product - follows. The dry antianemic product is packed in the polymer bags on the packaging unit, the concentrated antianemic (liquid) product is first poured into glass containers and then sealed on the glass containers sealing unit. After that the packaged product is delivered to the storage warehouse. The following storage parameters

should be maintained in the warehouse: the temperature of 4 ± 1 °C and relative humidity of air below 75%.

The formulations of an antianemic product are shown in Table 1. Developed products can be produced in 12 versions. The difference in this case is formed on the basis of raw materials, either cattle or pork blood, and also depends on the acid used in hydrolysis, either acetic at concentrations of 5% and 10%, or 10% citric acid.

Physicochemical, microbiological indicators, and the content of toxic elements are the main properties of food and preventive products, which determine good consumer quality indicators and cause high demand for the finished product. Therefore, the next stage of research was the study of physicochemical indicators of finished products.

The results presented in Tables 2 and 3 indicated the effectiveness of the selected antianemic product. At the same time, it should be noted that high indicators are characteristic for both dry and concentrated (liquid) product. The mean molecular weight of the aminopeptide complexes of dry and concentrated antianemic products corresponds to the 22.0 ÷ 25.5 kDa range. These values confirm the effectiveness of the hydrolysis carried out, since there are no whole proteins. The proportion of iron corresponds to a range of 4.0 ÷ 5.8% for the concentrated product and 5.5 ÷ 8.0% for the dry product. The higher iron content in the dry product compared to the concentrated product is due to the large amount of residual moisture in the finished product. It should also be noted that the iron content is greater in the product obtained on the basis of pork blood.

During the research of microbiological indicators, the goal was to control the technological process and the sanitary and hygienic conditions of production. The results are shown in Table 4 for the dry antianemic product and in Table 5 for the concentrated (liquid) antianemic product. The results show the compliance with all necessary standards, which also confirms the efficiency of the process and its hygiene.

In the studies of resistance to deterioration during the storage of the product, microbiological indices were studied. The results are presented in Table 8.

The dry antianemic product showed good resistance in terms of the index of pathogenic microorganisms. Bacteria of the Salmonella genus were not detected during the entire shelf life at a temperature of 20 ± 2 ° C. Coliforms were also not detected in 0.1 g throughout the shelf life, the same applies to sulfide-reducing clostridia in 1.0 g. Molds show growth on the fiftieth day of storage and, with continued storage, under equal conditions, the mass fraction of these indicators gradually increases over time. Considering that molds start exceeding the threshold level of permissible values on the fiftieth day of storage, the following conclusion can be drawn: the shelf life, taking into account the reserve ratio for perishable products, should not exceed 40 days.

Analysis of the data in Table 9 shows that for a concentrated (liquid) antianemic product, pathogenic microorganisms, including bacteria of the Salmonella genus in 25 g, are not detected during the entire storage period at a temperature of 20 ± 2 °C. The same applies to the storage period under consideration for coliforms in 0.1 g and sulfite-reducing clostridia in 1.0 g. The growth of the mass fraction of molds is observed on the thirtieth day of storage and then this index gradually increases over time. Considering that molds start exceeding the threshold level of permissible values on the thirtieth day of storage, the shelf life should not exceed 25 days.

5. Conclusion

There were selected the technological stages of obtaining an antianemic product from the blood of two species of animals: cattle and pork. When choosing the technology stages, a number of factors were taken into account. The blood should be stabilized by a specially developed stabilizer with preservation effect (4% sodium citrate solution, 99.965% mass fraction in the preservative stabilizer, and 10% citric acid solution, mass fraction in the preservative stabilizer - 0.035%. The dose of "preservative stabilizer" introduction into the blood equals to 1:10). Hydrolysis of erythrocytes was carried out with food acids (citric acid 10%, acetic acid 10%, acetic acid 5%); the drying - with the thermolabile way (sublimation).

The effectiveness of the technology was proved by the results of physical and chemical studies. The iron mass fraction was quite high (from 4% to 5.8%), and the average molecular weight of the aminopeptide complexes (22.0 ÷ 25.5 kDa) proved the effectiveness of the hydrolysis process. Microbiological indicators were within the normal range. Studies were conducted on the finished antianemic product shelf life calculation.

In the future, the developed antianemic product will be introduced into classical food products to give them functionality in the field of preventing iron deficiency states of the human.

The goals and objectives of this study have been achieved.

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