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Hydrolysis of the Red Blood Cells of Pig and Cattle to Ensure Optimum Conditions for the Manufacturing of Iron-Containing Products Having Maximum Heme Iron

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Abstract

The study parameters of the hydrolysis of red blood cells of pig's blood and the blood of cattle are presented. We investigated the dependence of the yield of amine nitrogen, the degree of hydrolysis in dependence of the duration of the process, the version of an acid used for the hydrolysis, and the temperature of the process. An analysis of the fractional composition of the amine-peptide mixture after hydrolysis of erythrocytes was conducted. The choice of the optimal parameters of the hydrolysis process for the extraction of iron-containing mixture with a maximum content of heme iron and the separating of the protein fractions of erythrocyte mass on the amino-peptide mixture was justified.

Keywords: Erythrocyte mass; Pig's blood; The blood of cattle; Heme iron; Amino nitrogen; Acid hydrolysis; Degree of hydrolysis; Fractional composition

Introduction

The development of new and improved traditional technologies in the meat processing industry is aimed at improving the quality and safety of products, giving them new properties and improving consumer properties, as well as reducing their cost.

An important factor in the sphere of processing of raw materials of the meat industry is the processing of nontraditional sources of raw materials [1,2], for example, processing the blood of slaughtered animals for obtaining products for the prevention of iron deficiency.

The iron-containing base in the blood is the erythrocytes, which contain the protein hemoglobin [3]. The difficulties that are characteristic of the methods of isolation of hemoglobin from the blood of slaughtered animals, hinder the extraction of hemoglobin of high purity, because it increases the cost of its extraction and as a consequence increases the cost of the final product, which is used for the treatment and prevention of anemias [4-7].

Many drugs that are based on the blood products of slaughtered animals (such as hematogenous and pantohematogen) and used for the prevention of iron deficiency do not use the hemoglobin extract but the nutritional albumin, which is derived from whole blood. It does not lead to high costs of production. However, in this case the human body is at risk of obtaining excessive amounts of allergenic components, represented by leukocytes (white blood cells) and their modifications, which are present in the blood of farm animals [8,9].

There's a remedy obtained by the hydrolysis of whole blood that contains leukocytes and their modifications. It is a mixture of peptides and amino acids. The hydrolysis is carried out by enzymatic way, preventing severe destruction of amino acids and removing toxic compounds. However, many technologically unjustified operations are used in this process, which lead to large economic costs for the implementation of this technology.

Free germs when released into the gastrointestinal tract of the body will not require the additional efforts of the digestive system for its release from the protein of hemoglobin. In addition, the use of hydrolysis will help to bypass a number of labor-intensive and costly operations and will improve the efficiency of production of iron supplements from the blood of slaughtered animals [10].

The aim of this work is to assist in the selection of parameters for the hydrolysis of red blood cells of pigs and cattle for the extraction of easy digestible heme iron.

Methods

Materials

The theoretical and experimental research was conducted in accordance with the tasks of the Research and Education Center and the Department of Bionanotechnology of a federal state budget educational institution of higher education, Kemerovo Technological Institute of Food Industry."

The following objects were used in the research:

- whole blood of cattle and pigs;
- dietary acetic acid according to the federal standard, GOST 6968 – 76;
- dietary citric acid according to GOST 908 – 2004;
- 5,5 aqueous nutritional sodium citrate according to GOST 31227 – 2004;
- drinking water according to GOST 2874-82;
- auxiliary raw materials that met the requirements of the applicable documents or materials produced by import (sodium hydroxide and potassium hydroxide, hydrochloric acid, citric acid, mineral salts) and were approved for application in the food industry.

Determination of amine nitrogen content and the degree of hydrolysis

For the quantitative determination of mass fraction of amine nitrogen, the method of Lowry was used. Two reactions are combined in this method. The first is the biuret reaction.

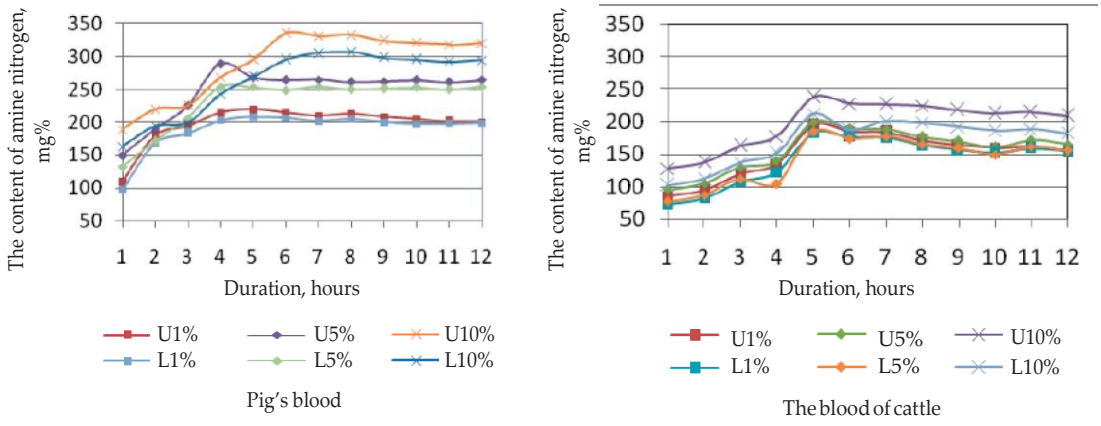

Figure 1: Output of amino nitrogen during hydrolysis

In the second reaction (Pholin's reaction) is involved a reagent discovered by Pholin-Ciocalteu. The blue-colored complexes of phosphorus-tungsten and phosphorus-molybdenum acid are formed under the action of tyrosine and tryptophan, the amino acids of the proteins. The degree of hydrolysis was determined as the ratio of amine nitrogen to the total nitrogen.

Determination of fractional composition

The fractional composition of the hydrolysate of red blood cells was assessed by the electrophoresis method in polyacrylamide gel (PAAG) by the method of Laemmli.

Results

Due to the fact that the main focus of the end product is the prevention of human iron deficiency, dietary acids were used for the hydrolysis. The final product should be a mixture of heme iron and amino-peptide complexes of high digestibility. The stage of neutralization and filtration is not usually used for reducing the loss of amine nitrogen and heme iron. The hydrolysis was carried out with acetic (U) and lemon (L) acids with concentrations of 1, 5, and 10%. The ratio of erythrocytes of blood to acid is 10:1. The efficiency of hydrolysis was controlled by the number of amine nitrogen, by the content of the fractions, and by the degree of hydrolysis.

The dynamics of amine nitrogen yield during the conduction of hydrolysis with the selected concentrations is presented in Figure 1.

Also, studies of polypeptide composition of the finalized hydrolysates by electrophoresis were conducted. The data is presented in Figures 2 and 3 and Tables 1 and 2.

Also the studies were aimed for the choice of a temperature mode of hydrolysis. Experiments were performed with the use of the most optimal variants of acids—L10%, U5%, and U10%. The following temperature mode was chosen, which is close to the recommended level: 30, 35, and 40^{8}C. The efficiency of the process was controlled by the degree of hydrolysis of erythrocytes.

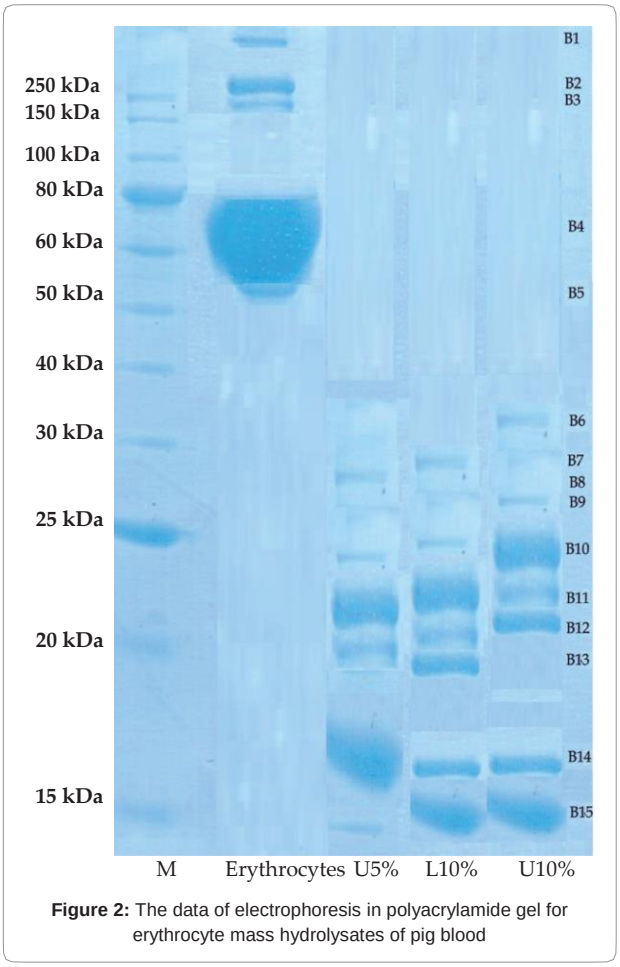

Figure 2: The data of electrophoresis in polyacrylamide gel for erythrocyte mass hydrolysates of pig blood

Discussion

Analyzing the data of Figure 1, we can draw the following conclusions.

In the acid hydrolysis of pig blood and cattle blood, the dynamics of amine nitrogen yield is characterized by two periods: the period of a constant increase in the quantitative content of amine nitrogen and the relatively stable period of this content for 6-8 h for pig's blood and 7 h for the blood of cattle. However, there is a difference in the dynamics of the release of the amine nitrogen by the different groups of animals. During the hydrolysis of the blood of cattle, there is the highest jump of the values corresponding to the duration of the process—5 h, followed

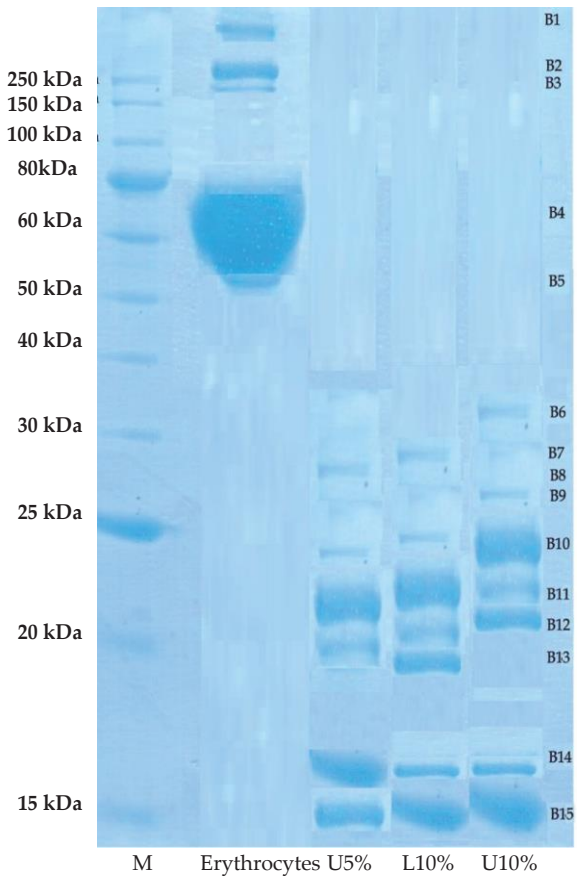

Figure 3: The data of electrophoresis in polyacrylamide gel for erythrocyte mass hydrolysates of cattle blood

Number of stripe	Molecular weight, kDa	% of the total protein content		
		U5%	L10%	U10%
B6	33.2	–	–	1.6
B7	28.6	–	6.8	0.6
B8	27.2	4.2	–	–
B9	26.3	3.7	–	1.4
B10	24.0	3.3	2.4	29.9
B11	22.1	29.0	25.5	19.1
B12	21.5	–	14.4	10.0
B13	19.5	20.5	13.1	–
B14	16.8	37.0	10.4	9.0
B15	14.0	2.3	27.4	28.4

Table 1: Actual values and a description of the detected fractions of the electrophoretic analysis for the hydrolysate of erythrocyte mass of a pig's blood

by the reduction of the quantitative content of amine nitrogen. The highest yield of amine nitrogen is observed in the hydrolysis by acids L10% at a level of 212 mg% and U10% at a level of 238 mg%. By the end of the hydrolysis process (12 h), the content of amino nitrogen is at a level of 183 mg% for L10% (fall of the amine nitrogen is 13.6%) and 209 mg% for U10% (fall of the amine nitrogen is 12.2%). Thus, for the blood of cattle, the maintenance of hydrolysis of more than 5 h leads to a decrease in the number of amine nitrogen, which is undesirable. For the pig's blood, the highest value of the amine nitrogen is also typical for

Number of stripe	Molecular weight, kDa	% of the total protein content		
		U5%	L10%	U10%
B6	32.3	–	–	2.1
B7	28.6	–	3.2	–
B8	27.9	2.5	–	–
B9	26.5	1.3	1.2	1.3
B10	24.0	2.4	2.8	28.8
B11	22.1	26.0	29.5	16.2
B12	21.5	–	14.4	12.8
B13	19.5	20.5	13.1	–
B14	16.3	25.0	9.4	9.4
B15	14.0	22.3	26.4	29.4

Table 2: Actual values and a description of the detected fractions of erythrocyte mass hydrolysates of cattle blood by electrophoresis

Acid	The consistency of the hydrolysate	The color of the hydrolysate	Smell
U5%	Viscous liquid	Brown with a red tint	Sour, without foreign odors
U10%	Viscous liquid	Dark chocolate brown	Sour, without foreign odors
L10%	Viscous liquid	Chocolate brown	Sour, without foreign odors

Table 3: The results of organoleptic evaluation of the finalized hydrolysates

acids L10% (307 mg%) with a runtime of 8 h and for U10% (335 mg%) with a runtime of 6 h. Then there is a slight decrease in the content of amine nitrogen. If the duration of hydrolysis is 12 h with the use of L10%, the amino nitrogen is at a level of 294 mg% (loss of the amine nitrogen compared to the maximum content is 4.2%), and with the use of U10%, the amino nitrogen is at a level of 320 mg% (amine nitrogen losses amount to 4.5%). Such losses of the amine nitrogen can be considered as noncritical. The results of the amine nitrogen release, obtained by the hydrolysis with L1%, L5%, U1%, and U5%, have a positive result but are less effective than with L10% and U10%.

During the study of polypeptide composition of the finalized hydrolysates, the amino-peptide mixture was considered, and in both cases, for pig's blood and for blood of cattle, it was characterized by the existence of fractions that did not exceed a weight of 35 kDa (Figures 1 and 2). The amino-peptide mixture obtained from pig's blood had fractions of different masses in its composition, depending on the acid used during hydrolysis. While using the acid U5% (Table 1), the mixture composed of the following fractions: 22.1 kDa (largest weight), 19.5 kDa, 16.8 kDa, and 14.0 kDa. The acid L10% was characterized by the presence of fractions with masses of 22.1 kDa, 21.5 kDa, 19.5 kDa, and 14.0 kDa. For acid U10%, the masses were 24.0 kDa, 22.1 kDa, and 14.0 kDa.

The amino-peptide mixture obtained from the blood of cattle (Table 2) has also in its composition fractions of different masses, depending on the acid used during hydrolysis. While using the acid U5%, the mixture composed of the following fractions: 22.1 kDa (largest weight), 19.5 kDa, 16.3 kDa, and 14.0 kDa. The acid L10% is characterized by the presence of fractions with masses of 22.1 kDa, 21.5 kDa, 19.5 kDa, and 14.0 kDa. For the acid U10%, the masses were 24.0 kDa, 22.1 kDa, 21.5 kDa, and 14.0 kDa (Table 1).

Temperature mode of red blood cell hydrolysis of pig's blood

At a temperature of 30ºC, the highest degree of hydrolysis was achieved with the use of acid U10% and was at the level of 29%, and for

acids L10% and U5%, the value of the degree of hydrolysis did not rise above 26.5% at this temperature. For all acids the maximum value of the degree of hydrolysis was reached within 10 h and had a relative stability during the subsequent 2 h of the process.

The results of changing the degree of hydrolysis at a temperature of 35ºC have the same effect as at 30ºC, and there are two periods: the first period has a constant increase and the second period has a relatively stable level of degree of hydrolysis. For the acid U5% the first period lasts for the first 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at the level of 29.8%. For the acid L10% the first period lasts for 10 h, the second period lasts over the next 2 h, and the maximum value of the degree of hydrolysis is at a level of 27.0%.

At a temperature of 40ºC a similar pattern is observed. For the acid U5% the first period lasts for 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at a level of 30.2%. For the acid L10%, the first period lasts for 10 h, the second period lasts over the next 2 h, and the maximum value of the degree of hydrolysis is at the level of 27.5%. For the acid U10% the first period lasts for 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at the level of 31.0%.

Temperature mode of red blood cell hydrolysis of cattle

At a temperature of 30ºC the highest degree of hydrolysis was achieved by using acid U10% with the level of 30.3%, and for acids L10% and U5%, the value of the degree of hydrolysis did not rise above 28.0% at this temperature. For all acids the maximum value of the degree of hydrolysis was achieved in 10 h and had a relative stability during the following 2 h.

The value of hydrolysis degree at a temperature of 35ºC is the same as at 30ºC. There are two periods: the first period has a constant increase and the second period has a relatively stable level of hydrolysis degree. For the acid U5% the first period lasts for 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at the level of 29.8%.

For the acid L10% the first period lasts for 10 h, the second period lasts over the next 2 h, and the maximum value of the degree of hydrolysis is at the level of 27.4%. For the acid U5% the first period lasts for 9 h, the second period lasts over the remaining 3 h, and the maximum value of the degree of hydrolysis is at the level of 31.8%.

At a temperature of 40ºC, any significant changes in the dynamics of the degree of hydrolysis were not available. For the acid U5% the first period lasts for 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at the level of 30.2%. For the acid L10%, the first period lasts for 10 h, the second period lasts over the remaining 2 h, and the maximum value of the degree of hydrolysis is at the level of 27.9%. For the acid U10% the first period lasts for 9 h, the second period lasts over the next 3 h, and the maximum value of the degree of hydrolysis is at the level of 32.3%.

By the end of the hydrolysis process in all three cases (L10%, U5%, and U10%), we have a viscous liquid with a characteristic sour odor without any additional smell. In the case of U5% the liquid had a brown color with a red tint, in L10% the liquid had a chocolate brown color, and in the case of U10% the hydrolysate had a dark chocolate brown color.

Conclusions

Due to the fact that the magnitude of the degree of hydrolysis depends on temperature, the difference in the conduction of a process at a temperature of 35 and 40ºC is not more than 2%. It can be considered more optimal to apply a temperature of 35ºC during hydrolysis of erythrocyte mass of pig blood.

For the hydrolysis it is possible to use the best three options. According to the first case the process of hydrolysis is conducted with 5% acetic acid, the ratio of the erythrocytes of blood to acid is 10:1, and the process temperature is 35ºC, with a duration of 12 h.

According to the second case, the hydrolysis is conducted with 10% citric acid, and the ratio of the erythrocytes of blood to acid is 10:1, at a temperature of 35ºC with a duration of 12 h.

According to the third case, the hydrolysis is conducted with 10% acetic acid, the ratio of the erythrocytes of blood to acid is 10:1, and the process temperature is 35ºC, with a duration of 12 h.

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