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PROCESSING OF SLAUGHTERHOUSE BLOOD FOR ANTIANEMIC FOOD PRODUCTS

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ABSTRACT: Currently, rational processing of blood from slaughterhouses remains as a waste fluid in many regions. Traditional approaches to use the blood for food are significantly limited because of specific and non-favorable organoleptic characteristics. Present study provides a comparison of various methods for modifying the red blood cell (RBC) mass of animals and a more in-depth study of acid hemolysis. The solution of ascorbic acid has been proposed as a hemolyzing agent. There has been established experimentally that the addition of equal volumes of RBC and a solution of an ascorbic acid with a concentration of 0.75 mol/dm^3 can effectively destroy the stroma more up 90% of red blood cells within 15 minutes. By this, the hemoglobin oxidation degree to methemoglobin is about 50%, which forms the desired color of the resulting hydrolysate. The dry semi-finished product has a neutral odor and brown color with high functional and technological properties. It also contains 0.9% organic iron with good biological value. Thus, the study shows that blood products can effectively use in various foods such as meat products, and also as a dietary supplement for various proposes. Consumption of these products has potent positive effect on hemoglobin levels and it is recommending for people with iron deficiency anemia.

Keywords: Antianemia products, By-products, Heme iron, Farm animals, Slaughterhouse.

INTRODUCTION

Slaughterhouse blood and blood products are using for various food products production (Bah et al., 2013; Chiroque et al., 2023). Currently, the food industry uses about 30% of the slaughterhouses' blood; Majority of this blood going to the meat industry for use as a gelling agent and natural coloring agent (Álvarez-Castillo et al., 2023; Chiroque et al., 2023). Various methods have been developed for obtaining dyes from blood to stabilize the color of meat products (Wismer-Pedersen, 1988; Ofori and Hsieh, 2012).

Clarified blood and formed elements in dry, liquid and frozen form are using together with soy protein or sodium caseinate (Lynch et al., 2017; Damba, 2017). One of the ways for using blood cells is as raw materials for protein hydrolysates, produced in the form of coagulate or dried form (Lynch et al., 2017).

The slaughtered animals' blood is a significant resource of highly digestible organic iron (Hertrampf et al., 2000; Alao et al., 2017). Consumption of foods with heme iron is an effective means for the prevention and treatment of iron deficiency anemia (Bak et al., 2018; Siti et al., 2021), which, according to WHO, affects more up 500 million people worldwide in 2020. The limiting factor to use the blood and its products is the hemoglobin heme component. It gives the final product an undesirable dark brown color, a specific blood odor, and a metallic taste (Lynch et al., 2017; Ofori and Hsieh, 2012). There are of existing approaches for reducing these undesirable characteristics boil down to separate the heme and globin protein. That is complicates the technological process and impairs the bioavailability of nutrients.

It can achieve discoloring blood formed elements by treating them with a strong oxidizing agent, which is a well-known and effective technique (Ofori and Hsieh, 2014). The resulting bleached blood is a highly effective emulsifier with good foaming properties, a source of essential amino acids, and can also be used as a fat substitute in foods (Kikafunda and Sserumaga, 2005). Strong oxidizing agents like acetone and hydrogen peroxide damage the heme part, affecting iron absorption. This makes it promising to use more gentle approaches to the treatment of blood and its formed elements.

Enzymatic hydrolysis breaks down erythrocyte membranes without harming the heme part. This method of hydrolysis neutralizes the bloody odor and increases the digestibility of heme iron by up to 30% (Musa and Idrus, 2021). The presence of low molecular weight peptides in the hydrolysate decreases the raw material properties by a third (Sanchez-Reinoso et al., 2021). Also, the red color of low oxidized hemoglobin restricts the use of this product in food technology.

One way to eliminate the characteristic color of blood can be considered the transformation of hemoglobin into methemoglobin, which occurs at a certain heme iron oxidation degree (Velusamy et al., 2022). This will allow the blood to

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turn brown, allowing it to be used not only in meat products but also in imitation confectionery products. To achieve this effect, it can use solutions of food organic acids. They will be able to cause as blood cells destruction and the iron oxidation with methemoglobin formation, without destroying the structure of the metallo-protein.

Thus, the aim of present study was to study the effectiveness of the use of acid hydrolysis in the modification of erythrocyte mass for its use in food technologies. The study objectives are to choose the acid and its quantity. Also, to assess the rate and hemolysis completeness and heme iron oxidation degree and the formed organoleptic and functional-technological characteristics.

MATERIALS AND METHODS

The study is aimed to investigate the effectiveness of hemolysis various methods for modifying the qualitative characteristics of the slaughtered animals' RBC mass. The explanatory research design was based on mixed methods and a constructivist view of veterinary and medical therapies. Use of bovine blood from animals were examined by a veterinarian and found to be healthy. The slaughter of animals was in accordance with the veterinary rules for the slaughter of animals approved by the Ministry of Agriculture of the Russian Federation (order No. 269 of April 28, 2022; ethical regulation). The slaughter was carried out in a modular slaughterhouse, which is a complex of technological lines equipped with units and devices that ensure humane slaughter. Animals were stunned mechanically, using a pneumatic pistol. The animals on the way of bleeding were an unconscious state. Blood collection carried out in a closed manner, by a hollow knife into a closed reservoir under a slight vacuum (according to GOST 33674-2015). A mixture of 4% sodium citrate solution and 0.75% disubstituted sodium phosphate solution in equal proportions was used as a stabilizer. The dose of stabilizer is 1:9. There are carried out the experimental studies in scientific laboratories of the Department of Technology of Production and Processing of Agricultural Products of the Stavropol State Agrarian University in the period 2022-2023. The main studied indicators were the rate and completeness of hemolysis, hemoglobin oxidation degree and the hemolysate resulting organoleptic assessment.

The study objects were hemolysates of erythrocyte mass (ascorbic acid). Materials were included of 1) cattle blood (pH 7.4, viscosity - 5.5 N-s/m^2, stabilizer - sodium pyrophosphate) and ascorbic acid (mass fraction of the main substance 99.00%).

Tools and techniques

- Blood fractionation into formed elements and plasma was carried out on a laboratory centrifuge Bios Neofuge 15 (Heal Force, China);
- The completeness of hemolysis was determined by determining the optical density of a mixture of 0.25 cm^3 of hemolysate and saline solution (84 times dilution) at a wavelength of 670 nm on a Unico S-1200 spectrophotometer (USA);
- Determination of the content of hemoglobin derivatives was carried out according to the Austin and Drabkin method based on the analysis of the absorption spectra of the two-component system of oxyhemoglobin and methemoglobin;
- Organoleptic assessment was carried out in accordance with GOST ISO 13299-2015;
- The mass fraction of moisture/dry substances was determined by drying a sample to constant weight at a temperature of 105 ºC;
- The mass fraction of protein was determined by the Kjeldahl method (GOST 25011-2017);
- Active acidity was determined by the potentiometric method on a pH meter / millivoltmeter pH-410 (Russia) (according to GOST R 51478-99);
- The mass fraction of ash was determined according to GOST 31727-2012 by the method of mineralization of a sample followed by its combustion in a muffle furnace SNOL 6-10 (Russia) at a temperature of 500 ºC;
- The mass fraction of iron was determined according to GOST 26928-86.

Procedure

Stabilized blood was centrifuged at 8000 rpm for 10 minutes in order to isolate the formed elements. Hemolysate were prepared by adding ascorbic acid to 50 ml of red blood cells with concentrations of 0.25; 0.5; 0.75 or 1.5 mol/dm^3. The ratio of PE and acid solution varied 1:0.5; 1:1 and 1:1.5. The completeness of hemolysis was assessed every 60 seconds by pipetting a sample from the sample volume. The study of the effect of ascorbic acid included the addition of its solution with concentrations of 0.25, 0.75, 1.0 and 1.5 mol/dm^3 in 3 ratios: 1:0.5; 1:1; 1:1.5. Organoleptic studies included a descriptive assessment of the consistency, color and smell of the resulting hemolysate in liquid form.

Data analysis

Experiments and analytical determinations were carried out in triplicate. Only representative, reproducible data from each experiment are discussed. Statistical processing of experimental data performed by STATISTICA Base (Statsoft

products) and included the determination of the following values: arithmetic mean, quadratic dispersion, standard deviation of a single result, standard deviation of an average result, adequacy degree, confidence interval (P<0.05).

RESULTS AND DISCUSSION

Given the known strengths and weaknesses of RBC hemolysis methods, it was decided to conduct an in-depth study of acid hemolysis using organic acid used in the food industry. For these purposes, it was decided to use ascorbic acid, the addition of which will also provide fortification of the product.

The major criteria of hemolysis effectiveness are the speed and completeness of the process. For this, primarily, will be influence the ratio of volumes formed elements to the acid solution in the system and its concentration. In order to establish the optimal values of these parameters, a number of studies have been carried out. Working solutions of ascorbic acid had the following concentrations: 0.25 mol/dm^3, 0.5 mol/dm^3, 0.75 mol/dm^3 and 1.5 mol/dm^3.

Using an acid with a molar concentration of 0.25 mol/dm^3 showed a low efficiency of hemolysis over the studied time period (Figure 1), while a concentration of 1.5 mol/dm^3 causes the fastest and most complete destruction of red blood cells (Figure 4). Intermediate options for adding ascorbic acid with a concentration of 0.75-1 mol/dm^3 also causes almost complete hemolysis in a given period, but the intensity of its occurrence is lower (Figures 2-4).

Analysis of the experimental data obtained led to the conclusion that the optimal concentration of ascorbic acid solution in terms of the speed and completeness of hemolysis is 0.75 mol/dm^3. An increase in concentration leads to active acidity values below 5.0 units, the formation of a sour odor and astringent taste, which is unacceptable for a number of food technologies.

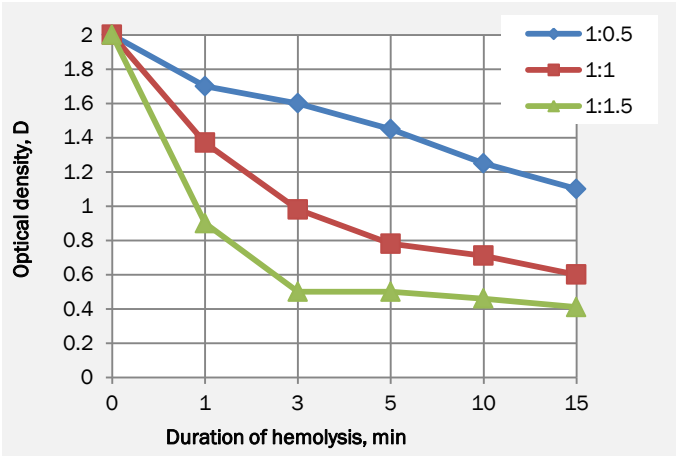

Figure 1 – The hemolysis effectiveness by ascorbic acid (concentration 0.25 mol/dm^3)

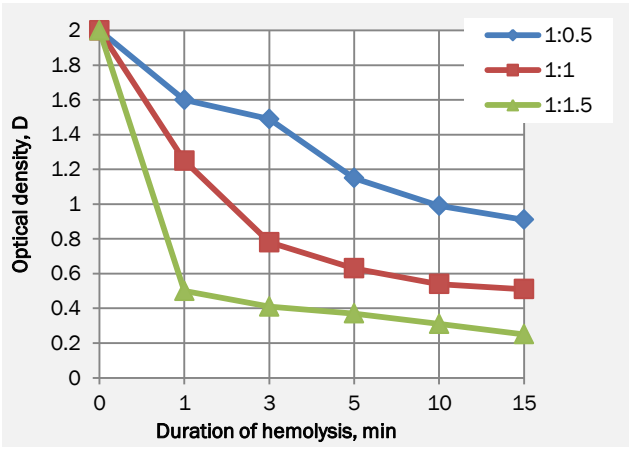

Figure 2 – The hemolysis effectiveness by ascorbic acid (concentration 0.5 mol/dm^3)

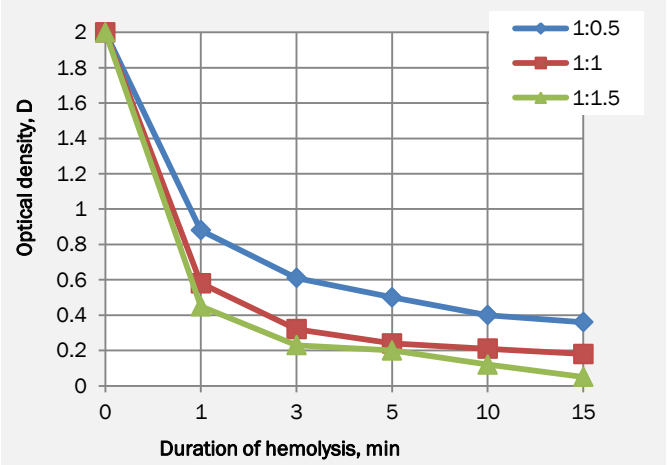

Figure 3 – The hemolysis effectiveness by ascorbic acid (concentration 0.75 mol/dm^3)

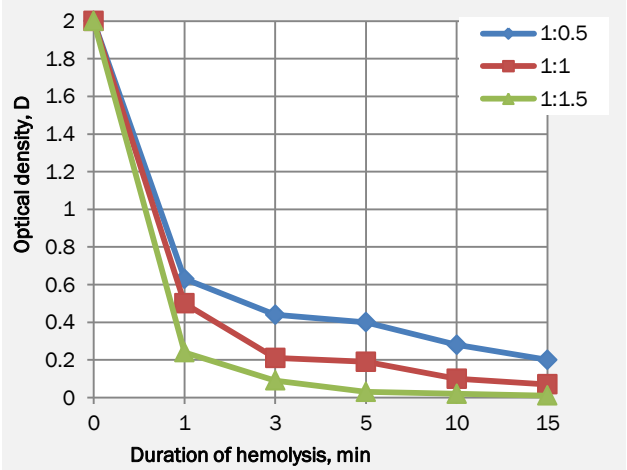

Figure 4 – The hemolysis effectiveness by ascorbic acid (concentration 1.5 mol/dm^3)

An assessment of the significance of the ratio of formed elements and the hemolyzing agent showed that, with an increase in the proportion of acid solution, the completeness of hemolysis increases. However, taking into account the undesirability of excessive dilution of the system, the optimal ratio of components is 1:1. Another factor confirming the optimal ratio of formed elements and ascorbic acid with a concentration of 0.75 mol/dm^3 was the study of the hemoglobin oxidation degree when varying the ratios of components and concentrations. Under the influence of ascorbic acid, hemoglobin is oxidized to methemoglobin, which significantly changes the color of the product from red to brown. Therefore, it is important to control the concentration of methemoglobin, as this shapes the organoleptic characteristics of the hemolysate (Figure 5).

The optimal color for hemolysate, from the point of view of its use in the formulations of meat products, as well as confectionery products, is chocolate brown. This color is achieved when about 50% of hemoglobin is oxidized to methemoglobin. The results presented in Figure 5 showed that to achieve the desired color, it is formed at an acid concentration of 0.75 mol/dm^3 and its equal volumetric ratio with formed elements. An increase in the concentration of methemoglobin leads to the formation of an excessively dark color, and a decrease in the concentration give the hemolysate a red tint.

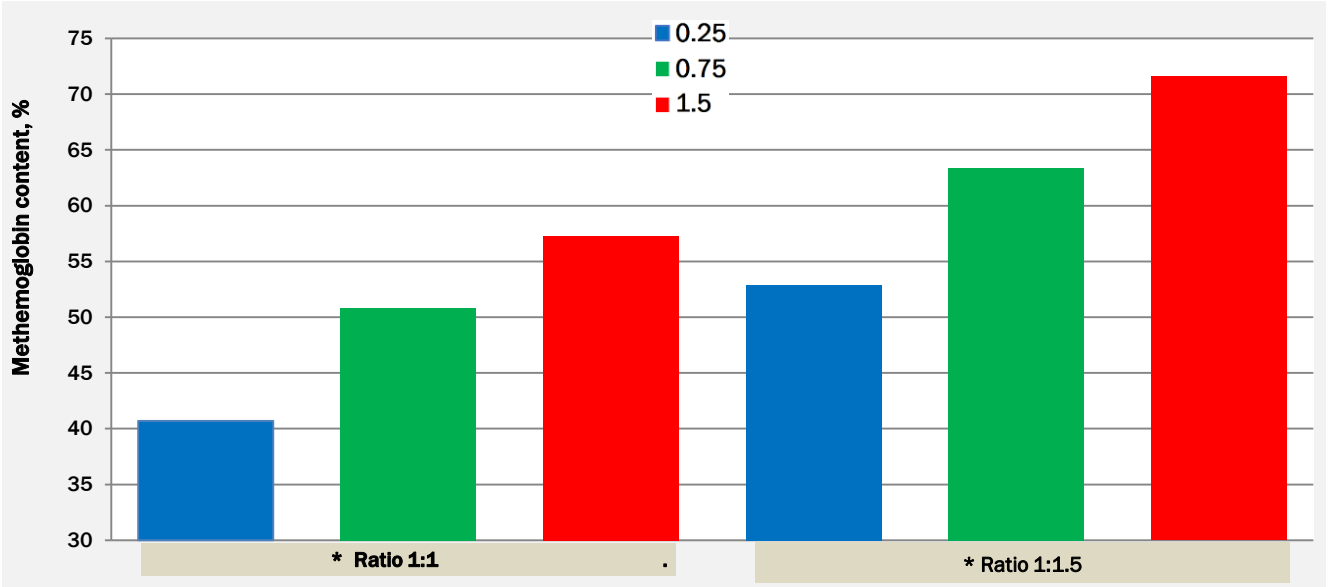

Figure 5 – Accumulation of methemoglobin in hemolysate when treated with ascorbic acid. * The ratio 1:0.5 had a very low effectiveness, so there are only 2 items for comparison in the graph.

Izgarishev et al. (2013) studied the use of acetic and citric acid for the hydrolysis of red blood cells. The authors suggest adding acids with a concentration of 5 to 10% in a ratio of an erythrocyte mass of 10:1. The process takes 12 hours at a temperature of 50 ℃, and the degree of erythrocyte hydrolysis does not exceed 32%. The authors did not study the oxidation degree of hemoglobin. A certain amount of blood plasma is always present in the separated mass of formed elements. The acid will affect globular proteins in the blood plasma, destroying their structure and causing irreversible transformation into the fibrillar type of proteins (Xing et al., 2022). The presence of modified blood plasma proteins in the hemolysate increases its structuring and binding properties, which are valuable in the development of polydisperse food systems.

Using the stronger citric and acetic acids will cause a decrease in acidity below 5 pH units, which will negatively affect the structuring properties of the hemolysate protein system. And the accumulation of many amines low molecular weight peptides will cause the formation of a bitter taste (Song et al., 2018; Bak et al., 2018). In addition to the destruction of red blood cells, hemolysis is accompanied by the destruction of hemoglobin molecules, which leads to the accumulation of free heme iron in the solution. This fact will help improve the bioavailability of iron and emphasize the antianemia nature of the resulting food component (Song et al., 2018). The resulting liquid hemolysate has a neutral odor and is brown (Powers and Buchanan, 2019; Evlash et al., 2022). However, in liquid form, it is a suitable nutrient medium for the development of microorganisms, which is due to the presence in the system of a significant amount of protein in hydrolyzed form. To solve this problem, it was decided to dry it in order to remove free moisture as much as possible. The hemolysate was dried at an air temperature of 120°C. The qualitative characteristics of the resulting dry product are presented in tables 1 and 2.

The dry semi-finished product has satisfactory organoleptic characteristics, as well as high levels of organic iron and animal protein. An assessment of the quality of the protein according to the profile of essential amino acids showed its

usefulness, and the limitation in threonine, methionine and lysine can be successfully leveled through the principles of food combinatorics, since the product is planned to be used mainly as an antianemia additive. It has also been established that dry hemolysate is well compatible with fats, forming with them fairly stable dispersion systems like emulsions. This makes its use promising in the formulations of emulsion meat products, as well as imitation confectionery products. Good solubility allows the hemolysate to be used in injection brines for injection of whole-piece meat products (Coker et al., 2002; Irinmwinuwa et al., 2023).

Table 1 - Qualitative indicators of dry ascorbic acid hemolysate (P <0.05)

Indicators	Value
Dry substances, %	80.3±1.2
Moisture, %	8.4±0.1
Protein, %	74.6±1.1
Fat, %	2.9±0.1
Iron, %	0.9±0.04
Ash,%	4.4±0.06
Active acidity, units	5.8±0.07
Emulsifying capacity, %	69.3%±0.9%
Emulsion stability, %	78.9%±0.85%
Solubility degree, %	95.8±1.2
Structure	Powdery, lumps break down easily
Color	Light brown, uniform throughout the product
Smell	Without smell

Table 2 - Results of studying the quality of the amino acid composition of dry hemolysate

Indicators / Amino acids	The content of amino acids in the protein of dry hemolysate, g/100 g of protein	Amino acid rate, %	Reference protein (indispensable amino acids), g/100 g of protein	SEM	P-value
Phenylalanine + Tyrosine	6.88	114.7	6.0	0.067	<0.01
Valyn	5.74	114.8	5.0	0.047	<0.01
Threonine	2.47	61.7	4.0	0.032	0.012
Leucine + Isoleucine	11.57	105.2	11.0	0.1157	0.01
Methionine + Cystine	1.58	45.1	3.5	0.0177	0.011
Lysine	4.96	90.2	5.5	0.059	0.12

Some of studies about of preparation of red blood cells for food use (Rossi et al., 2019; Chiroque et al., 2023), proposed adding lactic acid bacteria Enterococcus faecalis and Lactobacillus salivarius. This method allows you to suppress the development of pathogenic microorganisms and stabilize the level of hemolysis for 48 hours. However, maintaining red blood cells in their native state impairs the digestibility of the product, since the cell stroma is resistant to the action of digestive enzymes. In addition, the hemoglobin oxidation degree will be minimal, which will limit the use of the product because of its specific color and odor.

Heme iron is one of the most easily absorbed forms of iron in organic form, without the undesirable effects that are inherent in metallic iron and inorganic forms of iron (poor tolerance, organoleptic defects). The bioavailability of heme iron is almost 4 times greater than that of ferrous sulfate. This can be explained by the presence of two absorption pathways - binding to two types of receptors. The first type of DMT1 receptor, located in the duodenum, is for iron salts. A second type of receptor, PEPT1, located throughout the small intestine, is designed to bind peptides, resulting in significantly increased absorption (Ofori and Hsieh, 2012). It is important to keep in mind that the absorption of iron from a fortified product is largely determined by the composition of the food. In particular, ascorbic acid enhances the absorption of iron. Based on the quantitative content of iron in the resulting dry hemolysate, the level of its inclusion in the product for the prevention of nutritional anemia should be 1.5-2%.

CONCLUSION

Based on the studies conducted, it was concluded that acid hemolysis of red blood cells is the most rational way of processing it in preparation for use for food purposes. The use of ascorbic acid with a molar concentration of 0.75 mol/dm^3 can effectively destroy the stroma of more up 90% of red blood cells within 15 minutes. In this case, hemolysis

under the influence of acetic and citric acids ensures the destruction of no more than 32% of red blood cells and takes significantly more time. The resulting additive has acceptable organoleptic characteristics, good functional and technological properties and high nutrient status due to the high content of protein and organic iron. The brown color of the additive comes from a transformation of 50% of hemoglobin into methemoglobin. That makes it useful for anti-anemic meat products and chocolate-like confectionery products. 100 g of dry hemolysate contains 0.9 g of iron; therefore, to achieve a preventive effect, the dosage added to the product should be 1.5-2%. The high content of iron in organic form allows us to recommend blood products in the diets of children, pregnant women, and people with various types of anemia.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' Contribution

Both authors designed the study and manuscript writing. S. Shlykov collected samples and data. R. Omarov analyzed data and wrote manuscript. All authors drafted and revised the manuscript and approved the final manuscript.

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Competing interests

The authors have not declared any competing interests.

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