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# Innovative Approach to Fabrication of Blood Product of Farm Animals Intended for Prevention of Iron Deficiency

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**Abstract:** Parameters of stabilization of whole blood of farm animals have been selected aimed at increase in stability of erythrocyte membrane: separation coefficient 2000; process duration 6.0 min for pig blood and 5.5 min for beef cattle blood. The process of acid hydrolysis of packed red blood cells of farm animals using citric and acetic acids has been analyzed. It has been proven that hydrolysis with edible acids promotes separation of heme iron from total protein bulk as well as achievement of necessary hydrolysis degree and fabrication of amino-peptide complexes of protein fractions of packed red blood cells. Hydrolysis parameters have been selected with consideration for peculiarities of physicochemical properties of packed red blood cells.

**Key words:** Fractionation · Acid hydrolysis · Packed red blood cells · Amine nitrogen · Heme iron

## INTRODUCTION

A nation-wide health care problem of many countries is iron deficiency anemia: clinical hematological symptom complex, characterized with violation of hemoglobin generation as a result of iron deficiency in blood serum and bone marrow, as well as progression of trophic changes in organs and tissues.

According to data from World Health Organization (WHO), nowadays in the World with population approaching 7 billion inhabitants about 2 billion persons suffer from iron deficiency anemia [1]. Even in the developed countries of Europe and Northern America 7.5-11 % of women of reproductive age suffer from iron deficiency anemia and latent tissue iron deficiency is observed for 20-25 %. In developing countries every second pregnant woman and about 40 % of preschool children are exposed to iron deficiency anemia [2].

Iron deficiency anemia amounts to 80 % of all anemias. Especially susceptible category includes children and pregnant women. In the situation of demographic recession such trend of manifestation of this disease is undesirable, since it leads to risks of occurrences of dangerous consequences, resulting in decrease in birth rate and increase in child mortality rate [1].

According to WGO data, control of iron deficiency anemia should be performed within several scopes: improvement of human welfare, various food ration; development of health care system; fabrication of iron-containing medical and preventive products [3].

It is known that enrichment of food products is an efficient way to improve status of iron and iron deficiency anemia among population. Program of efficient enrichment of food products with iron requires for joint efforts of state, food industry and consumers. Population preferences should be considered within selection of products for enrichment. For instance, possible list of products can include wheat flour or pasta and flavorings, such as sugar, salt, curry, sodium glutamate, bouillon cubes and soy sauce [3].

Some iron-containing products are successfully applied in numerous national programs. Here are some examples in this regard. In Philippines rice is enriched with mixture of iron sulfates [4]. In Chile flour and bread are enriched with iron sulfates [5]. In some countries wheat or rice flour is used enriched with metallic iron (Sweden, Great Britain, United States) or ferrous fumarate (Venezuela) [6]. For children under one and preschool age products are developed on the basis of fine particles of metallic silver. In Sweden iron complex with sodium orthophosphate is successfully applied [7]. Iron pyrophosphates and orthophosphates are not used as a consequence of low biologic availability.

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In West European countries and United States the issues related with iron deficiency are solved mainly by implementation of the WHO program titled "Гемоглобиновое оздоровление населения". Such countries as United States, England, Sweden, the Netherlands approved nation-wide programs on prevention of iron deficiency by means of enrichment of bread, grain products, fruit juices, baby milk powders with mixtures of iron non-organic salts. However, this produced no visible results. Moreover, due to occurrence of side effects many countries (France, Belgium, Germany and others) prohibited usage of iron salts for enrichment of feed products [8].

US committee on food additives believes that iron is the only known mineral substance, the demand for which during pregnancy cannot be satisfied only by nutrition. In this country more than 25 million persons take iron-containing medicines permanently.

Some technologies of fabrication of products for elimination of iron deficiency are patented. A method is known (Patent #CN102766206) for purification of recombinant human lactoferrin in transgenic milk, which makes it possible to obtain product for elimination of iron deficiency (iron-containing recombinant human lactoferrin, FerhLF) [9].

Patent #US2009281021 describes a composition, containing heme iron or polypeptide with heme iron in combination with iron ions and chelated iron [10].

In general, analysis of global market of antianemic agents evidences that known iron-containing products have some disadvantages (low accessibility, high cost, bitter taste and others), which should be eliminated in order to intensify prevention and treatment of iron deficiency anemia.

This work is aimed at development of technology of fabrication of blood product of farm animals intended for prevention of iron deficiency.

## MATERIALS AND MTEHODS

*Weight fraction of iron* was determined according to Standard GOST 30648.3-99.

*Protein content* was determined on Rapid N Cube N/protein analyzer (Germany) using the Dumas combustion method. The method is based on combustion of sample with recording of total nitrogen in terms of thermal conductivity.

In order to determine *weight fraction of amine nitrogen* the Lowry protein assay was applied based on two reactions: biuret reaction and the Folin reaction.

*Hydrolysis degree* was determined as ratio of amine nitrogen to total nitrogen.

*Active acidity* was determined by activity of hydrogen ions using potentiometric analyzer.

## RESULTS AND DISCUSSION

Within blood fractionation it is important to prevent hemolysis of erythrocytes and penetration of hemoglobin molecules into blood plasma, which leads to decrease in its content in the extracted bulk. In addition, at low separation coefficient (centrifugal acceleration) a portion of erythrocytes remains in blood plasma. Exactly these principles are used in the studies described below. At the first stage iron content in blood plasma after fractionation as a function of separation coefficient was studied (Fig. 1).

Iron in blood plasma at low separation coefficient (up to 1000) is in the range from 1.1 to 8.0 mg% for pig blood and from 0.6 to 5.5 mg% for beef cattle blood. When separation coefficient is 1500÷2000 the iron content in extracted blood plasma is relatively stable and does not exceed 0.5 mg% for pig blood and 0.2 mg% for beef cattle blood. When separation coefficient increases, hemolysis is observed, which leads to increase in iron content in plasma.

Then, the influence of separation coefficient on separation time was studied. The obtained results are illustrated in Fig. 2.

At minimum separation coefficient (500) the time of fractionation is the highest and equals to 60 min for pig blood and 5.5 min for beef cattle blood. When separation coefficient is 2000, the process time is 6 min for pig blood and 5.5 min for beef cattle blood. The process time in the range of separation coefficient of 2000÷6000 is characterized with slight decrease: from 6 min to 3.5 min for pig blood and from 5.5 min to 3.0 min for beef cattle blood.

Therefore, optimal value of separation coefficient (Fr), which prevents preliminary hemolysis of erythrocytes and corresponds to minimum separation time, is 2000.

Since the blood temperature after stabilization is close to the temperature of animal, certain loss of iron occurs within fractionation, which is related with hemolysis of erythrocytes. In order to prevent undesirable loss of iron it is necessary to know, which temperature provides minimum loss. With this aim the influence of the process temperature on fractionation efficiency was studied, the obtained results are depicted in Fig. 3.

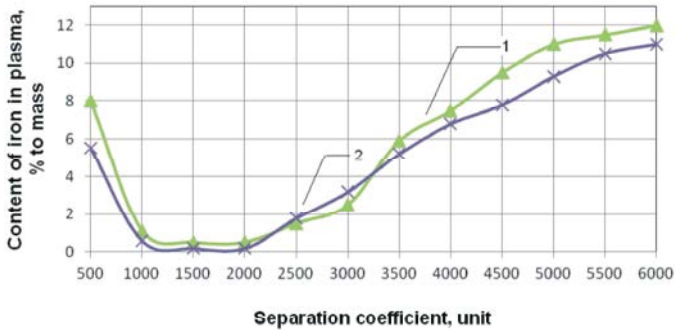


Fig. 1: Iron content in blood plasma after fractionation as a function of separation coefficient

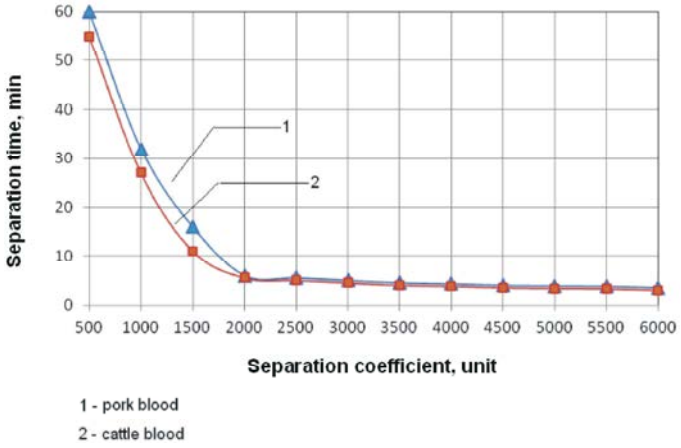


Fig. 2: Blood separation time as a function of separation coefficient

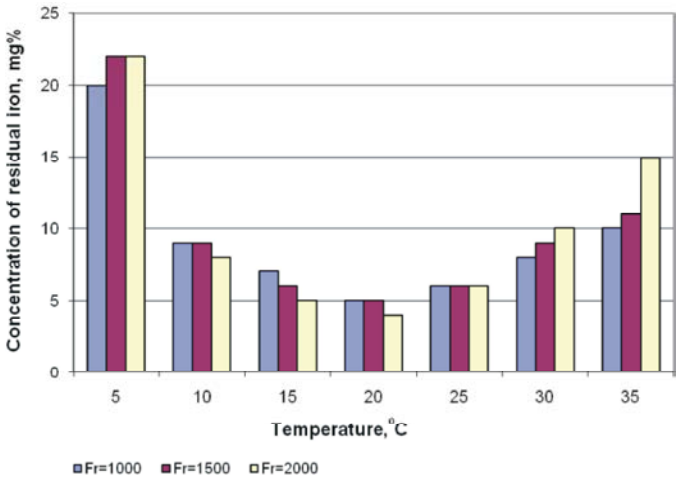


Fig. 3: Influence of process temperature on blood fractionation efficiency

The data in Fig. 3 show that the best result is achieved for 20°C and the minimum losses are observed at the separation coefficient of 2000.

Therefore, the applied approach makes it possible to use higher speed of rotation within fractionation without significant losses of packed red blood cells. The value of separation coefficient should not be higher than 2000 units. This value facilitates efficient and rapid separation of blood into fractions. The usage of one and the same value of separation coefficient for bloods of beef cattle and pig due to initially existing differences in physical properties stipulates application of different time of exposure: 5.5 min for beef cattle blood and 6 min for pig blood.

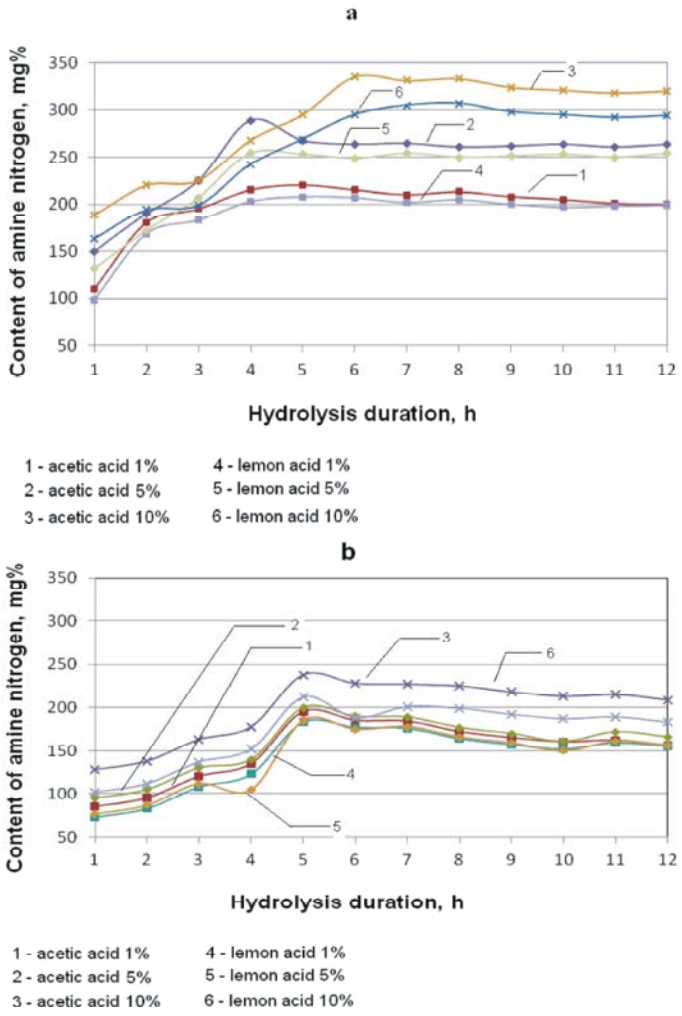


Fig. 4: Yield dynamics of amine nitrogen within hydrolysis: 1 - pig blood, 2 – beef cattle blood

Taking into consideration the main usage of final product for prevention of human iron deficiency, edible acids were used as hydrolyzing agents: citric and acetic. The final product will be a mixture of heme iron and amino-peptide complexes, obtained as a result of acid impact on protein. Herewith, the stage of neutralization and filtration should not exist with the aim of decrease in losses of amine nitrogen and heme iron. Hydrolysis was performed using acetic and citric acids at concentrations of 1 %, 5 % and 10 %. The dynamics of amine nitrogen yield within hydrolysis is illustrated in Fig. 4.

While analyzing the data in Fig. 4, the following conclusions can be made. The curves of content of amine nitrogen within hydrolysis by acids in various concentrations both for beef cattle and pig blood have two characteristic intervals: the region of constant increase in amine nitrogen and the region of its relatively steady content in 6÷8 hours for pig blood and 7 hours for beef cattle blood. However, there is a difference in the dynamics of amine nitrogen yield for different animal groups. Within hydrolysis of beef cattle blood there is a higher rise corresponding to process time of 5 hours, then there is a drop in terms of quantitative content of amine nitrogen.

Within hydrolysis of beef cattle blood the highest yield of amine nitrogen is observed at the use of 10 % citric acid (212 mg%) and 10 % acetic acid (238 mg%). After termination of the hydrolysis (12 hours) the content of amine nitrogen is about 183 mg% for 10 % citric acid (decrease in amine nitrogen content is 13.6 %) and 209 mg% for 10 % acetic acid (decrease in amine nitrogen content is 12.2 %). Therefore, for beef cattle blood the hydrolysis time in excess of 5 hours leads to decrease in content of amine nitrogen, which is unreasonable.

Table 1: Weight fraction of heme iron, %, for various variants of hydrolysis of pig blood

| | Content of free heme iron, % of hydrolysate | | | | | |
|---|---|---|---|---|---|---|
| Process duration, hours | Acetic acid, 1% | Acetic acid, 5% | Acetic acid, 10% | Citric acid, 1% | Citric acid, 5% | Citric acid, 10% |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 1 | 0.5 | 0.7 | 1.0 | 0.4 | 0.5 | 0.7 |
| 2 | 0.7 | 0.9 | 1.2 | 0.6 | 0.7 | 0.9 |
| 3 | 0.9 | 1.1 | 1.4 | 0.8 | 0.9 | 1.1 |
| 4 | 1.1 | 1.3 | 1.6 | 1.0 | 1.1 | 1.3 |
| 5 | 1.5 | 1.7 | 2.0 | 1.4 | 1.5 | 1.7 |
| 6 | 1.9 | 2.3 | 2.6 | 1.7 | 1.9 | 2.1 |
| 7 | 2.2 | 2.6 | 2.9 | 2.0 | 2.2 | 2.4 |
| 8 | 2.3 | 2.7 | 3.0 | 2.1 | 2.1 | 2.3 |
| 9 | 2.6 | 3.0 | 3.3 | 2.4 | 2.4 | 2.6 |
| 10 | 2.8 | 3.2 | 3.5 | 2.6 | 2.7 | 2.9 |
| 11 | 2.9 | 3.3 | 3.6 | 2.7 | 2.8 | 3.0 |
| 12 | 2.9 | 3.3 | 3.5 | 2.7 | 2.8 | 3.0 |

Table 2: Weight fraction of heme iron, %, for various variants of hydrolysis of beef cattle blood

| | Content of free heme iron, % of hydrolysate | | | | | |
|---|---|---|---|---|---|---|
| Process duration, hours | Acetic acid, 1% | Acetic acid, 5% | Acetic acid, 10% | Citric acid, 1% | Citric acid, 5% | Citric acid, 10% |
| 1 | 0.3 | 0.5 | 0.8 | 0.2 | 0.3 | 0.5 |
| 2 | 0.5 | 0.7 | 0.1 | 0.4 | 0.5 | 0.7 |
| 3 | 0.7 | 0.9 | 1.2 | 0.6 | 0.7 | 0.9 |
| 4 | 0.9 | 1.1 | 1.4 | 0.8 | 0.9 | 1.1 |
| 5 | 1.1 | 1.3 | 1.6 | 1.0 | 1.1 | 1.3 |
| 6 | 1.3 | 1.7 | 2.0 | 1.1 | 1.3 | 1.5 |
| 7 | 1.5 | 1.9 | 2.2 | 1.3 | 1.5 | 1.7 |
| 8 | 1.8 | 2.2 | 2.5 | 1.6 | 1.6 | 1.8 |
| 9 | 1.9 | 2.3 | 2.6 | 1.7 | 1.7 | 1.9 |
| 10 | 2.2 | 2.6 | 2.4 | 2.0 | 2.1 | 2.3 |
| 11 | 2.4 | 2.8 | 3.0 | 2.2 | 2.3 | 2.5 |
| 12 | 2.5 | 2.9 | 3.1 | 2.3 | 2.4 | 2.6 |

For pig blood the highest contents of amine nitrogen are characteristic also for 10 % citric acid (307 mg%) at 8 hours of hydrolysis and for 10 % acetic acid (335 mg%) at 6 hours of hydrolysis. Then there occurs slight decrease in content of amine nitrogen. The yields of amine nitrogen within hydrolysis of 1 % citric acid, 5 % citric acid, 1 % acetic acid and 5 % acetic acid can be considered as inefficient.

In addition, content of free heme iron was analyzed within hydrolysis using the aforementioned acids. The obtained results are summarized in Table 1 for pig blood and in Table 2 for beef cattle blood.

Iron content for all variants of hydrolysis increases in the overall progress of the process. In the last 2 hours of the process the iron content is relatively steady, variations are insignificant, which evidences that it is inexpedient to increase the process time in excess of 12 hours.

Within hydrolysis of packed red blood cells of pigs the highest iron yield (3.3 %) is observed at the use of 5 % acetic acid, 10 % acetic acid (3.5 %), as well as 10 % citric acid (3.0 %). Within hydrolysis of packed red blood cells of beef cattle the highest iron is observed at the use of 5 % acetic acid (2.9 %), 10 % acetic acid (3.1 %) and 10 % citric acid (2.6 %).

Based on the analysis of yield of amine nitrogen and content of heme iron within hydrolysis with various acids it has been established that it is expedient to perform hydrolysis in 12 hours. The most acceptable variants are as follows: 10 % citric acid, 5 % and 10 % acetic acid.

The choice of temperature mode of blood hydrolysis was carried out for the most optimal variants of acids: 10 % citric, 5 % acetic and 10 % acetic. The temperatures 30°C, 35°C and 40°C were analyzed. The process efficiency was monitored in terms of hydrolysis degree of packed red blood cells.

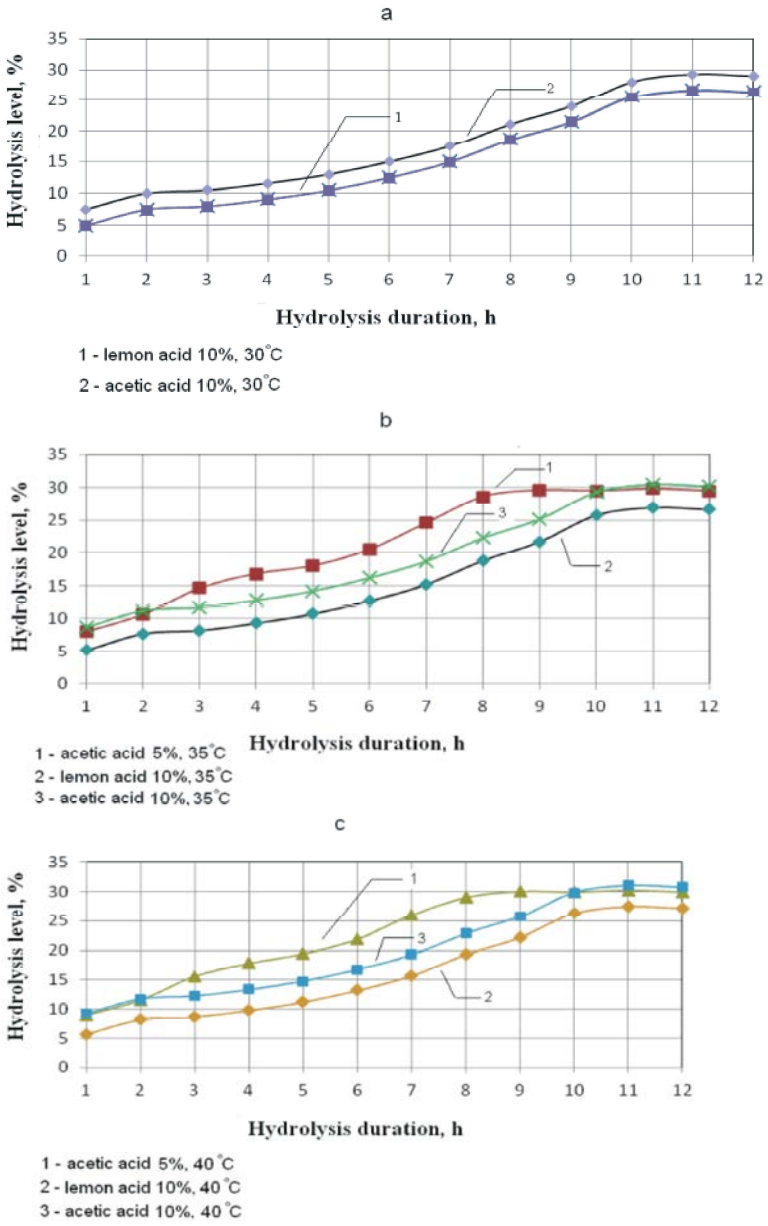


Fig. 5: Hydrolysis degree as a function of process time at various temperatures of hydrolysis for packed red blood cells of pigs: a) 30°C; b) 35°C; c) 40°C

It has been shown that at 30°C (Fig. 5a) the highest hydrolysis degree is achieved for 10 % acetic acid and amounts to 29 %; for 10 % citric acid and 5 % citric acid the hydrolysis degree is not higher than 26.5 % at this temperature. For all acids the maximum value of hydrolysis degree is achieved in 10 hours and is relatively steady in subsequent 2 hours of the process.

The curves of hydrolysis degree at 35°C (Fig. 5b) have the same pattern as at 30°C, 2 periods can be observed in them: 1) constant increase and 2) relatively steady level of hydrolysis degree. For 5 % acetic acid the first period amounts to 9 hours, the second amounts to 3 hours, maximum hydrolysis degree is about 29.8 %. For 10 % citric acid the first period is 10 hours, the second period is 2 hours, maximum hydrolysis degree is about 27.0 %.

For 10 % acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 30.5 %.

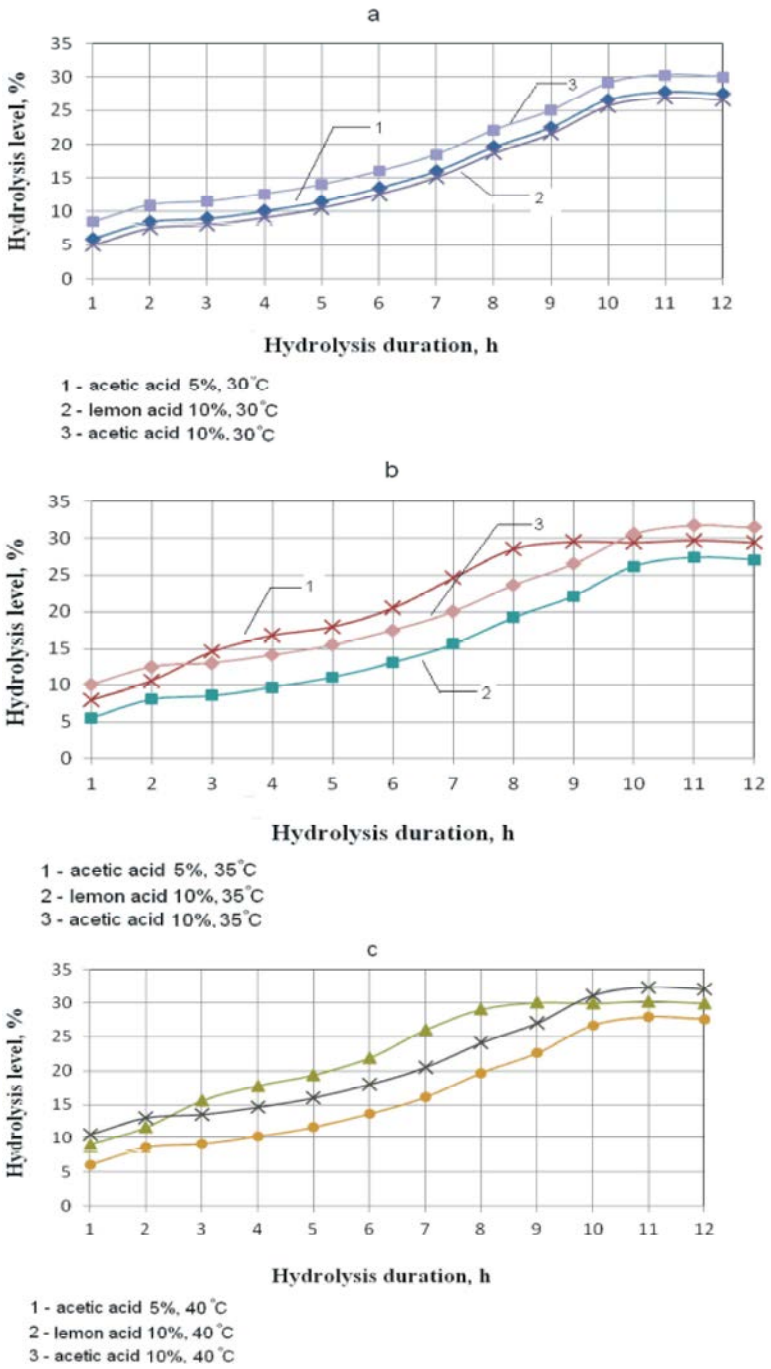


Fig. 6: Hydrolysis degree as a function of process time at various temperatures of hydrolysis for packed red blood cells of beef cattle: a) 30°C; b) 35°C; c) 40°C

At 40°C (Fig. 5c) a similar situation is observed. For 5 % acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 30.2 %. For 10 % citric acid the first period is 10 hours, the second period is 2 hours, maximum hydrolysis degree is about 27.5 %. For 10% acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 31.0 %.

The analysis of data in Fig. 5 evidences that the optimal temperature for hydrolysis of packed red blood cells of pig blood is 35°C.

At 30°C (Fig. 6a) the highest hydrolysis degree is achieved for 10 % acetic acid and is about 30.3 %; for 10 % citric acid and 5 % citric acid the hydrolysis degree is not higher than 28.0 % at this temperature. For all acids the maximum hydrolysis degree is achieved in 10 hours and is relatively steady in the subsequent 2 hours.

The hydrolysis degree at 35°C (Fig. 6b) is the same as at 30°C, there are 2 periods: 1) constant increase and 2) relatively steady level of hydrolysis degree. For 5% acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about is about 29,8%.

For 10% citric acid the first period is 10 hours, the second period is 2 hours, maximum hydrolysis degree is about 27.4 %. For 10 % acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 31.8%.

At 40°C (Fig. 6c) no significant variations in the dynamics of hydrolysis degree are observed. For 5 % acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 30.2%. For 10 % citric acid the first period is 10 hours, the second period is 2 hours, maximum hydrolysis degree is about 27.9%.

For 10 % acetic acid the first period is 9 hours, the second period is 3 hours, maximum hydrolysis degree is about 32.3 %.

Based on the analysis of Fig. 6 for hydrolysis of packed red blood cells of pig blood the optimal temperature is 35°C.

Organoleptic evaluation of ready hydrolysates have been performed. The results are summarized in Table 3.

**Final Remarks:** The obtained results make it possible to conclude as follows.

Technological parameters of beef cattle and pig blood fractionation have been selected. The fractionation is based on 2 main conditions. The first condition is maximum yield of iron and the second condition is minimum time of the fractionation. The conditions are satisfied at the following parameters: separation coefficient Fr=2000; process time 6.0 min for pig blood and 5.5 min for beef cattle blood.

The hydrolysis process of packed red blood cells of beef cattle and pigs has been analyzed. Based on the performed studies the technological modes of hydrolysis of packed red blood cells have been developed. The hydrolysis is performed in three variants:

- 5 % acetic acid, packed red blood cells / acid ratio 10:1, process temperature 35°C, process time 12 hours.
- 10 % citric acid, packed red blood cells / acid ratio 10:1, process temperature 35°C, process time 12 hours.
- 10 % acetic acid, packed red blood cells / acid ratio 10:1, process temperature 35°C, process time 12 hours.

## CONCLUSIONS

After analyzing of influence of hydrolysis parameters on the yield of amine nitrogen, content of free heme iron, as well as on hydrolysis degree, it is possible to conclude as follows. In order to perform hydrolysis of packed red blood cells of pigs and beef cattle it is possible to apply the following variants: 5 % acetic acid, 10 % citric acid and 10 % acetic acid. All variants provide positive effect of hydrolysis.

## ACKNOWLEDGMENTS

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