VOL. 15, NO. 21, NOVEMBER 2020 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

www.arpnjournals.com

THE LOW-TEMPERATURE TECHNOLOGY OF PROCESSING THE BLOOD OF SLAUGHTERED ANIMALS

I. A. Korotkiy[1], E. V. Korotkaya[2], E. N. Neverov[1], A. N. Rasshchepkin[1] and D. E. Fedorov[1]
[1]Department of Heat Engineering, Institute of Engineering, , Kemerovo State University, St. Krasnaya, Kemerovo, Russia
[2]Department of General and Inorganic Chemistry, Institute of Food Science and Technology, Kemerovo State University, St. Krasnaya, Kemerovo, Russia
E-Mail: i.a.korotkiy@mail.ru

ABSTRACT

In the food industry, the secondary raw material, such as blood, is obtained as a result of processing animal carcasses. This work has been aimed at developing a low-temperature technology for processing the blood of slaughtered animals. From such blood, the hemoglobin solution has been obtained by osmotic hemolysis. Next, separation freezing has been performed on a laboratory capacitive cryoconcentrator. The freezing experiments have been performed at various temperatures of the heat exchange surface and with various initial mass fractions of the solids. The highest efficiency of freezing has been observed with the temperature of the heat exchange surface of minus 2 ± 0.2 °C. Equations have been made for calculating the thermophysical properties of the hemoglobin solutions with various concentrations. A method of two-stage freezing of the hemoglobin solution has been developed. The expediency of the product sublimational dehydration with a layer thickness of 10 ± 1 mm has been proven. A technology has been developed for producing purified hemoglobin in the liquid, concentrated, and dry form.

Keywords: animal blood, cryoconcentration, hemoglobin, crystallizer, lyophilization.

INTRODUCTION

In the food industry, the secondary raw material, such as blood, is obtained as a result of processing animal carcasses. At most meat processing enterprises, the use of this raw material is not efficient enough. Less than 10 % of the animal blood is sent for processing, and the rest is disposed of as production wastes. With that, the processed blood is mainly sent for the production of feed products [1]. This is economically disadvantageous because the blood of slaughtered animals is a valuable raw material [2]. It contains a whole complex of useful micronutrients (Table-1) [3-9].

Table-1. The average chemical composition of the blood of farm animals, %.

Indicator name	In whole blood	In blood plasma	In the erythrocyte concentrate
1	2	3	4
Water	79.0 - 82.0	91.0 - 92.0	59.0 - 63.0
Dry matter,	18.0 - 21.0	8.0 - 9.0	37.0 - 41.0
including: proteins	16.4 - 18.9	6.8 - 7.3	30.3 - 32.7
fats	0.36 - 0.39	0.26 - 0.32	1.9 - 7.8
other organic matter	0.50 - 0.67	0.17 - 0.23	-
Minerals	0.8 - 0.9	0.85 - 0.87	0.7 - 1.0

The blood of slaughtered animals may be hydrolyzed for obtaining antanemic products [10, 11]. For example, this can be done with enzyme preparations. Another common method of processing the blood of slaughtered animals is drying; mainly spray drying [12-14]. In most cases, dried blood is used for adding it to hematogen, or for obtaining the feed for farm animals. This makes it possible to increase the biological and nutritional value of the feed.

Hematogen is one of the most common products made with the use of the blood of slaughtered animals. This product is recommended for anemia, treatment after serious diseases, in the post-surgery period, etc. Both whole stabilized blood and defibrinated animal blood may be used for the production of hematogen. Sugar syrup, condensed milk, vitamins, aromatic components, etc. may also be added to this product [15].

In obtaining food and medical products based on the erythrocyte concentrate, hemoglobin production is an integral technological process. This operation is performed by destructing the cell membranes of erythrocytes with the release of hemoglobin to the outside. The need for this procedure is determined by the low digestibility of the cell walls, which reduces the nutritional value of the product.

Various methods are used to purify the erythrocyte concentrate from the membranes. The simplest

ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

one is osmotic hemolysis. The essence of this method is diluting the erythrocyte concentrate with distilled water at a ratio of approximately 1:10. Due to the creation of a hypotonic environment, the membranes of erythrocytes burst. The resulting empty shells of erythrocytes (the stromal component) may be removed from the solution by centrifugation and filtering.

The purified hemoglobin solution is used not only for obtaining several functional products. It is also used for making reference samples in hematological studies. From the foregoing, a conclusion may be drawn about the feasibility of developing effective technologies for extracting hemoglobin from the blood of slaughtered animals.

The methods of concentrating the hemolyzed and purified hemoglobin solution should ensure the maximum degree of product preservation. First of all, this means the absence of a thermal effect on such a solution. The membrane methods satisfy this requirement; however, they have certain drawbacks. These include high operating costs of the process and of membranes removal/replacement.

A possible method of concentrating the hemoglobin solution may be separation freeze-out. The essence of this method is gradual freezing of the solution. With successive freeze-out, pure moisture crystallizes first, and the molecules of the dry matter remain in the nonfrozen part of the solution. With the moisture freeze-out, the cryoscopic temperature of the unfrozen solution gradually decreases. Thus, the liquid product becomes concentrated.

The advantage of the separation freeze-out method is the absence of thermal effects on the product, which is important for the hemoglobin solution. Besides, separation freeze-out features relatively low energy costs: the latent heat of melting is 332.43 J/kg. Given the refrigeration coefficient, the specific energy consumption for ice formation will be even lower.

This work was aimed at developing a low-temperature technology for processing the blood of slaughtered animals with the use of separation freeze-out.

OBJECTS AND METHODS

The object of the research was the hemoglobin solution obtained from the pig blood that had been collected following the Sanitary Rules and Norms SanPIN 2.3.2.1078-01 of the Russian Federation by introducing a hollow knife into the neck of the animals and cutting the blood vessels close to the right precardium. All the tools, including the hollow knife and the containers, had been previously sterilized. The animals were one-year-old pigs of the Sibirskaya Severnaya breed grown in the Kemerovo region.

The hemoglobin solution was prepared by osmotic hemolysis. For this purpose, the pig blood was subjected to defibrination followed by centrifugation with a separation factor of 2,000. The erythrocyte concentrate was subjected to double washing with physiological saline solution, after which it was diluted with distilled water in the ratio of 1:9. With this ratio, effective hemolysis of the erythrocytes was ensured. The erythrocyte shells were removed from the solution by centrifugation.

The mass fraction of the dry substances was determined following ISO 1442:1997 Meat and Meat Products - Determination of the Moisture Content (Reference Method).

The mass fraction of protein was determined on a Rapid N Cube protein nitrogen analyzer (ELEMENTAR Analysen systeme GmbH, Germany, manufactured in 2011) following the Dumas's method.

The mass fraction of ash was determined by completely burning the organic part of the weighted sample of the product and subsequent weighing.

The mass fraction of fat was determined by extracting the fat from the tested food concentrate with ethyl or petroleum ether in a Soxhlet extraction apparatus and subsequent gravimetric determination of the added fat by the difference between the weight of the weighted sample of the tested concentrate before and after extraction.

Separation freeze-out was performed in a laboratory capacitive cryoconcentrator, the layout of which is shown in Figure-1. This unit had been assembled at the laboratory of the HVAC Department of the Federal State Budget Educational Establishment for Higher Education (FSBEI HE) Kemerovo State University (KemSU).

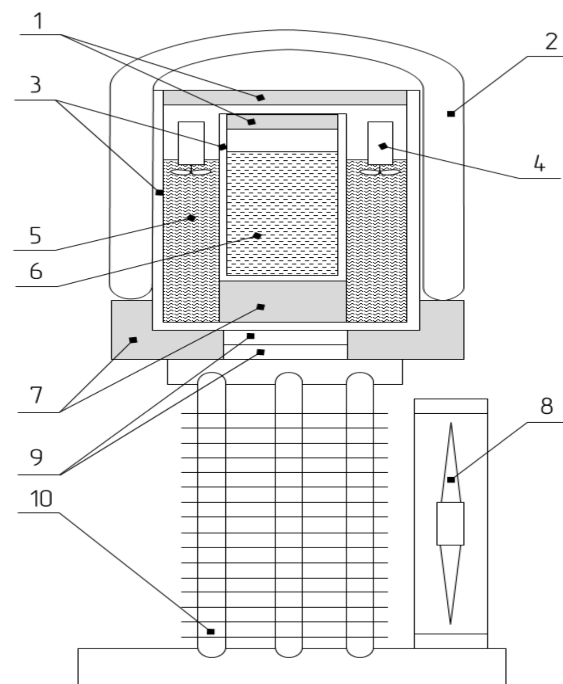

Figure-1. The layout of the laboratory cryoconcentrator: 1 - heat-insulating cover; 2 - Dewar vessel; 3 - aluminum container; 4 - stirrer; 5 - coolant; 6 - concentrated product; 7 - heat insulation; 8 - fan; 9 - Peltier elements; 10 - radiator.

VOL. 15, NO. 21, NOVEMBER 2020 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

www.arpnjournals.com

Working container 3, in which the product freeze-out occurred, was placed in a larger container filled with the coolant. Between these containers, heat insulation 7 was provided. Both containers were closed with heat-insulated covers 1. The heat was removed from the coolant using two series-connected Peltier elements 9. With the help of these, the heat was transferred from the coolant to radiator 10 blown by the air. To ensure uniform temperature distribution throughout the coolant volume, stirrers 4 were provided. The coolant temperature was maintained by varying the voltage at Peltier elements 9 by the temperature sensor.

The concentrated hemoglobin solution was dried on an Iney-6M lyophilic unit (the Institute for Biological Instrumentation of the Russian Academy of Sciences (IBI RAS), Russia, manufactured in 2010). In this lyophilic unit, the product was stacked on pallets set in vertical drying chambers. In these chambers, there were heat sources - infrared lamps. From the drying chambers, the air through a vacuum pump entered the desublimator. In it, the moisture was frozen out on the surface of the refrigeration machine evaporator at the temperature of minus 40 ± 2 °C. The residual pressure in the drying chambers was about 80 ± 2 Pa.

The experiments were repeated three times; the arithmetic mean of the three measurements was adopted as the final value.

RESULTS

The hemoglobin solution obtained by osmotic hemolysis in the hypotonic environment features relatively high moisture content - 95.8 %. This necessitated concentrating it for improving the biological potential.

The purified hemoglobin solution was frozen in a capacitive cryoconcentrator. First, the temperature of the heat exchange surface was experimentally chosen. This parameter in various experiments was minus 2 ± 0.2, minus 4 ± 0.2, and minus 6 ± 0.2 °C. The changes in the amounts of the crystalline phase during the hemoglobin solution separation freeze-out are shown in Figure-2. The experiments had been continued until the freeze-out degree of 90 ± 2 % was reached.

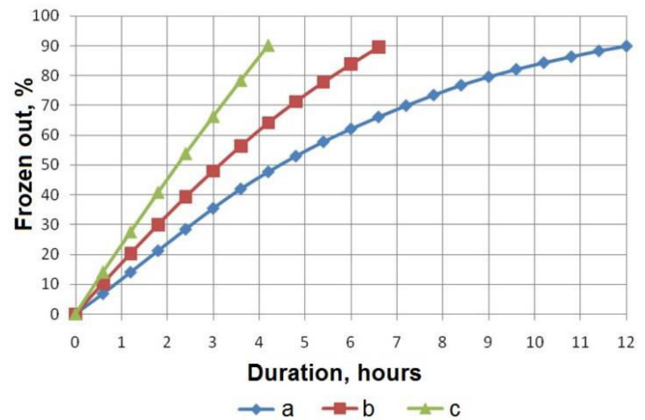

Figure-2. The dependency curves of the amount of frozen out moisture on the duration of the hemoglobin solution cryoconcentration with the heat exchange surface temperature equal to the following values:
a - minus 2 ± 0.2 °C, b - minus 4 ± 0.2 °C, and c - minus 6 ± 0.2 °C (curves accuracy ± 4 %).

To assess the effectiveness of separation freeze-out, the content of the dry matter in the unfrozen part of the concentrate was assessed. The results are shown as curves in Figure-3.

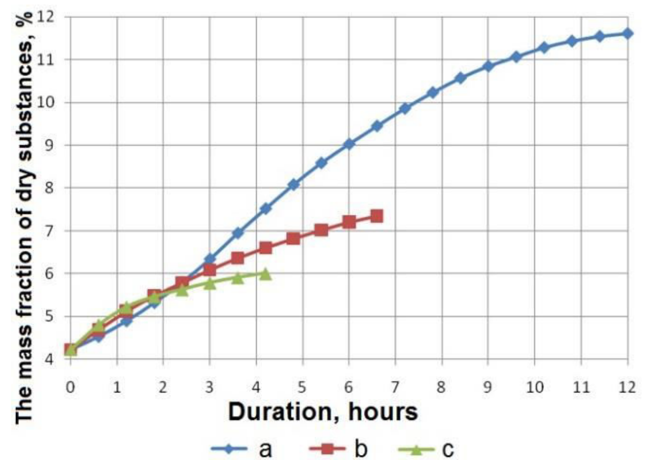

Figure-3. The dependency curves of the mass fraction of the dry matter in the unfrozen part of the hemoglobin solution during separation freeze-out with the heat exchange surface temperature equal to the following values:
a - minus 2 ± 0.2 °C, b - minus 4 ± 0.2 °C, and c - minus 6 ± 0.2 °C.

The mass fraction of the dry matter in the ice was also calculated by the known concentration of the dry matter in the liquid phase. Based on the data obtained, the dependency of the loss of the dry matter on the degree of freeze-out was calculated (Figure-4).

ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

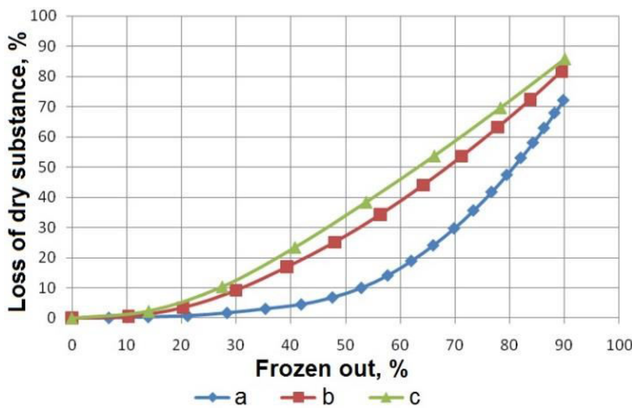

Figure-4. The dependency curves of the dry matter losses in the hemoglobin solution on the degree of freeze-out with the heat exchange surface temperature equal to the following values: a - minus 2 ± 0.2 °C, b - minus 4 ± 0.2 °C, and c - minus 6 ± 0.2 °C.

Next, separation freeze-out studies were performed with the initial mass fractions of the dry matter of (1 ± 0.1), (5 ± 0.1), (10 ± 0.1), and (15 ± 0.1) %. The amounts of the crystalline phase formed during this process are shown as curves in Figure-5.

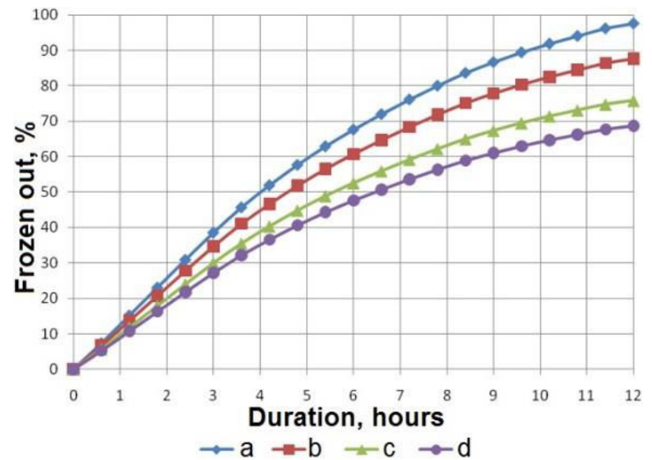

Figure-5. The curves of the amount of frozen product during the hemoglobin solution cryoconcentration with the initial mass fraction of the dry matter equal to the following values: a - 1 ± 0.1 %, b - 5 ± 0.1 %, c - 10 ± 0.1 %, and d - 15 ± 0.1 %.

The mass fractions of the dry matter during the freeze-out of hemoglobin solutions with various degrees of concentration are shown in Table-2.

Table-2. The concentrations of the liquid phase of the hemoglobin solution during separation freeze-out.

The duration of freezing, h	The initial mass fraction of dry substances, %			
	1	5	10	15
1	2	3	4	5
3	1.8	7.2	13.0	18.2
6	2.1	10.2	16.9	23.4
9	2.6	12.1	20.0	26.8
12	3.0	13.1	21.8	29.5

Next, experiments with freeze-drying of the (16 ± 0.1) % hemoglobin solution were performed with various thicknesses of the dried layer: 5 ± 1, 10 ± 1, 15 ± 1, and 20 ± 1 mm. The curves of the changes in the moisture mass fraction in the hemoglobin solution during lyophilization are shown in Figure-6.

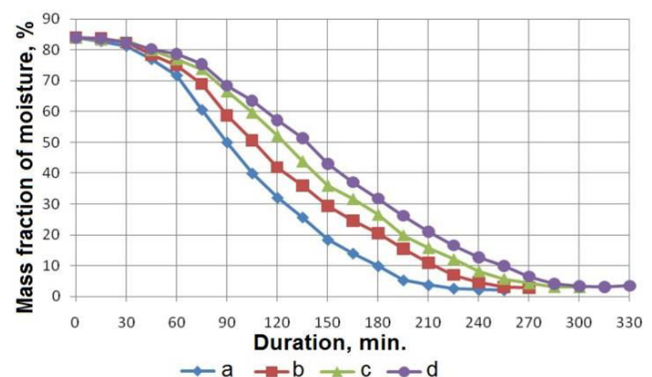

Figure-6. The curves of the hemoglobin solution lyophilization with the thicknesses of the product layer equal to the following values:
a - 5 mm, b - 10 mm, c - 15 mm, and d - 20 mm

The compositions of all three hemoglobin products are shown in Table-3.

©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

Table-3. The mass fraction of the main hemoglobin product components.

Component name	Product		
	16 % concentrate	**70 % concentrate**	**Dry powder**
1	2	3	4
Moisture	84.0 ± 0.5	30.0 ± 0.5	2.8 ± 0.2
Dry matter	16.0 ± 0.2	70.0 ± 0.5	97.2 ± 0.5
Protein	15.7 ± 0.2	69.0 ± 0.2	95.3 ± 0.5
Fat	not found	not found	not found
Ash	0.2 ± 0.1	0.8 ± 0.1	1.2 ± 0.1

RESULTS INTERPRETATION/DISCUSSIONS

The greatest moisture crystallization rate was observed during the first hour of the process; it ranged from 12 ± 2 %/h to 25 ± 2 %/h. With the growth of the ice layer, the thermal resistance between the wall of the crystallizer and the phase boundary surface increased. This was the reason for decreasing the rate of ice formation.

The higher the speed of ice formation is, the more linear the dependency of the amount of frozen moisture on the duration of cryoconcentration becomes. From the obtained data (Figure-2), the following equation was obtained in the MathCad application:

$$V = -2.222\tau t + 0.79t^2 + 5.576t + 8.045 + 8.747\tau - 0.483\tau^2, \quad (1)$$

where V was the amount of the frozen out moisture, %, τ was the duration of separation freeze-out, hours, and t was the heat exchange surface temperature, °C.

This equation allows calculating the amount of frozen moisture with various durations of the process and temperatures of the heat exchange surface. It should be noted that it is valid in the temperatures range from minus 2 °C to minus 6 °C since the equations were derived from the data obtained in this range.

The nature of the dry matter concentration in the liquid phase of the product during freeze-out depends on many factors. During slow freezing, correctly ordered hexagonal crystal structures are formed. During fast freezing, structures in the shape of irregular dendrites and spherulites are formed. In the latter case, the dry matter molecules are intensively captured by the formed crystalline phase.

As already noted, the rate of ice formation decreases during separation freeze-out. Moreover, the efficiency of cryoconcentration should increase with decreasing the rate of ice formation. However, it should be borne in mind that, relative to the unfrozen volume of the product, the freeze-out rate, on the contrary, increases. Besides, the content of the dry matter in the liquid phase of the product changes during the freeze-out. This also influences the degree of capturing the dry matter molecules by the crystalline phase.

In the first two hours at lower freeze-out temperatures, the content of the dry matter in the liquid phase was higher. This was due to greater degree of product freeze-out. However, further freeze-out at higher temperature was accompanied by higher concentration efficacy. This was due to the lower ice formation rate.

According to the data shown in Figure-3, the following mathematical relationship was derived using the MathCad application:

$$C = 0.157\tau t + 0.032t^2 + 0.034t + 3.735 + 1.36\tau - 0.0306\tau^2, \quad (2)$$

where C was the mass fraction of the dry matter in the hemoglobin liquid phase, %.

This equation is true in the temperature range from minus 2 °C to minus 6 °C with the initial mass fraction of the dry matter of 4.2 ± 0.1%, since the equations were derived from the data obtained in this range.

The lower the temperature is, the more linear the dependency of the amount of frozen moisture on the duration of freeze-out becomes. The highest efficiency of freezing is observed with the temperature of the heat exchange surface of minus 2 °C. In these conditions, the loss of the dry matter before freezing out of 50 ± 5 % of the product is less than 10 %. Thus, the expediency of separation freeze-out of the hemoglobin solution at the temperature of the heat exchange surface of minus 2 ± 0.2 °C was found. To increase the efficiency of cryoconcentration, this process should be studied with various initial mass fractions of the dry matter.

With greater initial mass fraction of the dry matter, the ice formation rate is slower. This is due to the lower cryoscopic temperature of the liquid phase. In the case of freezing out of the 1 ± 0.1 % hemoglobin solution, 97 ± 1 % of the product crystallized after 12 hours. In the case of freezing out of the 15 ± 0.1 % solution, only 68 ± 1% of the product crystallized over the same period.

With increasing the initial mass fraction of the dry matter, the efficiency of concentration decreased (Figure-5). In the case of freezing out of the 1 ± 0.1 % hemoglobin solution, the degree of concentration after 12 hours was about three. In the case of freezing out of the 5

ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

± 0.1 %, 10 ± 0.1 %, and the 15 ± 0.1 % hemoglobin solutions, it was possible after 12 hours to increase the degree of concentration 2.6, 2.2, and 1.9 times, respectively. This difference was not only due to the different amounts of frozen out moisture, but also due to the increased loss of the dry matter.

Based on the obtained data (Figure-5), the following equation was made in MathCad for calculating the mass fraction of the dry matter:

$$C = 0.078\tau C_H - 0.034C_H^2 + 1.527C_H - 1.656 + 0.56\tau - 0.0278\tau^2, \quad (3)$$

From equation (3), by expressing τ, an equation for calculating the duration of the hemoglobin solution freeze-out can be obtained.

Some important characteristics of the product during separation freeze-out are its thermal properties. They allow simulating the required processes with regard to the changes in the properties of the separated media.

To determine the thermophysical properties, the well-known calculation method can be used.

The specific heat capacity can be calculated following the additivity rule according to the following equation:

$$c = \sum_{k=1}^{n} (c_c \, \chi_c), \quad (4)$$

where c_c *is* the heat capacity of the component, kJ/(kg·K); and χ_c is the mass fraction of the component.

To calculate the thermal conductivity coefficient, the additivity method can also be used. This characteristic is calculated using the Lichtenecker's formula [16]:

$$\lambda_{eff} V = \sum_{k=1}^{n} \lambda_c V_c, \quad (5)$$

where λ_{eff} *is* the effective thermal conductivity of the product, W/(m·K); λ_c is the coefficient of thermal conductivity of the component, W/(m·K); V_c *is* the volume occupied by the component, and V is the total volume of the product.

The temperature conductivity coefficient can be found using the well-known formula:

$$a = \frac{\lambda}{c_p \rho}, \quad (6)$$

where λ *is the* coefficient of thermal conductivity of the product, W/(m·K); c_p is the isobaric specific heat capacity, J/(kg·K); and ρ is the product density, kg/m^3.

Using formulas 5, 6, and 7, curves were built based on the values of the specified heat capacity of the substance. After that, the following mathematical dependences were derived in the MS Excel application for calculating the thermophysical properties with the use of the trend curves (the linear approximation for equations 7 - 10 and the polynomial approximation for equations 11 - 12), based on the obtained curves:

$$c_l = -0.0293C + 4.19, \quad R^2 = 1, \quad (7)$$

where c_l *was* the specific heat capacity of hemoglobin liquid phase, kJ/(kg·K); and R^2 was the veracity of approximation (calculated in MS Excel);

$$c_c = -0.0104C + 2.3, \; R^2 = 1, \quad (8)$$

where c_c *was* the specific heat capacity of hemoglobin crystalline phase, kJ/(kg·K);

$$\lambda_l = -0.0056C + 0.597, \; R^2 = 1, \quad (9)$$

where λ_l *was* the conductivity coefficient of hemoglobin liquid phase, W/(m·K);

$$\lambda_c = -0.0022C + 2.24, \quad R^2 = 1, \quad (10)$$

where λ_c *was* the conductivity coefficient of hemoglobin crystalline phase, W/(m·K);

$$a_l = -C^3 \cdot 10^{-6} + 75.3 \cdot 10^{-6} C^2 - 0.0091C + 1.437, \; R^2 = 0.9996 \quad (11)$$

where a_l was the temperature conductivity coefficient of hemoglobin liquid phase, 10^{-7}, m^2/s; and

$$a_c = -0.00027C^2 - 0.0741C + 10.5, \; R^2 = 0.9997 \quad (12)$$

where a_c was the temperature conductivity coefficient of hemoglobin crystalline phase, 10^{-7}, m^2/s.

The hemoglobin production technology should include concentrating this solution until the mass fraction of the dry matter reaches 16 ± 0.1 %. This is due to the need for increasing the nutritional value of the product. Besides, the resulting 16 ± 0.1 % concentrate can be used not only in the food industry. It can also be used for obtaining reference solutions for hematological analyses. In the latter case, the 16 ± 0.1 % solution was divided into three parts in certain ratios. Two of them were diluted with distilled water until the 12 ± 0.1 % and 8 ± 0.1 % solutions were obtained.

Based on the foregoing, the technology of cryoconcentration can be represented as follows Figure-7.

©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

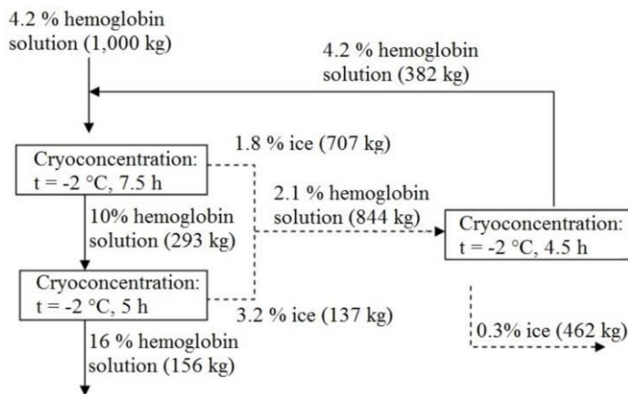

Figure-7. The process scheme of separation freeze-out of the hemoglobin solution.

For illustrative purposes, as an example, one ton was adopted as the starting weight of the 4.2 ± 0.1 % hemoglobin solution. This solution entered the first stage of cryoconcentration, where about 70 % of the product was frozen out. The obtained 10 % solution was sent to the second stage of freeze-out, where its concentration increased to 16 %. The resulting ice from the first and the second stages was melted and mixed, and entered the parallel freeze-out stage. The 4.2 % concentrate obtained in the parallel stage was returned to the first stage. This technology allowed reducing the loss of the dry matter to 3.6 % and increasing the concentration.

Separation freeze-out following the above technology may also be used as a preliminary stage of dehydration before freeze-drying. In this case, freeze-out allows reducing the cost of drying. The average energy consumption of a freeze-dryer is 2.0 - 5.0 kWh per kilogram of the removed moisture. For separation freeze-out with the temperature of the heat exchange surface of minus 2 ± 0.1 °C, this value is about 0.15 - 0.20 kWh/kg. Even with the parallel freeze-out stage, the energy consumption in the case of cryoconcentration is significantly lower than in the case of drying.

With increasing the thickness of the dehydrated layer, the duration of moisture removal as well as the final moisture content in the product increased (Figure-6). In the case of drying a layer 5 ± 1 mm thick, the mass fraction of moisture in the dry product was 2.2 ± 0.1 %. With the product layer thickness of 10 ± 1, 15 ± 1, and 20 ± 1 mm, these values were 2.8 ± 0.1 %, 3.1 ± 0.1 %, and 3.5 ± 0.1 %, respectively. The hemoglobin solution should be dehydrated with the layer thickness of 10 ± 1 mm. The dehydrated hemoglobin was dark red smooth free-flowing powder with a pronounced characteristic odor.

It should be noted that in the absence of separation freeze-out stage, the duration of dehydration would have increased 2 - 3 times.

Lyophilisation of the 16 ± 0.1 % hemoglobin solution might also be performed to achieve the mass fraction of 30 ± 1 % for obtaining the concentrate. The drying time, in this case, was 150 ± 15 min.

The presented data showed the relatively high purity of the hemoglobin products in terms of protein (Table-3).

Thus, the hemoglobin concentrate or dry powder production scheme may be the following (Figure-8).

After its collection, the blood of slaughtered animals is sent to a defibrinator to remove fibrin. This will prevent coagulation and increase the purity of the final product. Fibrin does not form a continuous network of fibers that can entrain the formed elements. Therefore, the erythrocyte concentrate remains almost unaffected during defibrination. Instead, fibrin is removed in the form of dense fibers. The filtered fibrin may be sent for further processing, for example, for the production of fibrin films. The defibrinated blood is to be centrifuged to separate the plasm from the erythrocyte concentrate. The separation factor indicating how many times the acceleration of the centrifugal field developed in the centrifuge exceeds the gravitational field acceleration should be around 2,000. The centrifugation time should be 5.5 - 6 min [17]. Plasm may also be sent for further processing. It may be used for enriching food products with protein, as a blowing agent for the production of medicinal products, etc. [18, 19].

ARPN Journal of Engineering and Applied Sciences
©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

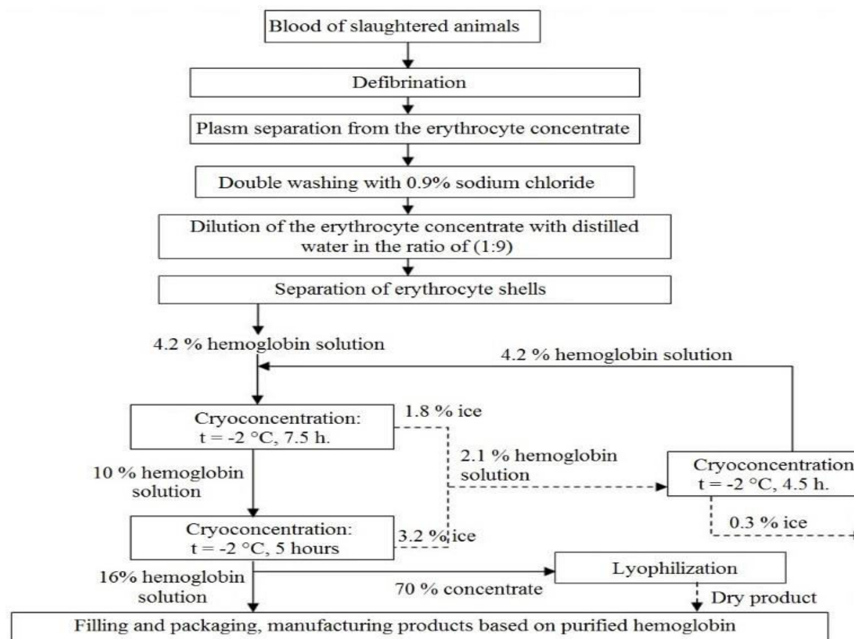

Figure-8. The scheme for extracting hemoglobin from the animal blood.

The erythrocyte concentrate is to be washed twice with the 0.9 % solution of sodium chloride. This removes the remaining plasm and other cells. Next, the erythrocyte concentrate is to be diluted with distilled water. The distilled water to the erythrocyte concentrate ratio is to be 9:1. This ensures effective hemolysis of the formed elements. The hemolysate is to be subjected to centrifugation with the separation factor exceeding 5,000, for 20 min. This would remove the empty membranes of the erythrocyte shells and other foreign components. The supernatant is the purified hemoglobin solution with the mass fraction of the dry matter of 4.2 ± 0.1 %.

The hemoglobin solution is to be subjected to two-phase separation freeze-out based on the principles described above. The hemoglobin concentrate may be sent to the production of hemoglobin reference kits. Otherwise, it may be subjected to lyophilization for obtaining the 70 ± 1 % concentrate or dry powder. These may be used, e.g., for the production of antanemic products [20, 21].

Thus, the scientific contribution of the work is in developing the technology for extracting hemoglobin from the blood of slaughtered animals using osmotic hemolysis, followed by the concentration through freezing out. The technology can be used not only for the pig blood but also for the blood of cattle, the only difference being the centrifugation conditions.

Comparing the obtained results to the existing ones, it may be said that this technology has certain advantages. In one of the works [22], the method for extracting hemoglobin by hemolysis in a membrane bioreactor was proposed. The pressure in the reactor was to be maintained by pumping the hypotonic buffer solution out at a certain rate. This method was characterized by a high degree of purity of the obtained hemoglobin; however, there was a need for periodic replacement of the membranes, which makes the technology more costly.

The most common method of concentrating hemoglobin is drying, mainly freeze-drying [23] or spraying [24]. Freeze-drying is characterized by high energy costs, and spraying envisages exposure to high temperatures, which may cause denaturation of the heat-labile components of the product. In this case, separation freeze-out has an advantage, since it is characterized by low energy consumption and the absence of exposure to high temperatures.

CONCLUSIONS

The developed technology for extracting hemoglobin by osmotic hemolysis followed by separation freeze-out may be used not only for obtaining reference hemoglobin solutions in hematolytic studies but also for obtaining antanemic products and biologically active supplements. Separation freeze-out is a promising concentration method. The possible disadvantage of introducing this technology is the initial cost of manufacturing the required equipment. However, the low operating costs ensure fast payback and the relatively high economic efficiency of the cryoconcentration technology.

REFERENCES

[1] Mandal P. K. and Biswas A. K. 2014. Animal by-products utilization, In: Animal Products Technoloy, Studium Press (India) Pvt. Ltd, New Delhi. pp. 586-608.

[2] Choe J. H., Choi M. H., Ryu Y. C., Lim K. S., Lee E. A., Kang J. H., Hong K.C., Lee S. K., Kim Y. T.,

©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

Moon S.S., Lee K.W., Rhee M.S. and Kim B.C. 2015. Correlations among various blood parameters at exsanguination and their relationships to pork quality traits. Animal Production Science. 55(5): 672-679. doi:10.1071/AN13424

[3] Dobrynina A. F., Mayakov G. A., Khomich Y. Y., Zaripova R. K. 2011. Ispolzovanie krovi zhivotnykh v myasopererabatyvayushchei promyshlennosti [The use of animal blood in meat industry]. Modern high technologies. 1: 80.

[4] Kriger O., Lisitsyn A. and Prosekov A. 2016. Characteristics of the molecular weight distribution of the prion protein fractions in blood and milk processing products. Biosciences Biotechnology Research Asia. 13(1): 85-90. doi:10.13005/bbra/2007.

[5] Izgarishev A. V., Kriger O. V. Prosekov A. Y., Babich O. O. and Dushlyuk L. S. 2013. Innovative approach to fabrication of blood product of farm animals intended for prevention of iron deficiency. World Applied Sciences Journal. 23(4): 532-540. doi:10.5829/idosi.wasj.2013.23.04.13082

[6] Lisitsyn A., Prosekov A. and Kriger O. 2016. Research of Methods of Identification and Quantitative Content of Prion Protein in Blood of Animals and Man. Research journal of pharmaceutical biological and chemical sciences. 7(2): 1723-1728. WOS:000410566700285.

[7] Perkel T. P. 2004. Fiziko-khimicheskie i biokhimicheskie osnovy proizvodstva myasa i myasnykh produktov [Physicochemical and biochemical principles of meat and meat products production]: The Kemerovo Institute of Food Technology. Kemerovo. p. 100.

[8] Nekrasova I. I. 2014. Belkovyi sostav syvorotki krovi zhivotnykh razlichnoi stressoustoichivosti [Protein composition of the blood serum of animals with various stress tolerances]. Agricultural sciences and the AIC at the turn of the century. 8: 124-127.

[9] Tae-Hwan Jung, Jae-Hwan Choi, Kyung-Chul Koh, Woo-Min Jeon and Kyoung-Sik Han1. 2017. Purification and Anti-pathogenic Properties of Immunoglobulin Concentrates from Porcine Blood. Korean J Food Sci Anim Resour. 37(5): 743-751. doi: 10.5851/kosf

[10] Izgaryshev A. V., Babich O. O., Karchin K. V., Bezyukov J. E. and Izgarysheva N. V. 2016. 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. Biology and Medicine. 8(6), article number 330. doi: 10.4172/0974-8369.1000330.

[11] Verma A. K., Chatli M. K., Kumar P. and Mehta N. 2018. Effects of inclusion of porcine blood hydrolysate on physico-chemical quality, oxidative and microbial stability of pork batter stored at (4 ± 1 °C). Journal of Food Science and Technology. 55(12): 4758-4769. doi: 10.1007/s13197-018-3409-2.

[12] Tran M. C., T'Jollyn I., Logie J., van den Broek M., De Paepe M. and Vanslambrouck B. 2016. Performance of heat pump drying system integrated into a blood dryer. Drying Technology. 34(14): 1677-1689. doi:10.1080/07373937.2016.1187627.

[13] Kumaki R., Moriya T. and Takahashi T. 2018. An expression model of blood drying process based on SPH method. International Workshop on Advanced Image Technology. pp. 1-4. doi:10.1109/IWAIT.2018.8369728.

[14] Zhang Y., Xiong W., Liu W., Wang X. and Zhang J. 2016. Effects of drying methods on the physicochemical properties of pig serum. Modern Food Science and Technology. 32(10): 220-225. doi:10.13982/j.mfst.1673-9078.2016.10.033.

[15] Fedorov D. E. 2014. Razrabotka nizkotemperaturnoi tekhnologii vydeleniya gemoglobina iz krovi uboinykh zhivotnykh [Development of a low-temperature technology for extracting hemoglobin from the blood of slaughtered animals]: Thesis ... Candidate of Technical Sciences. 05.18.04. Kemerovo. 136 p.

[16] Korotky I. A. 2009. Issledovanie i razrabotka tekhnologii zamorazhivaniya i nizkotemperaturnogo khraneniya plodovo-yagodnogo syrya Sibirskogo regiona [Research and development of the technologies for freezing and low-temperature storage of fruit and berry raw materials in the Siberian region]: Thesis ... Doctor of Technical Sciences. 05.18.04. Kemerovo. p. 410.

[17] Izgaryshev A. V. 2013. Issledovanie i razrabotka produkta iz krovi selskokhozyaistvennykh zhivotnykh

©2006-2020 Asian Research Publishing Network (ARPN). All rights reserved.

dlya profilaktiki zhelezodefitsita [Research and development of a product from the blood of farm animals for preventing iron deficiency]: Abstract of Thesis for Candidate of Technical Sciences. 05.18.04, Kemerovo. p. 18.

[18] Kriger O. V. 2014. Advantages of porcine blood plasma as a component of functional drinks. Foods and Raw Materials. 2(2): 26-31. doi: https://doi.org/10.12737/5456. (In Russ.).

[19] Torres M. R., Marn F. R., Ramos A. J., Soriano E. 2002. Study of operating conditions in concentration of chicken blood plasma proteins by ultrafiltration. Journal of Food Engineering. 54(3): 215-219.

[20] Izgarishev A. V., Izgarisheva N. V. and Ostroumov L. A. 2018. Development and study of food product with anti-anemic agent based on farm animal blood. Foods and Raw Materials. 6(1): 56-62. doi: https://doi.org/10.21603/2308-4057-2018-1-56-62. (In Russ.).

[21] Antipova L. V., Peshkov A. S., Toporkova A. E. and Kuznetsova E. I. 2008. Sozdanie antianemicheskikh produktov na osnove vtorichnykh produktov myasopererabatyvayushchei otrasli [Creating antanemic products based on byproducts of the meat processing industry]. Advances of modern natural science. 6: 133.

[22] Stojanović, Radoslava, Ilić, Vesna, Manojlović Verica, Bugarski Diana, Devic Marija, Branko Bugarski. 2012. Isolation of Hemoglobin from Bovine Erythrocytes by Controlled Hemolysis in the Membrane Bioreactor. Applied biochemistry and biotechnology. 166: 1491-506. doi: 10.1007/s12010-012-9543-9.

[23] Hou C., Song X., Li Z., Wang W., Shen Q. and Zhang D. 2019. Arginine improves the color stability of hemoglobin powder during freeze-drying and storage. Food Science and Nutrition. 7(5): 1677-1684. doi:10.1002/fsn3.1004.

[24] Drvenica I. T., Stančić A. Z., Kalušević A. M., Marković S. B., Dragišić Maksimović J. J., Nedović V. A., Bugarski B. M. and Ilić V. L. 2019. Maltose-mediated, long-term stabilization of freeze- and spray-dried forms of bovine and porcine hemoglobin. Journal of the Serbian Chemical Society. 84(10): 1105-1117. doi:10.2298/JSC190513067D.