

This paper reports the development of an express method for assessing the quality of biologically active substances derived from colostrum. We tested the hypothesis that there may be a dependence between the amount of protein that is part of the colostrum and its characteristic (a difference in molecular masses) and electrical conductivity.

It has been shown that the colostrum contains several hundred proteins: it depends on the individual characteristics of cattle. The removal of lipids was accompanied by an increase in electrical conductivity from 5 % to 18 % compared to the whole colostrum while the subsequent removal of high-molecular proteins increased the electrical conductivity by 50–100 % compared to skimmed colostrum: this depends on the individual characteristics of cattle. Such an individual feature of the colostrum composition reflects the uniqueness of the individual animal's metabolism. A mathematical model has been built for the dependence of the content of charged molecules in the solution of proteins on the molecular mass of proteins, which explains the relationship between electrical conductivity and the molecular mass of proteins.

It was shown that there is a direct correlation between the colostrum electroconductivity and the temperature in a measuring cell in the range of temperatures from 14 °C to 19 °C. The electrical conductivity of colostrum components increased by no more than 20 % during storage (at a temperature of 3–4 °C) up to 18 days, which is associated with protein degradation. The electrical conduction method could be used to assess the colostrum composition during storage.

Technology for obtaining different colostrum components (skimmed fraction and a fraction of low-molecular components) has been devised, as well as a method for assessing the quality of products based on the characteristics of electrical conductivity.

Electrical conductivity is a promising method for assessing the quality of products that are derived from colostrum, at different shelf life at different stages of production: raw materials, fat removal, obtaining a fraction with a predefined composition of proteins

Keywords: *electrical conductivity, colostrum, biologically active compounds, low-molecular proteins, lipids, temperature, storage*

DEVISING AN EXPRESS METHOD FOR ESTIMATING THE QUALITY OF COLOSTRUM AND ITS COMPONENTS BASED ON ELECTRICAL CONDUCTIVITY

V. Kozheshkurt

Researcher*

E-mail: v.kozheshkurt@karazin.ua

Ie. Ivanov

PhD, Commercial Director

«Alpha» Farm Enterprise

Titkova str., 60, Odnorobivka, Zolochiv distr.,

Kharkiv reg., Ukraine, 62210

E-mail: Ivanovevg321@gmail.com

Ye. Antonenko

Senior Lecturer, Researcher*

E-mail: antonenko@karazin.ua

V. Katrich

Doctor of Physical and Mathematical Sciences, Professor*

E-mail: vkatrich@karazin.ua

A. Bozhkov

Doctor of Biological Sciences, Professor

Department of Molecular Biology and Biotechnology

V. N. Karazin Kharkiv National University

Svobody sq., 4, Kharkiv, Ukraine, 61022

E-mail: bozhkov@univer.kharkov.ua

T. Gromovoy

PhD, Senior Researcher

Laboratory of Mass Spectrometry of Surface of Nanosystems

Chuiko Institute of Surface Chemistry of

National Academy of Sciences of Ukraine

Generala Naumova str., 17, Kyiv, Ukraine, 03164

E-mail: grot@ukr.net

*Department of Physical and Biomedical Electronics and

Complex Information Technologies

V. N. Karazin Kharkiv National University

Svobody sq., 4, Kharkiv, Ukraine, 61022

Received date 25.01.2021

Accepted date 12.02.2021

Published date 19.02.2021

Copyright © 2021, V. Kozheshkurt, Ie. Ivanov, Ye. Antonenko, V. Katrich, A. Bozhkov, T. Gromovoy

This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0>)

1. Introduction

Colostrum is a natural complex, unique in composition and biological activity, which regulates the activity of the immune system. It is a fluid that is formed in mammalian

breasts within a few days after birth. Colostrum includes proteins, hormones, lipids, amino acids, cytokines, and a wide variety of low-molecular peptides [1]. The transfer factor contained in the colostrum is of much interest as a factor in the modulation of the immune system [2, 3].

Along with the transfer factor, colostrum contains a large number of other low-molecular biologically active compounds that remain under-researched and could be of great interest to the pharmacy.

The low-molecular components of colostrum (whose molecular mass is less than 10 kDa) are capable of eliminating the manifestation of oxidative stress in experimental animals with induced liver fibrosis [4]. In addition, low-molecular components of the colostrum have a modulating effect on the immune system [5] and affect other biochemical characteristics of the body. It could be argued that colostrum is a promising object for deriving biologically active compounds with a wide range of action. Despite this, there is no technology to obtain preparations from cow colostrum, except for the transfer factor [6]. However, in the processing of colostrum, researchers face a number of unresolved issues and, above all, the standardization of finished products, which makes it difficult to devise new technologies for processing products with complex composition. This issue's essence is to obtain a substance represented not by one but by a large number of different components. Determining their full composition is quite a complex task, which requires large time costs. Moreover, the composition of the components and the physical and chemical characteristics of colostrum could vary for different individual animals, depending on the timing after calving, and could change in the process of processing and storage. To address issues related to the standardization of colostrum components, it is necessary to devise control methods that would make it possible to evaluate the overall integrated indicators of the final product, to be express, reliable, and affordable. These criteria could be met by such a biophysical indicator as the electrical conductivity of liquid biological samples, in particular, colostrum.

It could be argued that the search for natural biologically active compounds that could regulate the activity of the body's immune system is an acute task for pharmacy and medicine; however, there are no drugs derived from colostrum, which is associated with the lack of methods to standardize and control the substances derived from colostrum.

2. Literature review and problem statement

It is known that the electrical conductivity of solutions is determined by the number of ions, their charge, and mobility [7]. The charge of an ion is a multiple of the charge of an electron, and the modulus of charge of an ion is equal to an integer, most often a small integer number of elementary charges, which introduces an integer coefficient into the value of electrical conductivity. The mobility of ions is defined by both the properties of the ion itself (mass, size, the formation of structures with surrounding solvent molecules) and solvent properties (viscosity, temperature, component composition). More than mobility, the concentration of ions can vary in solutions. A simple model is environments with a small number of components, which are a solution of inorganic electrolytes in water. Thus, electrical conductivity is a characteristic of the ion composition of these environments – both the total content of ions and, to a lesser extent, the ratio of different ions.

In multi-component, different-in-composition molecules of biological environments, electrical conductivity depends not only on the ion composition but also on the number and characteristics of macromolecules. It has been shown that

charged organic molecules could form current-conducting structures [8], and uncharged lipid components could increase the electrical resistance of the environment and, as a result, affect electrical conductivity [9]. Of great interest is the influence of both the content and composition of proteins on electrical conductivity, as in biological environments there are always proteins that show the greatest biological activity. At the same time, the contribution of proteins to electrical conductivity remains insufficiently studied. Currently, there is no universal model of electrical conductivity for biological environments. Existing analytical methods may not always be used to determine the complex composition of biological environments. Therefore, the indicator of electrical conductivity is promising for the integrated characteristic of complex biological environments with dynamic composition.

Colostrum contains a wide variety of biologically active compounds: hormones, cytokines [10], peptides, proteins with a variety of properties [11, 12]. Colostrum can be seen as a source of a variety of compounds that could be used in pharmacy. However, the issue of colostrum processing is associated with the lack of deep colostrum processing technology, which is due to several reasons. The colostrum composition depends on the individual characteristics of producers [13]; it is not subject to standardization. At the same time, the dependence of the composition of individual components of colostrum, in particular, low-molecular components of proteins, which are of interest as biologically active compounds, on individual characteristics of cows (producers) remains unexplored. The composition of colostrum proteins and peptides is extremely diverse [14] and has not been fully studied. Such a complex and heterogeneous protein composition could only be determined by the modern methods of mass spectrometry [15]. However, these methods are not affordable in conventional production, they require the training of highly qualified professionals, and are expensive. In this regard, it is necessary to search for simple, adequate, and fast methods for analyzing the composition of the colostrum components. A physical method such as the electrical conductivity of bodily fluids [16] could be used as an express method for analyzing the composition of colostrum and its components. This method is based on determining the number of charged ions and molecules [17]; their mobility is influenced by the temperature, environmental viscosity, and concentration of molecules, as well as the presence of molecules with dielectric properties [18]. However, there are no experimental studies of the relationship between the colostrum proteins and electrical conductivity.

3. The aim and objectives of the study

The aim of this work was to devise an express method for assessing the quality of biologically active substances derived from colostrum.

To achieve the set aim, the following tasks have been solved:

- to investigate the effect of temperature in a measuring cell on the electrical conductivity of different fractions of colostrum (whole colostrum and skimmed colostrum);
- to determine the electrical conductivity of different fractions of colostrum (whole colostrum, skimmed colos-

trum, and colostrum that contains only low-molecular proteins) obtained from different cows;

- to determine the composition of colostrum proteins by a mass spectrometry method;

- to build a model of the relationship between low-molecular (with a molecular weight of less than 10 kDa) and high-molecular (with a molecular weight of more than 10 kDa) proteins and electrical conductivity;

- to determine the electrical conductivity of different fractions of colostrum in the process of storage at 3 °C for up to 18 days.

4. Methods to derive the colostrum fractions and to study the composition of proteins and electrical conductivity

4.1. The working hypothesis of research

The working hypothesis assumed that there was a connection between the amount of protein, which is part of the colostrum, and its characteristic (a difference in molecular masses) and electrical conductivity.

4.2. The rationale for the experimental approach

Colostrum samples were received at the Alfa Farm in Kharkiv Oblast (Ukraine) from three Ukrainian milk-speckled cows named Aurora, Barynya, and Mukha. Colostrum samples were kept under the same conditions; colostrum of the second milk yield was adopted for the research. The whole colostrum was used to prepare fat-free colostrum. To this end, the whole colostrum was centrifuged at 3,000 *g* for 15 minutes at room temperature. Lipids were removed, and centrifuge was repeated one more time, followed by removal of residual lipids. We removed high-molecular proteins (exceeding 10 kDa) from skimmed colostrum by the membrane filtration, with a diameter of pores of ~10 μm, and thus we received the so-called low-molecular fraction of colostrum.

4.3. Determining the specific electrical conductivity of the samples being examined

Parts of the samples of whole colostrum, skimmed colostrum, and a solution of low-molecular colostrum fractions (LCF) in the amount of 1 ml were selected daily, during the study period, from the total volume of the samples stored at a temperature of 3 °C. Samples were brought to the Teflon cell, which was connected to two ports of the vector analyzer ZNB 40 “Rohde & Schwarz” (Austria). The specific electrical conductivity was measured in the 100 kHz–10 MHz range at an interval of 50 kHz.

The temperature of the samples was controlled by a thermometer at the thermocouple, with an accuracy of up to 0.1 °C. To avoid interfering with the conductivity measurement, the sample temperature was measured between conductivity measurements.

The ZNB 40 “Rohde & Schwarz” analyzer software makes it possible to acquire automatically calculated values for the actual (Z_{re}) and imaginary (Z_{im}) part of the measuring cell impedance.

To calculate the electrical conductivity of the cell suspension, the transition is made from the impedance of the measuring cell to the equivalent parameters of the electrical circuit. We shall represent the equivalent scheme of the measuring cell as a parallel connection between the capacitor and the resistor. Then the equivalent capacity C and the equivalent resistance R of the circuit could be calculated

through the actual and imaginary parts of the impedance from the following formulae:

$$C = -\frac{1}{\omega \cdot \left(\frac{Z_{re}^2 + Z_{im}^2}{Z_{im}} \right)}, \quad (1)$$

$$R = Z_{re} + \frac{Z_{im}^2}{Z_{re}}, \quad (2)$$

where ω is the cyclical frequency of the electric current.

The electrical conductivity of the contents of the measuring cell, in addition to the properties of the substance, is determined by the geometric parameters of the cell. To eliminate the influence of geometric parameters, the results were represented in the form of specific electrical conductivity values σ :

$$\sigma = k \cdot \frac{1}{R}. \quad (3)$$

The factor $k = 175 \text{ m}^{-1}$ was calculated for the 1 ml cylindrical cell used applying calibration solutions with a known specific electrical conductivity.

4.4. Determining the content and composition of colostrum proteins

The content of common proteins was determined by the Lowry et. al. method [19].

To study the composition of proteins in whole colostrum and LCF, we prepared samples so that 1 ml contained 2 mg of protein from each fraction. Mass spectrometric studies were carried out at the device Autoflex II LRF 20 “Bruker Daltonics” (Germany), equipped with a pulsed nitrogen laser ($\lambda = 337 \text{ nm}$, pulse duration – 3 ns). The samples of proteins, after mixing with the matrix, which was prepared according to the standard procedure: 12 mg of synapctic acid (Fluka) dissolved in 1 ml of the mixture water–isopropanol alcohol (1:2 V/V) with the addition of 0.1 % trifluoroacetic acid were applied to a steel target and dried at room temperature. The analysis was carried out under a linear mode of device operation with the detection of positive and negative ions. The results were analyzed using the open software ProteoWizard (<http://proteowizard.sourceforge.net>) mMass (<http://mmass.org>).

4.5. Statistical treatment of results

Reliable differences between the groups were determined by the non-parametric test of Mann-Whitney. All statistical analyses were performed using the software Statistica 8.0 (StatSoft Inc., USA). Differences between control and experimental groups were accepted as reliable at $p < 0.05$.

5. Results of studying the electrical conductivity of colostrum components at different temperatures and storage duration

5.1. The temperature dependence of the electrical conductivity of whole and skimmed colostrum

It is known that the electrical conductivity is affected by the temperature in the measuring cell. We found that with

an increase in temperature in the range of 14 °C to 19 °C, there was a linear increase in electrical conductivity, and this was manifested in the same degree, both in the case of whole and skimmed colostrum (Fig. 1). Hereafter, all measurements of electrical conductivity were carried out at a temperature of 18 °C.

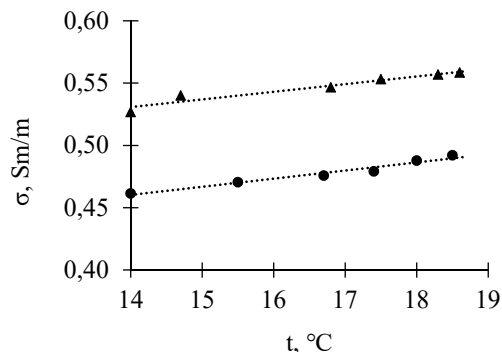


Fig. 1. Electric conductivity of whole colostrum (●) and skimmed colostrum (▲) in the temperature range from 14 °C to 19 °C at an electric current frequency of 0.49 MHz. Shown are the typical conductivity curves from three independent replicates

5. 2. The electrical conductivity of whole colostrum and colostrum components, derived from different cows

The electrical conductivity of the whole colostrum, which was measured at 18±1 °C, varied slightly for different cows from 0.37 to 0.43 S/cm and did not depend on the current frequency in the 100 kHz–10 MHz frequency range (Fig. 2, a–c).

Lipids exhibit the properties of dielectrics [20] and could affect electrical conductivity in multi-component environments such as colostrum.

The removal of lipids from the whole colostrum was accompanied by an increase in electrical conductivity compared to the whole colostrum; however, for Aurora, the increase amounted to 17.0 % of the original level, while for Barynya and Mukha – to only 5.6 and 2.9 %, respectively (Fig. 2, a–c). These results suggest that the amount of fat in the colostrum from different cows varies, or the contribution of fats to the electrical conductivity of the colostrum is not significant.

5. 3. The protein composition of whole colostrum and colostrum components and its relationship with electrical conductivity

As noted, colostrum contains a large number of proteins with different molecular weights, which could also vary in charge and exert an impact on electrical conductivity.

Thus, our determining the composition of proteins by a mass spectrometry method has revealed that the whole colostrum composition includes several hundred proteins with a molecular weight from 4 to 20 kDa (Fig. 3). Proteins with a molecular weight from 4 to 9 kDa and a fraction of caseins with a molecular weight from 19 to 20.5 kDa (Fig. 3) are represented in the largest quantities in colostrum. Along with the similarity of the protein composition of colostrum derived from different cows, the quantitative and qualitative differences were revealed (Fig. 3).

The removal of most high-molecular proteins (exceeding 10 kDa) by membrane filtration has made it possible to identify 27 fractions of proteins with a molecular weight from 4,835 to 9,470 Da for Aurora, and 30 fractions of proteins in the same range of molecular weight for Barynya (Fig. 4).

It should be noted that for the colostrum proteins obtained from Aurora and Barynya, there is no complete overlap of spectral characteristics by molecular weight, and only 5 fractions of proteins were identical (Fig. 4). Therefore, we can argue about the presence of the expressed individual characteristics of the colostrum protein spectra.

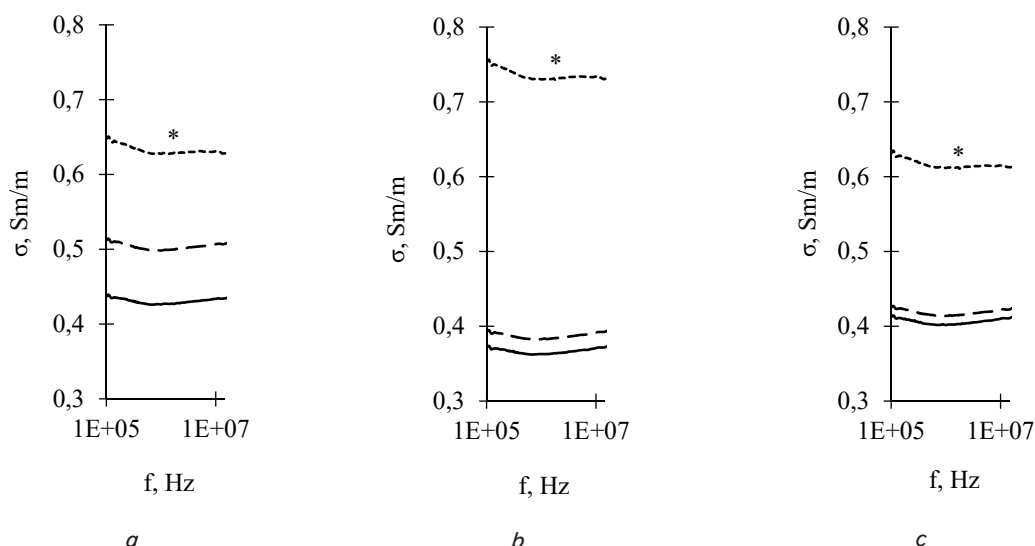


Fig. 2. The electrical conductivity of whole colostrum (————), skimmed colostrum (— — —), and low-molecular colostrum components (· · · · ·) in the 100 kHz–100 MHz frequency range: a – colostrum and colostrum products derived from Aurora; b – colostrum and colostrum products obtained from Barynya; c – colostrum and colostrum products obtained from Mukha. Shown are the typical electrical conductivity curves from three independent replicates; * – options for which p<0.05 compared to whole colostrum

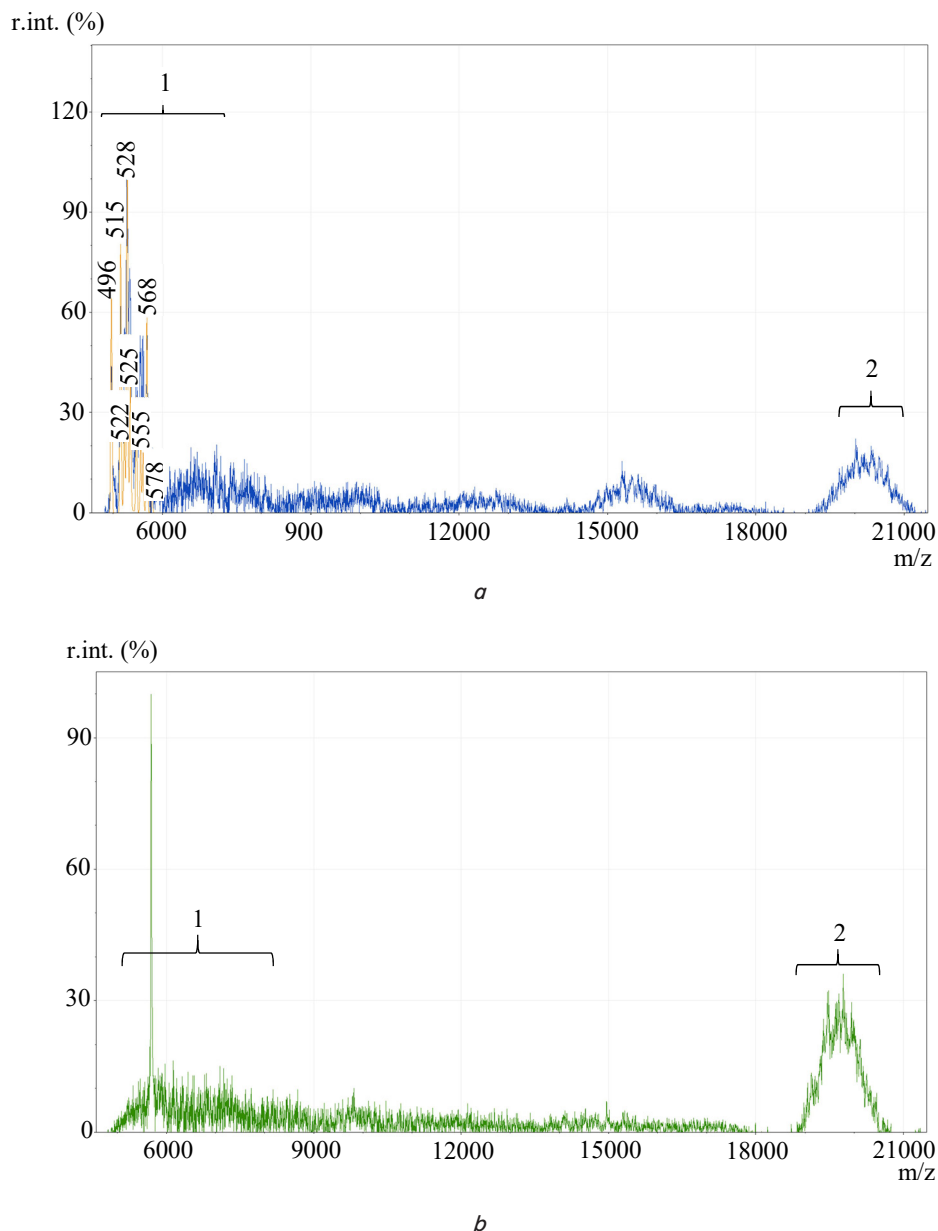


Fig. 3. The composition of skimmed colostrum proteins according to mass spectrometry: *a* – colostrum derived from Aurora; *b* – colostrum derived from Barynya; 1 – the composition of proteins with a molecular weight from 4 to 9 kDa; 2 – the fractions of colostrum with a molecular weight from 19 to 20.5 kDa. Typical protein spectra are represented

The high variability in the composition of colostrum proteins is also evidenced by data reported in other studies. The most interesting are the proteins of colostrum with a molecular weight of about 5 kDa – the so-called antigen-specific transfer factor – SFT [21]. The molecular analysis of antigen-specific colostrum proteins was for the first time reported in work [22], which showed that more than 200 different colostrum proteins possessed the immunomodulatory and immuno-replacement properties [14].

The removal of high-molecular proteins from skimmed colostrum was carried out by membrane filtration; the content of proteins reduced by 3.3 times compared to the original, almost equally for all samples. The removal of high-molecular proteins was accompanied by a significant increase in electrical conductivity for all three variants

under study (Fig. 2). For example, Aurora’s electrical conductivity of low-molecular colostrum components increased by 40.5 % and 47.4 % compared to skimmed and whole colostrum, respectively (Fig. 2, *a*). The Barynya’s electrical conductivity increased by 96.1 % and 101 % compared to skimmed and whole colostrum, respectively; that of Mukha – by 50.1 % and 52.3 % (Fig. 2, *b, c*).

It should be noted that the greatest increase in electrical conductivity after the removal of high-molecular colostrum proteins was observed for the colostrum derived from Barynya; it revealed the largest range of low-molecular proteins (Fig. 4, *b*).

Consequently, the high-molecular proteins (whose molecular mass exceeds 10 kDa), which made up most of the colostrum proteins, reduce the mobility of ions in the electric field.

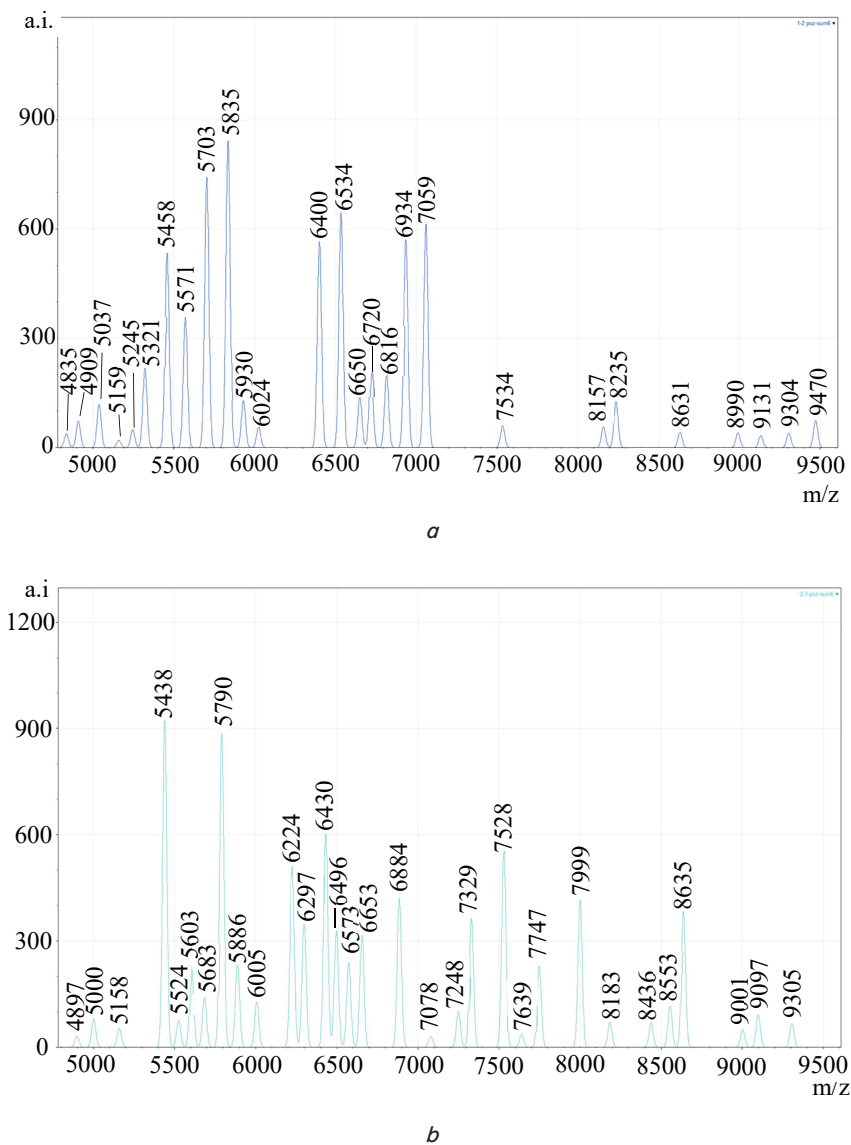


Fig. 4. The composition of proteins of low-molecular fractions of colostrum according to mass spectrometry: *a* – colostrum derived from the cow “Aurora”; *b* – colostrum derived from the cow Barynya. Typical protein spectra are represented

5. 4. Mathematical model of the number of free charge carriers in protein solutions

The ability of water solutions to conduct electric current depends on the number of charge carriers, especially ions, as well as their mobility. If the charge carriers are represented by proteins with a large molecular weight, their mobility would be low due to the resistance caused by other polymers contained in the colostrum. Consequently, the electrical conductivity of such multi-component mixtures with a large number of not only ions but also amino acids and proteins would depend on the molecular mass, size of molecules, their charge, and the ability to ionize.

To verify this position, a model was built for the relationship of molecular mass with the potential charge of the molecule. Since the protein molecule contains a different amount of amino acids with positive, negative charges, or neutral, proteins could be carriers of charges, and it depends on the composition of amino acids.

In this paper, the proteins are conditionally divided into low-molecular fractions with a molecular weight of about 6 kDa and fractions with a molecular weight of approximately up to 18 kDa. Assume that the average molecular mass of an amino acid equals 120 Da. Thus, the molecules of proteins of different fractions in the solutions studied contain, respectively, about 6,000/120/50 and about 18,000/120/150 amino acids.

We shall assess the probability of an event in which a protein molecule is neutral and an event in which a protein molecule has an uncompensated charge.

Denote the amount of amino acids in protein via *N*. Each amino acid is neutral, positively charged, and negatively charged, with probabilities, respectively, *p*₀, *p*₊, and *p*₋

$$p_0 + p_+ + p_- = 1. \tag{4}$$

If there are two types of amino acids with probabilities *p*₁, and *p*₂, *p*₁+*p*₂=1, the probability of an event in which the protein from *N* amino acids would have a certain sequence is:

$$P_{config} = p_1^{n_1} \cdot p_2^{n_2}, \tag{5}$$

where *n*₁ and *n*₂ are the numbers of amino acids of the first and second type, *n*₁+*n*₂=*N*.

The total charge of a protein molecule is determined by the number of amino acids of each type without taking into consideration their sequence. We shall take into consideration the number of different configurations of protein with the same amounts *n*₁ and *n*₂:

$$P_{rel} = p_1^{n_1} \cdot p_2^{n_2} \cdot \frac{N!}{n_1! \cdot n_2!}. \tag{6}$$

We shall apply the resulting formula to the protein molecule, adopting as the first and second type of amino acids the neutral amino acids and charged amino acids (regardless of the charge sign).

$$P_i = p_0^{i_0} \cdot (p_+ + p_-)^{N-i_0} \cdot \frac{N!}{i_0! \cdot (N-i_0)!}. \tag{7}$$

This equation shows the probability of an event in which a protein molecule contains the number *i*₀ of neutral amino acids.

At a predefined amount of neutral amino acids *i*₀, the amount of charged amino acids is equal to *N*-*i*₀. Consider the case of a molecule with a compensated charge:

$$n_+ = n_- = \frac{N - i_0}{2}. \tag{8}$$

The probability of an event in which the charge of a molecule at the predefined i_0 is compensated equals:

$$P' = p'_+ \frac{N-i_0}{2} \cdot p'_- \frac{N-i_0}{2} \cdot \frac{(N-i_0)!}{\left(\frac{N-i_0}{2}\right)! \left(\frac{N-i_0}{2}\right)!}, \quad (9)$$

where p'_+ and p'_- are the probabilities of events p_+ and p_- , recalculated given that the event p_0 has not already taken place (that is, the remaining amino acids under consideration are not exactly neutral), $p'_+ + p'_- = 1$.

The probability of events in which a protein molecule contains equal amounts of positive and negative amino acids at each certain amount of neutral amino acids i_0 is equal to:

$$P_0 = p_0^{i_0} \cdot (p_+ + p_-)^{N-i_0} \cdot \frac{N!}{i_0! (N-i_0)!} \cdot p'_+ \frac{N-i_0}{2} \cdot p'_- \frac{N-i_0}{2} \times \frac{(N-i_0)!}{\left(\frac{N-i_0}{2}\right)! \left(\frac{N-i_0}{2}\right)!} = p_0^{i_0} \cdot (p_+ + p_-)^{N-i_0} \cdot p'_+ \frac{N-i_0}{2} \times \frac{N!}{i_0! \left(\frac{N-i_0}{2}\right)! \left(\frac{N-i_0}{2}\right)!} \quad (10)$$

when summed:

$$P_{neutr} = \sum_{i_0=0}^N p_0^{i_0} \cdot (p_+ + p_-)^{N-i_0} \cdot (p'_+ \cdot p'_-)^{\frac{N-i_0}{2}} \cdot \frac{N!}{i_0! \left(\left(\frac{N-i_0}{2}\right)!\right)^2} \quad (11)$$

The software calculations took into consideration that in the case of an odd amount $n_+ + n_- = N - i_0$, the probability of compensation for the charge is zero.

Let us compare the concentration of free charge carriers in solution in the presence of the high-molecular (150 amino acids) and low-molecular (50 amino acids) proteins at the same mass of proteins in the solution.

Accept that the presence of the amino acid positive and negative charge is equally likely, $p_+ = p_-$. Consider three cases: a zero-chance of amino acid neutrality ($p_+ = 0, p_+ = p_- = 0.5$), with a 50 % chance of amino acids neutrality ($p_+ = 0.5, p_+ = p_- = 0.25$), and a 90 % chance of amino acids neutrality ($p_+ = 0.9, p_+ = p_- = 0.05$).

Our calculations according to formula (11) have shown that in all three cases, a protein molecule with 150 amino acids is more likely to be charged than a protein molecule containing 50 amino acids (accordingly, $P_{50} = 88.77\%$, $P_{150} = 93.50\%$ for the case $p_+ = 0, p_+ = p_- = 0.5$; $P_{50} = 99.99\%$, $P_{150} \approx 100\%$ for the case $p_+ = 0.5, p_+ = p_- = 0.25$; $P_{50} = 99.44\%$, $P_{150} \approx 99.99\%$ for the case $p_+ = 0.9, p_+ = p_- = 0.05$).

The molecular mass of high-molecular fractions is three times higher than the molecular mass of low-molecular fractions. This means that with the same protein content m in the solution, the number of molecules of low-molecular frac-

tions is three times greater than the high-molecular fractions since $N' = m/M$.

Even in the case of the maximum difference in the probabilities of the charge of the molecule ($P_{50} = 88.77\%$ and $P_{150} = 93.50\%$), the estimated average concentration of charge carriers in the solution of low-molecular fractions is larger than 2.85 times:

$$\frac{C_{50}}{C_{150}} = \frac{N'_{50} P_{50}}{N'_{150} P_{150}} = \frac{3 \cdot 0.8877}{0.935} \approx 2.85. \quad (12)$$

In addition to the concentration of free charge carriers, electrical conductivity is affected by their mobility. In the model examined, low-molecular fractions, due to the smaller size of the molecule, have greater mobility in the solution, which, at a qualitative level, also confirms their greater electrical conductivity.

5.5. The effect of colostrum shelf life on electrical conductivity

An important task in the practical use of colostrum is to define the conditions of storage before the processing of colostrum. Since biologically active compounds may lose their activity during freezing and defrosting, it was interesting to estimate the electrical conductivity in the storage of colostrum at 3–4 °C.

It turned out that the electrical conductivity during the storage of different fractions of colostrum (whole, skimmed, and low-molecular) increased from day 1 to day 18 of storage at 3–4 °C (Fig. 5).

We show the averages and a standard error of three independent experiments. * – options for which $p < 0.05$ compared to day 1.

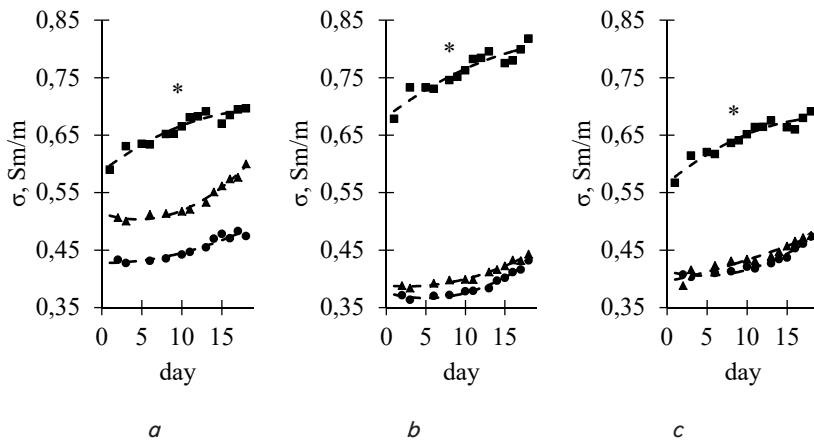


Fig. 5. The electrical conductivity of whole colostrum (●), skimmed colostrum (▲), and low-molecular colostrum components (■) at an electric current frequency of 0.49 MHz at 18 °C from day 1 to day 18 of storage at 3 °C: a – colostrum derived from the cow Aurora; b – colostrum derived from the cow Barynya; c – colostrum derived from the cow Mukha

However, the dynamics of the increase in electrical conductivity were different for different fractions of colostrum. Thus, the electrical conductivity of the whole and skimmed colostrum remained unchanged from day 1 to day 10 of storage, and only after that, there was a slight increase in electrical conductivity (Fig. 5). At the same time, the electrical conductivity of the low-molecular fraction of colostrum components increased linearly from day 1. On day 18, this

parameter increased by 18, 20, and 22 %, compared to the original value for Aurora, Barynya, and Mukha, respectively (Fig. 5).

Consequently, in the whole and skimmed colostrum, electrical conductivity remains relatively stable even with long-term storage of up to 18 days at 3 °C. At the same time, if fats and high-molecular proteins are removed from the colostrum, the electrical conductivity in the process of storage linearly increased during storage from day 1 to day 18. This may indicate a higher rate of degradation of low-molecular proteins compared to the whole and skimmed colostrum.

6. Discussion of results of studying the electrical conductivity of colostrum components

Our results suggest that electrical conductivity is a convenient, fast, and adequate method for assessing the quality of colostrum and its components. A given method could be used in technologies to derive biologically active substances from multicomponent biological objects.

Electrical conductivity is determined by the number of charged particles in the liquid environment and their ability to move in an electric field [17]. The increase in temperature in the measuring cell accelerates the mobility of ions and molecules; this dependence was linear in the temperature range of 14–19 °C (Fig. 1). This dependence holds for both the whole colostrum and skimmed (after lipid removal) colostrum (Fig. 1). The increase in the electrical conductivity of skimmed colostrum compared to whole colostrum is due to that lipids have the properties of dielectrics [20].

It turned out that the removal of lipids from colostrum, derived from different cows, was accompanied by an increase in electrical conductivity compared to whole colostrum, to varying degrees (Fig. 2). This is likely explained by that the ratio of lipids and proteins in colostrum, derived from different producers, is different. This fact is important in understanding the biological value of colostrum. This issue requires more research. However, our results indicate the possibility to determine this indicator (the ratio of proteins/lipids) in the colostrum by the method of electrical conductivity. The results obtained confirm the data on the diversity of colostrum proteins, so it can be argued that colostrum is characterized by a unique, individual protein profile (Fig. 3, 4). The electrical conductivity of the colostrum depends on the composition of proteins. The removal of high-molecular proteins was accompanied by a significant increase in electrical conductivity; this was manifested to varying degrees for colostrum derived from different producers (Fig. 2).

The fact that the electrical conductivity is affected not only by the charge but also by the size and likely the geometry of the protein molecule is evidenced by the increase in

electrical conductivity in the process of storing colostrum components (Fig. 5). Since protein molecules are hydrolyzed with their confirmative characteristics altered during storage [23], this was accompanied by an increase in electrical conductivity. The method of electrical conductivity could be used in the assessment of the nativity of colostrum components.

The results reported here allow us to recommend that the electrical conductivity of colostrum and its components should be used for their integrated evaluation in the technology of colostrum processing. The method is unparalleled and could complement those methods of measuring the physical and chemical characteristics of colostrum that are currently in use.

However, this approach has its own peculiarities and limitations, in particular, there are no data on the effect of specific indicators (ions, amino acids, the full composition of proteins, and others) on electrical conductivity. Further research involving model experiments should be aimed at devising the theory of electrical conductivity of complex biological solutions.

7. Conclusions

1. There is a direct correlation between the temperature in a measuring cell and electrical conductivity in the temperature range from 14 to 19 °C, due to the increased mobility of charged ions and molecules.

2. The electrical conductivity of colostrum depends on the ratio of components that make up its composition (ion, lipid, and protein components), as well as the producer of colostrum. Such an individual feature of the colostrum composition reflects the uniqueness of the metabolism of an individual animal.

3. Our analysis of the colostrum composition by a mass spectrometry method has made it possible to identify several hundred individual proteins, which are individual, which confirms the fact of the uniqueness of this biological liquid.

4. The removal of high-molecular proteins from colostrum was accompanied by a significant increase in electrical conductivity (by 50–100 %) that depends on the producer of colostrum. The built model of the dependence between the size of the protein molecule and the charge of molecules indicates that electrical conductivity depends not only on the charge of the molecule but also on its size and the presence of factors impeding the movement of ions and molecules in multi-component biological environments.

5. Electrical conductivity is a promising method for assessing the quality of products that are derived from colostrum, at different storage duration, at different stages of production: raw materials, fat removal, deriving a fraction with a certain composition of proteins.

References

1. Li, M., Li, Q., Kang, S., Cao, X., Zheng, Y., Wu, J. et. al. (2020). Characterization and comparison of lipids in bovine colostrum and mature milk based on UHPLC-QTOF-MS lipidomics. *Food Research International*, 136, 109490. doi: <https://doi.org/10.1016/j.foodres.2020.109490>
2. Vetvicka, V., Vetvickova, J. (2019). Effects of Transfer Factor Supplementation on Immune Reactions in Mice. *Journal of Nutrition and Health Sciences*, 6 (3), 301.
3. Borad, S. G., Singh, A. K. (2018). Colostrum immunoglobulins: Processing, preservation and application aspects. *International Dairy Journal*, 85, 201–210. doi: <https://doi.org/10.1016/j.idairyj.2018.05.016>

4. Bozhkov, A. I., Nikitchenko, Y. V., Lebid, K. M., Ivanov, E. G., Kurguzova, N. I., Gayevoy, S. S., Al Begai M. A. Y. (2017). Low molecular weight components from various sources eliminate oxidative stress and restore physiological characteristic of animals at early stages of Cu- induced liver fibrosis development. *Translational Biomedicine*, 8 (2). doi: <https://doi.org/10.21767/2172-0479.1000107>
5. Bozhkov, A. I., Ivanov, E. G., Begai, M., Alsardia, M., Kurguzova, N. I. (2017). Low-Molecular Weight Cow Colostrum Components in Functional Nutrition. *Journal of Nutritional Therapeutics*, 6 (1), 11–17. doi: <https://doi.org/10.6000/1929-5634.2017.06.01.2>
6. Ascher, M. S., Gottlieb, A. A., Kirkpatrick, C. H. (Eds.) (1976). *Transfer factor: Basic properties and clinical applications*. Academic Press, 780. doi: <https://doi.org/10.1016/c2013-0-07152-6>
7. Berkowitz, M., Wan, W. (1987). The limiting ionic conductivity of Na⁺ and Cl⁻ ions in aqueous solutions: Molecular dynamics simulation. *The Journal of Chemical Physics*, 86 (1), 376–382. doi: <https://doi.org/10.1063/1.452574>
8. Becker, F. F., Wang, X. B., Huang, Y., Pethig, R., Vykoukal, J., Gascoyne, P. R. (1995). Separation of human breast cancer cells from blood by differential dielectric affinity. *Proceedings of the National Academy of Sciences*, 92 (3), 860–864. doi: <https://doi.org/10.1073/pnas.92.3.860>
9. Rosenberg, B., Jendrasiak, G. L. (1968). Semiconductive properties of lipids and their possible relationship to lipid bilayer conductivity. *Chemistry and Physics of Lipids*, 2 (1), 47–54. doi: [https://doi.org/10.1016/0009-3084\(68\)90034-0](https://doi.org/10.1016/0009-3084(68)90034-0)
10. Hagiwara, K., Kataoka, S., Yamanaka, H., Kirisawa, R., Iwai, H. (2000). Detection of cytokines in bovine colostrum. *Veterinary Immunology and Immunopathology*, 76 (3-4), 183–190. doi: [https://doi.org/10.1016/s0165-2427\(00\)00213-0](https://doi.org/10.1016/s0165-2427(00)00213-0)
11. Sinanoglou, V. J., Cavouras, D., Boutsikou, T., Briana, D. D., Lantzouraki, D. Z., Paliatsiou, S. et. al. (2017). Factors affecting human colostrum fatty acid profile: A case study. *PLOS ONE*, 12 (4), e0175817. doi: <https://doi.org/10.1371/journal.pone.0175817>
12. Puppel, K., Gołębiewski, M., Grodkowski, G., Slósarz, J., Kunowska-Slósarz, M., Solarczyk, P. et. al. (2019). Composition and Factors Affecting Quality of Bovine Colostrum: A Review. *Animals*, 9 (12), 1070. doi: <https://doi.org/10.3390/ani9121070>
13. Elfstrand, L., Lindmark-Månsson, H., Paulsson, M., Nyberg, L., Åkesson, B. (2002). Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal*, 12 (11), 879–887. doi: [https://doi.org/10.1016/s0958-6946\(02\)00089-4](https://doi.org/10.1016/s0958-6946(02)00089-4)
14. Sánchez-González, D. J., Sosa-Luna, C. A., Vásquez-Moctezuma, I. (2011). Factores de transferencia en la terapéutica médica. *Medicina Clínica*, 137 (6), 273–277. doi: <https://doi.org/10.1016/j.medcli.2010.05.002>
15. Mesmin, C., Fenaille, F., Becher, F., Tabet, J.-C., Ezan, E. (2011). Identification and Characterization of Apelin Peptides in Bovine Colostrum and Milk by Liquid Chromatography–Mass Spectrometry. *Journal of Proteome Research*, 10 (11), 5222–5231. doi: <https://doi.org/10.1021/pr200725x>
16. Kozheshkurt, V., Antonenko, Y., Shtoda, D., Slipchenko, O., Katrych, V. (2018). Possibilities of Impedance Spectroscopy for the Study of Bioliquids. 2018 9th International Conference on Ultrawideband and Ultrashort Impulse Signals (UWBUSIS). doi: <https://doi.org/10.1109/uwbuis.2018.8520236>
17. Qian, X., Gu, N., Cheng, Z., Yang, X., Wang, E., Dong, S. (2001). Methods to study the ionic conductivity of polymeric electrolytes using a.c. impedance spectroscopy. *Journal of Solid State Electrochemistry*, 6 (1), 8–15. doi: <https://doi.org/10.1007/s100080000190>
18. Maskow, T., Röllich, A., Fetzer, I., Ackermann, J.-U., Harms, H. (2008). On-line monitoring of lipid storage in yeasts using impedance spectroscopy. *Journal of Biotechnology*, 135 (1), 64–70. doi: <https://doi.org/10.1016/j.jbiotec.2008.02.014>
19. Lowry, O., Rosebrough, N., Farr, A. L., Randall, R. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193 (1), 265–275. doi: [https://doi.org/10.1016/s0021-9258\(19\)52451-6](https://doi.org/10.1016/s0021-9258(19)52451-6)
20. Gramse, G., Dols-Perez, A., Edwards, M. A., Fumagalli, L., Gomila, G. (2013). Nanoscale Measurement of the Dielectric Constant of Supported Lipid Bilayers in Aqueous Solutions with Electrostatic Force Microscopy. *Biophysical Journal*, 104 (6), 1257–1262. doi: <https://doi.org/10.1016/j.bpj.2013.02.011>
21. Gómez Vera, J., Chávez Sánchez, R., Flores Sandoval, G., Orea Solano, M., López Tiro, J. J., Santiago Santos, A. D. et. al. (2010). Transfer factor and allergy. *Revista alergía Mexico*, 57 (6), 208–214. Available at: <https://www.scopus.com/record/display.uri?eid=2-s2.0-84979819260&origin=inward&txGid=8315279df09e01107c79deb948eab9cb#>
22. Rozzo, S. J., Kirkpatrick, C. H. (1992). Purification of transfer factors. *Molecular Immunology*, 29 (2), 167–182. doi: [https://doi.org/10.1016/0161-5890\(92\)90098-i](https://doi.org/10.1016/0161-5890(92)90098-i)
23. D'Amici, G. M., Rinalducci, S., Zolla, L. (2007). Proteomic Analysis of RBC Membrane Protein Degradation during Blood Storage. *Journal of Proteome Research*, 6 (8), 3242–3255. doi: <https://doi.org/10.1021/pr070179d>