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Original article

SELECTIVE-INTEGRATIVE TECHNOLOGY FOR THE SEPARATION OF COLOSTRUM INTO COMPONENTS AND THE POSSIBILITIES OF OBTAINING PROTEIN SUBSTANCES FROM DIFFERENT SOURCES

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Background. Obtaining biologically active natural compounds involved in the regulation of metabolism is an important goal in biotechnology. Colostrum is a unique natural source of various biologically active compounds. However, the extremely high natural variability of colostrum composition does not meet the existing requirements for standardization in pharmaceutical preparations.
Objective. To develop a method for separating colostrum into its basic components (lipids, casein, and protein fractions), thereby reducing the variability of whole colostrum composition, obtaining several target products, and demonstrating the possibility of acquiring new protein substances from different sources.
Methods. Colostrum separation was carried out through centrifugation and membrane filtration. Plant proteins (sunflower) and milk proteins were used to obtain protein substances from different sources. The composition of proteins, carbohydrates, and nucleic acids was determined using mass spectrometry, centrifugation, and membrane filtration.
Results. The proposed method for obtaining basic substances from colostrum significantly reduced the variability in composition compared to whole colostrum. The efficiency of protein sedimentation in concentrated protein solutions by centrifugation and ultrafiltration was shown to depend on protein concentration. Additionally, the formation of non-specific protein aggregates in the centrifugal field allowed the extraction of protein substances from various natural sources, which is relevant for functional nutrition.
Conclusions. The proposed selective-integrative technology for obtaining different substances from colostrum significantly reduces the high variability of whole colostrum composition. It increases the efficiency of component separation into lipid, casein fractions, low molecular weight protein fractions, and ultrafiltrate, while also enabling the acquisition of protein substances from diverse sources.
Keywords: colostrum; composition variability; fractionation; ultrafiltration; mass spectra; biologically active compounds.

Introduction

There is a growing interest in obtaining biologically active compounds of natural origin, which can be used not only in pharmacology but also as functional food products [1]. There are several reasons for this: as a rule, they are non-toxic; they have a "soft", specific regulatory effect on the body; they can have a multifunctional effect on metabolic processes; the variety of natural substances is huge, which can provide solutions to a wide range of medical and biological problems; and the development of biotechnology modern methods allows to significantly expand the possibilities of biological origin obtaining substances [2].

Despite the importance and potential advantages of natural products over synthetic drugs, the pharmaceutical biotechnology development is not as intensive as might be expected. An example of this is such a unique biological product as colostrum. Although colostrum has been called "liquid gold" or "immune milk", and Gil Hardy stated in 2000 that colostrum is "the most important food in the world" [3], its use as a substance for functional foods or pharmaceuticals is limited for a number of reasons: 1 – problems that arise during storage and processing; 2 – instability and high variability of colostrum composition, which depends on the combination of a large number of factors [4, 5]; 3 – when developing functional food products, it is often necessary to include additional components in their composition [6], and there are no effective methods yet to address this issue.

In order to eliminate the existing limitations in obtaining biologically active substances from natural sources, including colostrum, it is necessary to develop new approaches and technological methods that can allow, on the one hand, to provide stan-

dard products and, on the other hand, to enrich natural substances with the necessary compounds in the production of functional foods.

In this regard, the aim of the present work was to develop a relatively simple technology of colostrum processing that will provide: firstly – to obtain various "basic" substances satisfying the conditions of standardization, which can be used as finished products and/or as raw materials for obtaining additional mono-compounds; secondly – to increase the shelf life of the obtained products; and thirdly – to provide an opportunity to "enrich" the obtained substances with additional protein components in the development of functional food products.

Materials and Methods

Experimental design

Colostrum was collected from cows ("Ukrainian Black and White") without pathology. It is a viscous, oily fluid. The collected samples, taken after the first milking from cows of the same age, of the same breed, on the same farm and in the same season, were stored at –15 °C for a maximum of 30 days.

Separation of lipid fraction

Colostrum was defrosted by slow thawing at room temperature, then all samples were combined into one batch and diluted 3-fold with distilled sterile water as determined experimentally, centrifuged at 3000 g for 15 min, and then cooled to 6 °C. During centrifugation of colostrum three phases are formed: interphase on the surface of the aqueous layer (lipids); aqueous phase, which includes all water-soluble components of colostrum and sediment, which is represented by cells, their fragments and aggregates. Under these centrifugation conditions, the lipid components floated and formed a dense fat layer on the surface of the aqueous phase, which was easily separated from the liquid phase. The cells contained in the colostrum migrated to the precipitate. For complete separation of lipids, the samples were centrifuged twice under the same conditions. The crude lipid yield was determined as the lipid content per liter of colostrum. The aqueous phase was transferred into clean sterile containers and used to obtain low-molecular-weight proteins and ultrafiltrate. For this purpose, casein was removed beforehand.

Separation of casein fraction

The liquid, defatted fraction of the colostrum was transferred to a clean sterile container and the cell precipitate was removed. Total lipids were determined on an analytical balance. In the next step, after defatting, the casein fraction was separated. For this purpose, the container with defatted milk was heated up to 32 °C, acetic acid was added with constant stirring until reaching pH 4.6, i.e. to the isoelectric point for casein, then the temperature of samples was led to 45 °C, casein in the residue was precipitated by centrifugation at 3000 g for 15 min. The yield of casein by crude matter per liter of colostrum was determined. Depending on the way of its further use, the obtained casein can be lyophilized or stored in a moist state at –15 °C until use.

Obtaining of the post-casein fraction ("LMWC") and ultrafiltrate

The post-casein fraction ("LMWC") can be used as a target product, for this purpose it was dried on a rotary evaporator at 37 °C and the dry samples were stored at 4–6 °C until use and/or it can be used to obtain peptides and low molecular weight components of colostrum by ultrafiltration (ultrafiltrate). For this purpose, before drying, the liquid fraction "LMWC" was subjected to sequential filtration through membrane filters with a pore diameter of 85→60→45→22 μm, which allows to obtain components with different molecular weights. The obtained fraction – "ultrafiltrate" was dried under the same conditions as "LMWC".

The yield of "LMWC" and ultrafiltrate was determined by dry residue per liter of colostrum.

Protein co-precipitation procedure

In experiments to determine the influence of initial protein concentration in solution on the increase of separation efficiency, we used additional protein precipitation (co-precipitation) solution of plant proteins, which were obtained from sunflower meal by salt extraction, as described in [7]. The solution of plant proteins was additionally supplemented with a solution of proteins, which was obtained from the colostrum – "LMWC" to a final concentration of 5.5 g/l. The solution of proteins was added to the solution of plant proteins. To form protein aggregates, the obtained protein solutions were autoclaved for 30 min at 1.5 atm. Protein aggregates were collected by centrifugation

at 3000 g for 15 min, dried to constant mass and the amount of precipitated protein determined before and after adding milk proteins.

Determination of protein, RNA, and carbohydrate concentrations

The quantification of protein and RNA in the obtained samples was carried out as described previously [8], the amount of carbohydrates [9]. The absorption values for the determination of protein, nucleic acids and carbohydrates using the specified methods were measured spectrophotometrically (Shimadzu, Japan).

Determining the composition of proteins

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) with a pulsed nitrogen laser ($\lambda = 337$ 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 synaptic 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) and mMass (http://mmass.org).

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).

Bioethics

The study was conducted in accordance with the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.

Results

Colostrum is a specific biological substance containing an extremely wide range of cells and compounds, and the ratio between these components varies in different colostrum samples. The solution to the problem of standardisation is to obtain substances with the same or similar composition, i.e. to eliminate variability in composition.

As the colostrum composition, as well as other biotechnological substances, is influenced by a wide range of exogenous and endogenous factors, the standardisation process may consist of several steps. In order to partially eliminate the variability in composition in the first stage, it is necessary to select colostrum samples according to the main characteristics that may influence the colostrum composition (Fig. 1).

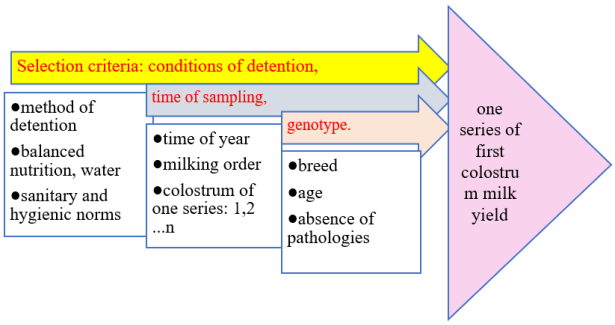

Figure 1: Schematic diagram that demonstrates the principle of colostrum selection whereby colostrum from producers of the same breed, of close age, from animals without pathologies, which are kept under the same conditions on the same farm, in the same season, are selected and only after the first milk yield are they combined into a single series for further processing

The optimal way of separating the colostrum into basic substances is a combination of centrifugation and membrane filtration (ultrafiltration) methods. By regulating the centrifugal acceleration and viscosity of the colostrum it is possible to separate the colostrum into such basic substances: lipids, casein, post-casein or a fraction of relatively low molecular weight proteins ("LMWP"), which can be subjected to a further separation procedure by sequential ultrafiltration using filters with different pore diameters (80, 60, 45, and 22 μm) and obtain protein components with different molecular weights.

In the second step, the samples of colostrum that have undergone "selection", and their number should be at least 10 or more, are combined ("integrated") into one series. The resulting substances are then documented and used as representatives of the single series (see Fig. 1) and are separated into separate basic substances: lipid components; casein; low molecular weight components of the colostrum ("LMWCs"); and ultrafiltrate. Centrifugation and ultrafiltration methods are used for this purpose.

This approach of fractionation of colostrum into basic substances can make it possible: to reduce

the variability of the obtained components composition; to have not one, but several products with different biological activity; to store them for a longer time; basic substances can be the basis for further fractionation and obtaining mono-compounds; in the process of sedimentation, additional proteins can be introduced, forming protein aggregates and enriching the obtained products with additional components, which is necessary for the making of products.

The results of colostrum fractionation are the following. It can be seen that 94.7 ± 0.34 g of lipids can be obtained from 1 liter of whole colostrum of the first milk yield obtained in the spring period from cows of the breed "Ukrainian black and brown". After the removal of lipids from colostrum in the form of a dense white sediment casein (35 ± 13.6 g by dry weight). The LMWC fraction, isolated after casein removal, accounts for 30.0 ± 3.6 g by dry weight (the amount of ultrafiltate by dry matter was 19.1 ± 3.1 g).

The fraction included a considerable number of various proteins with molecular masses ranging from 5 to 8 kDa (Fig. 2). It was demonstrated that the "LMWC" fraction exhibited immunotropic, antioxidant, and hepatotropic effects, was not toxic, and could also eliminate the toxic effect of copper ions [1, 10–12].

It is important to note that the composition of the "LMWC" fraction obtained from cows of the same breed and close age in the same season, after the first milking and kept in one farm, exhibited significant individual differences (Fig. 2). This confirms the high variability of colostrum components.

From one liter of colostrum, following the separation of "LMWC", it is possible to obtain a relatively small amount (19 g/l) of peptides, amino acids, growth factors and other low molecular weight components that are part of the "ultrafiltrate", including the so-called transfer factor [13].

Consequently, colostrum can be divided into at least four different fractions, which are represented in relatively large quantities, but which have pronounced individual differences in both qualitative and quantitative terms.

It is therefore important to note that the standard errors, which reflect the variability of indicators, were different for different fractions of colostrum. The greatest variability between samples was manifested for the lipid fraction. Thus, out of the six colostrum samples, the lowest amount of lipids was 31.7 g/l, while the highest amount was 107.5 g/l. This represents a difference of 3.4 times in the amount of lipids between the samples (Fig. 3).

At the same time, the differences in casein content in the six colostrum samples were not as pronounced as those observed for lipids, with no more than a 30% difference between the different samples (see Fig. 3).

The differences between samples for "LMWC" and ultrafiltrate were even less pronounced than for casein (Fig. 3).

It is therefore evident that the fractions obtained from different colostrum samples exhibited quantitative variability in their component composition. The greatest variability was observed in the amount of lipids present in the colostrum samples. This may be attributed to a number of factors.

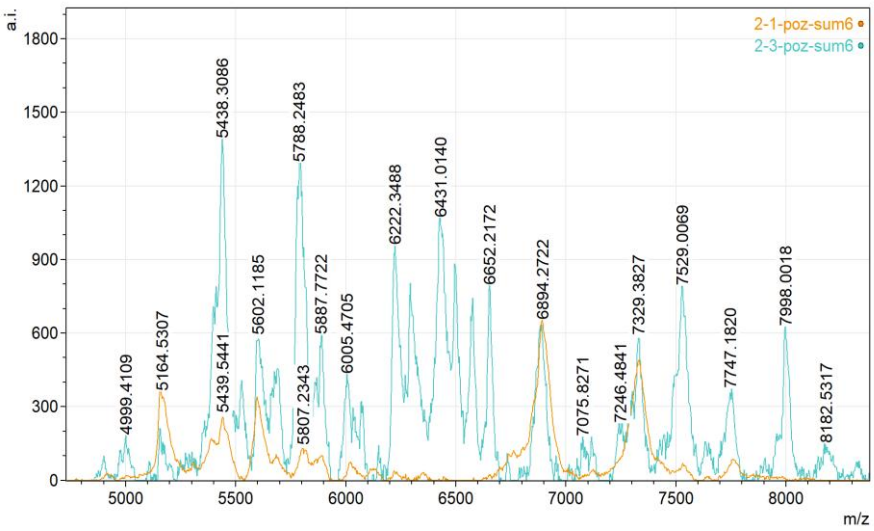

Figure 2: Protein composition of the "LMWC" fraction, when separated by mass spectroscopy, obtained from two different cows. The results are shown in different colors after the first milking of the same breed and similar physiological characteristics

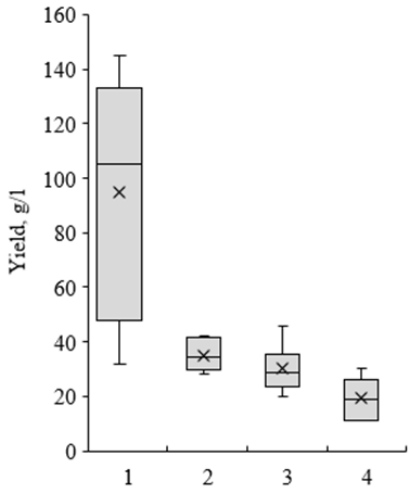

Figure 3: Variability of content of different fractions of colostrum components (lipids-1, casein-2, "LMWC"-3, and ultrafiltrate – 4 g/l in dry matter), which were obtained from the first colostrum of the first milk yield from 6 cows of the breed "Ukrainian black and brown" in the spring period

Firstly, lipid metabolism is regulated by unique features of genetic, trophic and exogenous factors, which may lead to pronounced individual variability in lipid content in colostrum. Secondly, it should be noted that fat separations from solutions by centrifugation and/or separation with high protein content, as in the case of colostrum, do not allow for their effective (complete) separation. At the same time, fractionation of colostrum into basic components can significantly reduce the variability at least for quantitative characteristics when obtaining "LMWC" and ultrafiltrate.

Along with the fractionation of colostrum, which can be defined as a "differential" method of obtaining components, another way to reduce the variability in the colostrum components composition can be combine colostrum obtained from different cows before its further fractionation, or conditionally "integrative" method of obtaining colostrum basic fractions. The concept of the "integrative" approach is predicated on the understanding that the separation efficiency of components in a centrifugal field is not solely dependent on the size of molecules and their density, but also on the concentration of the separated components in the initial solution. Thus, in dilute solutions, a much higher acceleration and/or centrifugation time is required to reach thermodynamic equilibrium. As the concentration of the substance increases, especially in multi-component aqueous systems such as colostrum, the rate at which the system reaches equilibrium is accelerated, increasing the efficiency of the separation into fractions.

In the next step of the work, the separation of colostrum into the same four basic substances was carried out after preliminary pooling of the colostrum samples. It was assumed that two approaches could be used in the technology to obtain different substances. In the first case, different colostrum samples are separated into components and then the resulting fractions are combined into a single series, i.e. the "differential approach". In the second case, the different colostrum samples are combined before fractionation, i.e. the "integrative approach".

Here is an example of the effectiveness of the "integrative" approach to obtaining and standardizing colostrum components compared to the "differential" approach. Thus, if from three different colostrum samples it is possible to obtain respectively 31.7, 52.9, and 107.5 g/l, and after mechanical pooling of these three samples – 192.1 g of dry matter (Table 1), if all three samples are pooled before lipid extraction, 325.3 g of lipids can be obtained from the same amount of colostrum, or 1.7 times more in the case of the "integrative" approach compared to the "differential" approach.

If we compare the yield of casein, "LMWC" fraction from different colostrum samples in the "integrative" approach with the "differential" approach, it can be observed that it was higher by 10 and 12%, respectively, in the case of using the "integrative approach" (see Table 1).

It is also worth noting that the amount of ultrafiltrate in the integral separation approach was slightly higher, by 6,6 g or 8.8% compared to the differential approach (Table 1). The lower yields of the components included in the ultrafiltrate indicate fundamental differences in the separation methods in the gravity field and membrane filtration.

It is therefore possible that the separation of colostrum into lipid, casein and "LMWC" fractions by centrifugation based on the "integrative" approach may yield a higher quantitative yield compared to the "differential" approach.

As it was noted, the components of "LMWC" have been observed to exhibit a variety of actions on biological systems [14]. Such polyfunctional action could be explained by the fact that the composition of "LMWC" includes a variety of compounds. Therefore, it would be of interest to determine the composition of "LMWC". It was found that the composition of "LMWC" includes approximately 14.0 mg/ml of proteins, which represents approximately 30% of the total protein content of whole colostrum (46.2 mg/ml). A relatively small amount of carbohydrates (up to 1.7 mg/ml) was present in the "LMWC" fraction along with pro-

Table 1: Lipid, casein, "LMWC" and ultrafiltrate yield from different colostrum samples, using "differential" and "integrative" colostrum fractionation approaches

Colostrum components	Sample variants			Yield from "differential" approach	Yield from "integrative" approach
	1	2	3		
Lipids	52.9	107.5	31.7	192.1	325.3
Casein	42.1	28.3	41.3	111.7	123.1
"LMWC"	28.1	20.0	32.3	80.4	90.0
Ultrafiltrate	25.4	18.0	25.0	68.4	75.0

Note. The amounts of lipid, casein, "LMWC" and ultrafiltrate are in gram per liter on the dry residue.

Table 2: Effect of additionally added exogenous colostrum protein on the total precipitable protein in variants with different dissolved endogenous protein content using thermal-aggregation method

Experiment variants	Amount of sunflower meal proteins, g/l	Amount of colostrum protein, g/l	Theoretically expected protein yield, g/l	Amount of experimentally obtained protein, g/l
1	6.6 ± 0.8	5.5 ± 0.1	12.1	13.1 ± 0.5
2	7.3 ± 0.9	5.5 ± 0.2	12.8	16.7 ± 0.8

Note. Mean values from three experiments and their standard errors are presented.

teins, which constituted the main amount. It should be noted that trace amounts of RNA (1.8 μg/ml) were also found in the composition of "LMWC", which is an important criterion for the use of these components as products of functional nutrition.

Consequently, the composition of "LMWC" is represented by proteins with different molecular masses, from 5 to 8 kDa, it included about 12% of the protein content and a small amount of RNA. Such heterogeneous composition of "LMWC" can provide its polyfunctionality of action.

The method of "co-precipitation", which is based on the formation of complexes between different proteins, can be applied not only to increase the yield of protein products, but also in obtaining functional foods. At present, methods for obtaining proteins from vegetable raw materials, in particular, from sunflower meal, peas, yeast, etc., are being developed [15]. At the same time, plant-derived proteins are deficient in essential amino acids and cannot fully substitute for animal proteins. Therefore, the enrichment of vegetable proteins with colostrum proteins is of significant practical interest.

In this context, we investigated:

1 – the influence of protein concentration in the solution on the increase in the amount of precipitated protein;

2 – the possibility of enriching sunflower meal proteins with colostrum proteins.

It was found that if the solution containing 6.6 g/l of sunflower meal proteins was supplemented with a solution of colostrum proteins in the amount of 5.5 g/l by dry matter and the proteins were precipitated by centrifugation, 13.1 g/l by dry residue was obtained, but not 12.1 g/l as could be expected (Table 2). In the case when the content of sunflower meal proteins was increased to 7.3 g/l in the solution and 5.5 g/l by dry matter of colostrum proteins were additionally added, the amount of precipitated proteins was 16.7 g/l and not 12.8 g/l as might be expected (Table 2).

Consequently, while in the first case there was an increase in the yield of precipitated protein by 8.3%, in the second case, by increasing the amount of protein in solution, the yield was increased by 30% compared to the amount of protein obtained separately.

Consequently, the yield of protein at precipitation by centrifugation is dependent on the initial concentration of protein in solution. With this approach it is possible to "enrich" vegetable proteins with proteins of animal origin, which is important for obtaining protein composites of high-grade composition when developing products of functional nutrition.

Discussion

It is well known that colostrum is a mammary gland secretion produced by female mammals within 72 h of giving birth [16]. Colostrum not only provides the newborn with nutrients, but also shapes the epigenetic characteristics of the organism and thus can influence the quality and duration of life [17]. The composition of colostrum includes not only a large number of nutrients (pro-

teins, lipids, vitamins, amino acids, peptides, trace elements) and an extremely wide range of biological regulators (cytokines, growth factors, hormones, immunoglobulins, vitamins, trace elements), but also a relatively large number of cells, up to 106 cells per ml (leukocytes, erythrocytes) [18, 19]. Recently, it has been shown that breast milk also contains stem cells capable of proliferation and differentiation into other cell types [20, 21]. Current evidence suggests that colostrum can be used as a source of an extremely wide range of bioactive substances and cells that not only have important properties, but can also be used as unique "carriers" or co-compounds.

It has been found that both colostrum and milk proteins, especially casein molecules, are capable of forming specific supramolecular complexes [22, 23] and can be used as agents for encapsulation of a variety of biologically active compounds [23]. An important role in the "structuring" of colostrum components is also played by lipids, which form globules with an average diameter of about 4 μm. As noted by the authors of a number of works, protein shells – membrane formations (MFGM) are formed around such lipid globules [24].

At the same time, when using colostrum as a finished product and/or raw material for isolation of biologically active substances from it, specialists face a number of objectively existing problems.

In particular, even if the strict requirements for milking are met, contamination occurs, which requires additional removal of microorganisms [25]. It known on the effect of different thermal and non-thermal processing techniques on the structural and functional modifications of milk proteins [26]. Another reason to specifically focus on milk proteins is that the major class of milk proteins, the caseins, respond differently to heating than most other food proteins [27]. It has been shown that the physicochemical parameters of milk changed under the influence of colostrum, and some of them changed under the influence of heat treatment, and this affected the efficiency of pasteurization [28]. Furthermore, the high density of colostrum makes it challenging to separate it into individual components, indicating that the technological processing has not yet been completely resolved.

The issues of standardization, especially of medical products obtained by biotechnological methods, are no less complicated in their production, since they are always a mixture or composition of different compounds and it is impossible to identify the "active ingredient" among them, which is most often the basis for the standardization of medical preparations, since such substances have a synergistic effect, and in this respect new approaches are needed to solve this issue.

Currently, the development of functional food products that can occupy an "intermediate" position between pharmaceuticals and diet products is gaining popularity. In doing so, at least two issues need to be addressed. Firstly, to eliminate the potentially allergenic properties that dairy products may have, and secondly, to be able to supplement functional food components with the necessary ingredients.

We believe that the solution to these practically important problems of biotechnology can be based on the use of an integrated approach that combines both methods of selection and various methods of separation and the possibility of combination (formation of new associates) of various compounds, including proteins.

The results of the work can be summarized in a few general points:

1 – the combination of centrifugation and membrane filtration methods allows the separation of four basic substances from colostrum, which can be used as target products or as raw materials for further purification to mono-compounds (lactoferrin, immunoglobulins, etc.);

2 – the pooling of colostrum obtained from different cows before its separation into fractions, i.e. the "integrative" method, combined with preliminary selection of producers or "selection", allows to significantly reduce the variability of the obtained fractions composition. The "integrative" method, in conjunction with preliminary selection of producers or "selection", permits a considerable reduction in the variability of the obtained fractions composition and an increase in the completeness of individual fractions isolation;

3 – the proposed method of separation of colostrum into components can be employed in obtaining balanced protein products, which are utilized in functional nutrition;

4 – casein, "LMWC" and ultrafiltrate after lyophilic drying have a long shelf life.

Dwelling on the discussion of the obtained results it is necessary to note that the most widespread application in the separation of multicomponent mixtures, are found in various methods of chromatography, centrifugation, membrane filtration, and their combinations [29]. Centrifugation methods are of particular interest in this respect, as it is: simple in execution, sufficiently efficient, and allow for the processing of relatively large amounts of samples. This is due to the fact that the ability

to adjust the centrifugal acceleration within wide limits allows for the precipitation of particles of different sizes. The rate of precipitation of components contained in the solution depends on the geometric shape of the particles, their density, the viscosity of the solution and the acceleration of gravity, which can be expressed by the generalized Stokes formula:

$$\nu = \lambda \cdot {}_{\delta}\rho \cdot g \cdot \omega^{2/3} \cdot \mu^{-1},$$

where ${}_{\delta}\rho = \rho_a - \rho_{f_s}$ is density of precipitated particles and density of the liquid phase, μ is aqueous phase viscosity, ω is particle volume, λ is shape coefficient, g is acceleration of gravity.

However, in the case of macromolecules separation in a centrifugal field, which can form supramolecular complexes, there is a deviation from the Stokes formula, which characterizes the deposition of mechanical particles. This provides an explanation for the absence of a unified theory of separation in the centrifugal field of high-molecular complex compositions characteristic of biological fluids. In particular, we know little about the influence of concentrations of these or those substances on the efficiency of their separation, the peculiarities of intermolecular complexes formation in the gravitational field and the influence on these processes of the carbohydrate's presence and other macromolecules, which can affect the efficiency of separation and purity of the obtained products, remain unexplored.

On this basis, it can be concluded that the use of a centrifugal field in solving the problems of separation of complex biological mixtures and their standardization has a number of disadvantages. At the same time, if a number of conditions are met, this method could allow not only to divide mixtures into individual components, but also provides an opportunity to obtain supramolecular protein complexes, which could be used in the development of functional food products. Thus, in particular, the present study demonstrated the potential for obtaining associations between proteins of animal and plant origin.

When considering the separation of proteins from colostrum by centrifugation, it is important to consider a number of key factors. Firstly, colostrum contains an extremely high concentration of proteins with varying molecular weights and properties. Secondly, it is highly viscous and does not separate into components during centrifugation. Thirdly, in the centrifugal field, different types of proteins move at different speeds and are able to form intermolecular interactions.

It is important to note that the process of protein aggregation [30], which is ensured by the formation of a large number of hydrogens, electrostatic, hydrophobic interactions and Van der Waals forces, depends on a large number of diverse factors and plays a key role in the structural and functional organization of the cell. The so-called native (intracellular) protein aggregation is currently under consideration [31]. Native protein aggregation is a dynamic process that can be regarded as a transition from the "unfolded" to the aggregated state and back to the unfolded state. The first data on the presence of native unfolded regions of proteins in a cell were obtained in 1978 [32].

The process of native protein aggregation may be determined by the heterogeneity of the structural organization of the cell, but these mechanisms are still only beginning to be investigated. It was found that between 35 and 51% of proteins in a eukaryotic cell are in the native unfolded state (the length of unfolded sites can be about 50 amino acid residues) [33]. Such extended regions of the unstructured state can facilitate intermolecular interactions between proteins.

During the process of protein isolation and transfer into solution, the majority of proteins undergo a transition to the unfolded state, which increases the probability of non-specific protein aggregation in the centrifugal field. It is quite obvious that non-specific aggregation leads to the formation of irreversible states in contrast to specific aggregation, which takes place in the cell.

It is known that large aggregates in a gravitational field settle at a higher rate than small aggregates [34]. Given that the composition of multicomponent mixtures, including colostrum, includes proteins with varying characteristics and molecular masses, they will exhibit different speeds of movement along the force fields within the centrifugal field. Large protein molecules "colliding" in the process of movement with small molecules will form non-specific aggregates and "enlarge" in the process of sedimentation.

As is known, protein aggregation processes in solution are determined by a number of factors, including the volume of molecules, molecular weight, concentration, temperature, viscosity and centrifugal acceleration, as well as the presence of unfolded sites.

Since, along with the concentration and characterization of proteins, the viscosity of the medium plays a significant role in the formation of aggregates and the rate of sedimentation in the centrifugal field. In this context, we have experimentally selected the viscosity of colostrum that provides the most effective precipitation of protein complexes, i.e., by observing these experimentally selected conditions, one can obtain good reproducibility of the results.

It can be concluded that the centrifugation method in combination with membrane filtration using a selective-integrative approach allows for partial standardisation of the obtained fractions, which can be used as target products. It has been demonstrated that the casein fraction of colostrum can be employed as a carrier (matrix) for the stabilization and delivery of polyphenolic compounds into the body [35], and "LMWC", has an immunomodulatory effect in animals with liver fibrosis. It has the capacity to normalize liver function at the early stages of fibrosis, and thus represents a promising candidate for functional nutrition products [36]. Currently, our laboratory is engaged in research on the production of hydrogels based on the colostrum lipid fraction. In addition, colostrum fractions can be employed to synthesize a range of mono-compounds, including lactoferrin, lysozyme, lactoperoxidase, immunoglobulins, and other biologically active compounds.

As it has already been mentioned, the formation of specific and non-specific protein aggregates in the cell and organism as a whole is one of the most important mechanisms of functioning of biological systems. The role of nonspecific protein aggregates in the mechanisms of age-related pathologies cannot be excluded. The study of the mechanisms of protein aggregates formation in the centrifugal field can be useful "old tool" in solving "new" problems in the emerging field of supramolecular biology.

The method of protein aggregates formation from proteins of different origin and differing in amino acid composition in a centrifugal field can be no less useful in the new methods development of obtaining functional food products. Obtaining of protein supramolecular complexes can provide: the protein products formation with a given amino acid composition; obtaining of stable and sustainable in storage food substances.

Consequently, the selective-integrative approach allows for the following: the obtaining of several substances from colostrum, which can be used as target products; a significant reduction in the variability of the obtained substances composition; an increase in the "completeness" of separation into components; and the composites formation with the required amino acid composition when obtaining products of functional nutrition.

Conclusions

A method of obtaining lipids, casein, low-molecular protein fractions and ultrafiltrate from colostrum, which can be used as target products or as raw materials for further purification to mono-compounds, has been developed. Combining colostrum obtained from different cows before its fractionation, i.e. the "integrative" method, combined with preliminary selection allows to partially reduce the variability of the composition of the obtained target products and increase the completeness of the extraction of individual fractions. The proposed method of separating colostrum into components can be used in the production of balanced protein products that are used in functional nutrition.

Interests disclosure

The authors declare no conflict of interests.

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СЕЛЕКТИВНО-ІНТЕГРАТИВНА ТЕХНОЛОГІЯ ПОДІЛУ МОЛОЗИВА НА КОМПОНЕНТИ ТА МОЖЛИВОСТІ ОТРИМАННЯ БІЛКОВИХ РЕЧОВИН ІЗ РІЗНИХ ДЖЕРЕЛ

Проблематика. Отримання біологічно активних природних сполук, які беруть участь у регуляції метаболізму, є важливим завданням біотехнології. Унікальним природним джерелом різноманітних біологічно активних сполук є молозиво. Однак надзвичайно висока природна варіабельність складу молозива не відповідає існуючим вимогам до стандартизації фармацевтичних препаратів.

Мета. Розробити метод поділу молозива на основні компоненти (ліпіди, казеїн і білкові фракції), тим самим зменшити варіабельність складу молозива, отримати декілька цільових продуктів і показати можливість отримання нових білкових речовин із різних джерел.

Методика реалізації. Поділ молозива здійснювали шляхом центрифугування та мембранної фільтрації. Для отримання білкових речовин із різних джерел використовували рослинні білки (соняшник) і молочні білки. Для визначення складу білків, вуглеводів і нуклеїнових кислот застосовували мас-спектрометрію, центрифугування та мембранну фільтрацію.

Результати. Показано, що запропонований метод отримання основних речовин із молозива значно зменшує мінливість їхнього складу порівняно з цільним молозивом. Ефективність осадження білків у концентрованих білкових розчинах шляхом центрифугування та ультрафільтрації залежить від концентрації білка. Крім того, утворення неспецифічних білкових агрегатів у центрифужному полі дає змогу отримувати білкові речовини із різних природних джерел, що є актуальним для функціонального харчування.

Висновки. Запропонована селективно-інтегративна технологія отримання різноманітних субстанцій із молозива значно зменшує високу варіабельність складу молозива. Вона підвищує ефективність поділу компонентів на ліпідну, казеїнову фракції, фракцію низькомолекулярних білків та ультрафільтрат, а також дає змогу отримувати білкові речовини з різних джерел.

Ключові слова: молозиво; варіабельність складу; фракціонування; ультрафільтрація; мас-спектри; біологічно активні сполуки.