

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 is 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:

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

where $\delta\rho = \rho_a - \rho_f$ 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 multi-component 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.

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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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С.Г. Іванов¹, А.В. Голтвянський¹, А.І. Божков¹, Т.Ю. Громовий²

¹Харківський національний університет імені В.Н. Каразіна, Харків, Україна

²Інститут хімії поверхні ім. О.О. Чуйка НАН України, Київ, Україна

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.