

The object of this study is the nutritional and biological value of whey subjected to nanofiltration treatment. The problem addressed is the insufficient efficiency of traditional methods of whey processing to increase its nutritional and biological value without losing useful components.

The study found that the use of nanofiltration significantly affects the consistency of whey, which becomes thicker and more viscous. The taste and smell change from sour-milk to sweet, with pleasant whey notes. Nanofiltration leads to an increased concentration of dry matter, by 3.3 times; protein, by 3.8 times; and minerals, by 2 times. Active acidity also increases, which indicates changes in acid-alkaline balance. Analysis of the amino acid composition revealed an average increase in both essential (threonine +31.24%, leucine +31.13%, methionine +24.12%, lysine +28.65%) and non-essential amino acids (serine +69.08%, glycine +54.43%, alanine +67.36%, and aspartic acid +21.46%). The amino acid score of most essential amino acids exceeded 110% while the coefficient of variation decreased, indicating a more balanced composition.

The increase in the biological value of whey to 60.4% indicates its increased nutritional value and potential as a functional ingredient in food products. The results obtained are explained by the selective permeability of nanofiltration membranes. In particular, those that retain protein substances and allow water and low-molecular components to pass through.

The results have practical significance since the ability of nanofiltration to selectively separate substances enables effective processing of whey, while preserving its nutritional and biological properties

Keywords: whey, membrane technologies, nanofiltration, biological value, amino acids, waste-free technologies

DETERMINING THE EFFECT OF NANOFILTRATION ON THE NUTRITIONAL AND BIOLOGICAL VALUE OF WHEY

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1. Introduction

Given current conditions for the development of food industry, the rational use of agricultural raw materials, as well as the processing of by-products of production, is of particular importance. One of such resources is whey – a by-product of cheese, fermented milk cheese, and casein production. Traditionally, whey is used as a feed additive or is partially utilized, while a small part of it is processed to obtain a dry product [1]. Given the significant concentration of beneficial nutrients in whey, it acts as a promising raw material for the manufacture of products with increased biological and functional value [2].

Whey contains up to 0.8–1.0% proteins, represented mainly by β -lactoglobulin, α -lactalbumin, and other bioactive proteins. It also contains about 4.5% lactose, as well as a wide range of minerals, vitamins, and enzymes. Whey proteins are characterized by high digestibility, immunomodulatory and antioxidant properties [3]. Therefore, whey is considered a valuable component for making functional products, particularly in the areas of sports, children's, and dietary nutrition.

One of the most effective methods for processing whey is the use of membrane technologies, in particular nanofiltration. This method makes it possible to selectively separate and concentrate the components of the raw material, preserving their natural structure. Unlike other techniques such as vacuum thickening, nanofiltration eliminates the risk of destruction of the components of the raw material due to exposure to high temperatures [4].

Therefore, it is a relevant task to carry out studies on the influence of nanofiltration treatment on the composition and biological value of whey. The results of such studies could contribute to the rational use of secondary raw materials, expanding the range of functional food products, and implementing the principles of sustainable development of the food industry.

2. Literature review and problem statement

Work [3] reports the results of studies on the chemical composition of whey. It is shown that whey, as a by-product of cheese production, consists of 94% water. The dry residue,

which is approximately 6%, contains valuable components that are important for the food industry. In particular, whey contains complete proteins (β -lactoglobulin, α -lactalbumin, immunoglobulins, lactoferrin), milk sugar (lactose), B vitamins, as well as macro- and microelements, in particular calcium, potassium, magnesium, and phosphorus. The authors note that modern scientific and technological advancements have already made it possible to create a wide range of ingredients based on whey. At the same time, it is emphasized that the molecular profile of this by-product still remains incomplete. In addition, the work does not consider the impact of modern membrane technologies on the preservation or change of the nutritional and biological value of whey. The unresolved nature of these issues is due to the complexity of the chemical matrix of whey, the dominance of lactose, which complicates the identification of small molecules.

Despite its significant nutritional potential, in practice, whey is mostly used to produce a dry or condensed product or is simply disposed of or drained. Techniques for sustainable use of whey are discussed in [5, 6]. In particular, the authors focus on the application of innovative technological solutions to obtain food products with added value. It has been shown that this reduces the environmental impact and contributes to the economic sustainability of industry. However, the papers do not assess in detail the impact of specific technologies, in particular membrane technologies (nanofiltration, ultrafiltration), on preserving the nutritional and biological value of whey. The objective reason is the technological complexity and significant cost of highly efficient membrane methods.

More technological solutions for the use of whey are highlighted in [7]. Attention is drawn to the fact that before use in production processes, it is necessary to pre-treat whey, in particular, to remove excess water and minerals. The possibilities of using membrane technologies are considered: ultrafiltration, microfiltration, nanofiltration. At the same time, the work does not investigate in detail the impact of membrane processes on the preservation of the nutritional and biological value of whey, in particular proteins and low-molecular bioactive components. The issue of integrating membrane technologies with other processing methods, such as thermal or enzymatic, for the development of specialized ingredients is also not considered. The subjective reason is the industry's focus on the production of standardized products, such as dry or thickened concentrates. This reduces the interest of the scientific community in a deeper study on the impact of whey processing technologies on its bioactive properties.

Work [8] shows possible areas for using whey-based products when making functional additives for sports nutrition, infant formulas, dietary products, and the pharmaceutical industry. At the same time, issues related to the complex processing of whey remain open. In particular, optimal technological schemes for simultaneous preservation of nutritional and biological value, effective removal of excess water and minerals, as well as obtaining target bioactive components on an industrial scale have not been studied in detail. This is due to the complex chemical interactions between proteins, peptides, minerals, and lactose, which create difficulties for selective separation of components. According to [9], about 40% of the world's whey production is disposed of by draining, which causes a significant negative environmental impact. A likely reason is the specific properties of whey, in particular its organoleptic properties and chemical composition (significant content of mineral components).

Partial demineralization of whey may be an option to overcome the difficulties. This approach was used in [10]. The authors found that whey contains many minerals, such as sodium, potassium, calcium, and phosphorus, which can give it a bitter or salty taste. In different types of whey, in particular cheese ("sweet") and fermented milk ("sour") whey, the chemical composition varies significantly, which affects the organoleptic properties. Due to the demineralization process, which reduces or completely removes minerals, the taste and aroma of the product can be significantly improved. This expands the possibilities of using whey, in particular in such sensitive industries as the production of baby food. Large-scale application of demineralization of "sour" whey under industrial conditions remains limited for several reasons. First, this is due to variations in the chemical composition of whey. The industry has traditionally preferred to process "sweet" whey as it already has established markets and proven quality assessment methods. Second, there are economic and regulatory barriers that make it difficult to implement new technologies for "sour" whey products on an industrial scale.

One of the options for overcoming the difficulties of whey processing may be the use of membrane technologies, in particular nanofiltration. As noted in [11], nanofiltration membranes have the ability to selectively remove mineral compounds and low-molecular substances while preserving valuable organic components. According to [12], there is no denaturation of proteins in the nanofiltration process since the technology does not involve high-temperature treatment, which makes it possible to preserve the native structure of proteins and, accordingly, their biological activity. This is a fundamental advantage compared to temperature treatment – thickening. The authors did not consider the issue of a comprehensive combination of nanofiltration with other technological methods to achieve maximum separation of target whey components. In addition, the impact of different nanofiltration modes on the stability and biological activity of low-molecular compounds and minerals in the final product has not been studied in detail. This is explained by the objective need for the use of highly sensitive analytical methods to control the levels of proteins, peptides, and minerals.

In [13] it was established that during the nanofiltration process, there may be losses of soluble vitamins (especially B1, B2) and a decrease in the content of some ions (Ca^{2+} , Mg^{2+}), which partially pass through the membrane. This may affect the overall nutritional value of the product. However, the work did not investigate the effect of nanofiltration treatment on the amino acid profile of whey. In particular, in [14], the authors emphasized the need for a more detailed study of the preservation of essential amino acids, such as lysine, methionine, and isoleucine, which are of key importance for the normal functioning of the human body. The proposed methodology for assessing membrane performance based on the Steric Pore Model and the Nernst-Planck diffusion equation takes into account concentration polarization and mass transfer coefficient. It has been shown that low molecular weight cut-off (MWCO) membranes are capable of separating glucose and glycine, which makes it possible to control the preservation of amino acids, in particular essential ones (lysine, methionine, isoleucine), during nanofiltration. Despite the proposed methodology, the comprehensive impact of nanofiltration on the entire amino acid profile of whey under real production conditions has not yet been investigated and remains insufficiently studied. The main reasons are

the complexity of whey as a multicomponent matrix and the technical difficulties that make it difficult to simultaneously monitor all important components.

The lack of such data limits the possibility of a comprehensive assessment of the impact of nanofiltration on the nutritional and biological value of the product. All this gives grounds to argue that it is advisable to conduct a study aimed at determining the impact of nanofiltration on the nutritional and biological value of whey, which could open up prospects for its more effective use when making functional food products.

3. The aim and objectives of the study

The aim of our study is to determine the impact of nanofiltration on the nutritional and biological value of whey. This will provide an opportunity to substantiate the feasibility of using nanofiltration technologies for processing whey in order to optimize its chemical composition for further use in food products.

To achieve the goal, the following tasks were set:

- to investigate the quality indicators of whey before and after nanofiltration treatment: organoleptic and physicochemical;
- to establish the amino acid profile of whey before and after nanofiltration treatment;
- to calculate the biological value of whey before and after nanofiltration treatment.

4. The study materials and methods

4.1. The object and hypothesis of the study

The object of our study is the nutritional and biological value of whey subjected to nanofiltration treatment.

Subjects of the study: organoleptic and physicochemical indicators, amino acid composition of whey samples.

Research hypothesis: the application of nanofiltration treatment to whey contributes to an increase in its nutritional and biological value by concentrating the main components. The positive functional properties of the processed whey determine the expansion of the range of products using them. It is assumed that the study would make it possible to assess the effectiveness of nanofiltration technologies for increasing the nutritional and biological value of whey and provide scientific justification for the feasibility of introducing membrane technologies in whey processing.

Assumptions accepted: it is assumed that the nanofiltration process does not cause significant denaturation of protein molecules, and changes in the composition of components occur mainly as a result of selective distribution through the membrane.

Simplifications adopted: analysis covers only the determination of the main indicators of the chemical composition of whey – proteins, carbohydrates, fats, and amino acid profile; the nanofiltration process and assessment of its efficiency involves the use of the average operating mode of the production plant, which excludes the analysis of variable operating modes and their impact on the results.

4.2. Raw materials and finished products used in the experiment

Whey was obtained during the production of hard cheeses (sample A1) and fermented milk cheese (sample B1); whey

was condensed by membrane separation on a nanofiltration unit of type AB (Automatic Basic) (samples A2 and B2). The samples were provided by the enterprise LLC “Bogodukhiv Dairy Plant” (Bogodukhiv, Kharkiv oblast, Ukraine).

4.3. Methodology for determining sample quality indicators

The quality assessment of whey samples was carried out according to generally accepted methodologies.

The acidity (pH) of whey samples was determined by the potentiometric method according to DSTU 8550:2015, DSTU 8551:2015. The density was determined by the areometric method according to DSTU 6082:2009.

The mass fraction of dry matter in whey samples was determined by the method of drying to a constant value of the indicator according to DSTU 8552:2015, DSTU 8574:2015. The mass fraction of protein substances was determined by the Kjeldahl method according to the recommendations from DSTU EN ISO 8968-4:2022. The mass fraction of fat was determined by the gravimetric method according to DSTU ISO 7208-2002.

Organoleptic indicators of whey samples were determined according to DSTU 7515:2014, DSTU 4553:2006.

Analysis of amino acids in whey samples was performed by ion-exchange liquid column chromatography using an automatic amino acid analyzer “T 339” (Prague, Czech Republic).

The amino acid score (AAS) was calculated according to formula (1). The reference composition of each essential amino acid, approved by the international organizations FAO/WHO, was used to calculate the amino acid score

$$AAC = \frac{\text{Amino acid content in sample (mg/g protein)}}{\text{Amino acid content in reference protein (mg/g protein)}} \cdot 100\%. \quad (1)$$

The biological value (BV) of whey, which is an indicator of the degree of protein assimilation taking into account their amino acid composition, was determined based on the amino acid score discrepancy coefficient (AASDC) according to formula (2)

$$BV = 100 - AASDC, \quad (2)$$

where the amino acid score divergence coefficient (AASDC) was calculated using formula (3)

$$AASDC = \frac{\sum_{j=1}^n \Delta AASD}{n}, \quad (3)$$

where n is the amount of essential amino acids;

$\Delta AASD$ – amino acid score difference of an amino acid, which is calculated from formula (4)

$$\Delta AASD = AAC_i - AAC_{\min}, \quad (4)$$

where AAS_i – excess score of the i -th essential amino acid, %;

AAS_{\min} – minimum of the scores of the essential amino acid of the studied protein relative to the standard, %.

Our experimental data are represented in units of the international system SI. The studies were carried out with threefold repeatability. All results are represented in the format: mean \pm standard deviation. Statistical processing was carried out using the Student's t -test. The difference was considered statistically significant at $p \leq 0.05$.

The experimental data were processed using Microsoft Excel software (Microsoft Office 2016, Microsoft Corporation, Washington, USA). All indicators of the studied whey samples were entered into a spreadsheet and analyzed according to a specific algorithm. First, the average values and standard deviations were calculated for each indicator based on several repetitions of the experiment, which allowed us to assess the stability of the obtained data. To compare groups of samples, the difference in mean values was calculated and its statistical significance was determined. The reliability of the results was assessed by determining the significance level (p), with a value of $p \leq 0.05$ being considered a criterion for confirming a statistically significant difference between groups. Plots constructed in Microsoft Excel were used to visually represent changes in indicators during whey processing.

5. Results of investigating the influence of nanofiltration on the nutritional and biological value of whey

5.1. Results of investigating the organoleptic and physicochemical parameters of whey before and after the application of nanofiltration

The results of organoleptic and physicochemical parameters of whey samples before and after the application of nanofiltration (NF) are given in Tables 1, 2.

Samples of natural whey obtained during the production of rennet cheese and fermented milk cheese demonstrate characteristic organoleptic properties inherent in this type of product.

The use of nanofiltration significantly affects the organoleptic characteristics of whey. The consistency becomes thicker and more viscous; the color acquires a rich yellow hue. The taste and smell change from fermented to sweet, with pleasant whey notes.

Native whey samples in terms of physicochemical parameters corresponded to the regulatory values.

The value of active acidity of whey samples after nanofiltration increases, which indicates changes in the acid-alkaline balance, which will potentially affect the taste or preservation of the product.

The use of nanofiltration led to an increase in the concentration of dry substances by 3.3 times in both the case of cheese whey and fermented milk cheese. At the same time, the protein content increased by 3.8 times, and the content of mineral substances (ash) by 2 times. This indicates that nanofiltration demonstrates high efficiency in removing water and low-molecular compounds, contributing to an increase in the concentration of useful components. This approach can significantly increase the nutritional value of whey.

5.2. Results of research on the amino acid profile of whey before and after the application of nanofiltration

The amino acid profile of the studied whey samples before and after the application of nanofiltration was analyzed chromatographically. The averaged results are represented by the diagram in Fig. 1.

During the study, 17 amino acid residues were identified in whey samples before and after nanofiltration.

Table 1

Organoleptic characteristics of whey samples

Indicator ID	Normative value	Actual value before NF		Actual value after NF	
		Sample A1	Sample B1	Sample A2	Sample B2
Appearance, consistency	Homogeneous liquid without foreign inclusions, with a permissible slight sediment	The liquid is homogeneous, transparent, without sediment	The liquid is cloudy, with a finely dispersed proteinaceous sediment	Thick, homogeneous liquid, without sediment	Thick, homogeneous liquid, without sediment
Color	From light yellow to greenish yellow, uniform throughout the mass	Light yellow	Light yellow with a greenish tint	Yellow, uniform throughout	Yellow with a greenish tint, uniform throughout the mass
Taste	Clean, typical of whey, without foreign flavors	Sweet, without foreign flavors	Sour, typical of whey, without foreign flavors	Sweet, with a slight sour-milky flavor, without foreign flavors	Sweet, with a noticeable sour-milky flavor, without foreign flavors
Odor	Clean, typical of the type of whey, without foreign odors	Fresh, with a characteristic faint cheese odor	Sour milk, clean, without foreign odors	Clean, sour-cheesy, without foreign odors	Clean, sour-milky, without foreign odors

Table 2

Physicochemical parameters of whey samples ($n = 3$, $p \leq 0.05$)

Indicator ID	Normative value	Actual value before NF		Actual value after NF	
		Sample A1	Sample B1	Sample A2	Sample B2
Active acidity, pH units	4.2...6.0	6.2 ± 0.1	4.5 ± 0.1	6.4 ± 0.1	4.9 ± 0.1
Density, kg/m ³	1025...1032	1026 ± 1.0	1028 ± 1.0	1075 ± 1.0	1080 ± 1.0
Dry matter content, %	5.0...6.5	6.1 ± 0.1	5.8 ± 0.1	20.0 ± 1.5	19.0 ± 1.0
Protein content, %	0.5...1.0	1.0 ± 0.05	0.8 ± 0.05	3.8 ± 0.05	3.1 ± 0.05
Fat content, %	0.1...0.5	0.1 ± 0.05	0.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.05
Lactose content, %	3.5...4.5	4.2 ± 0.1	4.0 ± 0.1	14.5 ± 0.2	14.2 ± 0.1
Ash content, %	0.4...0.7	0.6 ± 0.05	0.8 ± 0.05	1.2 ± 0.05	1.6 ± 0.05

Note: according to DSTU 7515:2014.

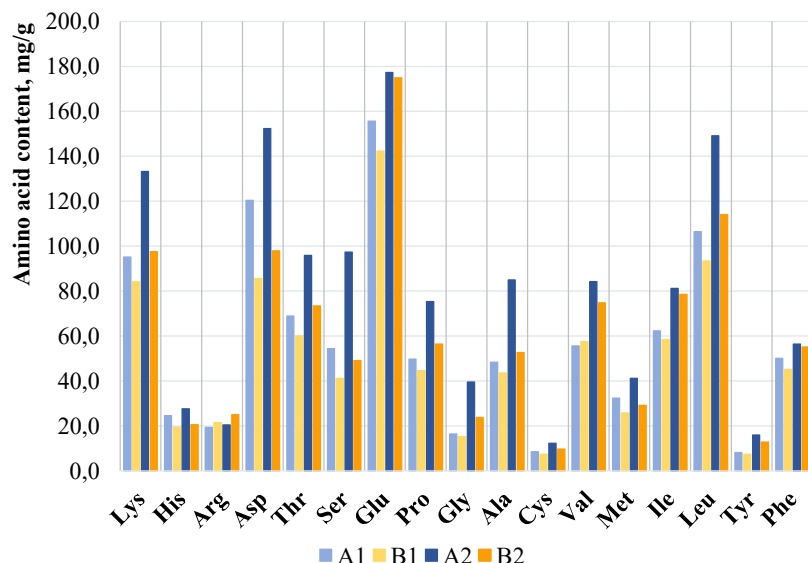


Fig. 1. Averaged amino acid profile of whey samples

Before treatment, whey samples (samples A1 and B1) were characterized by a high content of essential amino acids. In particular, leucine (106.5 and 93.4 mg/g), lysine (95.2 and 84.2 mg/g), phenylalanine (50.1 and 45.2 mg/g), threonine (68.9 and 60.1 mg/g), valine (55.6 and 57.6 mg/g), histidine (24.6 and 19.5 mg/g) predominated. Among the essential amino acids, the highest concentrations were recorded for glutamic acid (155.6 and 142.3 mg/g), aspartic acid (120.4 and 85.6 mg/g), proline (49.8 and 44.6 mg/g), and serine (54.4 and 41.2 mg/g).

After the application of nanofiltration in the whey samples (samples A2 and B2), an increase in the content of most amino acids was noted. The largest increase among the essential amino acids was observed for threonine (+31.24%), leucine (+31.13%), methionine (+24.12%), lysine (+28.65%), and isoleucine (+28.74%). Among the essential amino acids, a significant increase was noted for serine (+69.08%), glycine (+54.43%), alanine (+67.36%), and aspartic acid (+21.46%).

Our results indicate that nanofiltration contributes to the fortification of the protein composition of the samples with both essential and non-essential amino acids. The most pronounced positive effect was observed for threonine, leucine,

and serine, indicating an improvement in the amino acid profile and a potential increase in the biological value of the product.

5. 3. Results of determining the biological value of whey before and after nanofiltration treatment

Table 3 gives the results of determining the amino acid score (AAS) for essential amino acids of whey samples before (A1, B1) and after (A2, B2) nanofiltration treatment, as well as the calculated values of the amino acid score discrepancy coefficient (AASDC) and protein biological value (BV).

In the whey samples before nanofiltration (samples A1, B1), the amino acid score of most essential amino acids exceeded 100%, except for the total index for phenylalanine and tyrosine, which were limiting. The amino acid score coefficient of variation (AASDC) was 45.6% and

38.6%, respectively, and the biological value of the protein was 54.4% and 61.4%.

In the whey samples after nanofiltration (A2, B2), an increase in the amino acid score was recorded for almost all amino acids. The largest increase was noted for threonine (239.8% and 183.5%), lysine (242.2% and 177.5%), and leucine (213.0% and 163.0%). In samples A2 and B2, the values for methionine and cysteine also increased to 152.9% and 111.4%, respectively, which reduced their limiting effect. The AASDC coefficient of variation in sample A2 decreased to 39.6%, while in B2 it was 42.6%. The biological value of the protein after treatment increased to 60.4% in sample A2 but decreased slightly in B2 (57.4%) compared to the control sample B1.

Nanofiltration contributes to the concentration of essential amino acids in whey, which leads to a decrease in the discrepancy of the amino acid composition with respect to the "reference protein" and an increase in the biological value of the protein, especially in cheese whey (sample A2). The most pronounced increase is observed for threonine, lysine, and leucine, which indicates an improvement in the amino acid profile.

Table 3

Assessment of protein quality and biological value of whey samples

Essential amino acid	Sample A1		Sample B1		Sample A2		Sample B2	
	AAS, %	Δ AASD	AAS, %	Δ AASD	AAS, %	Δ AASD	AAS, %	Δ AASD
Histidine (His)	164.0	66.8	130.0	42.2	184.7	63.9	137.3	25.9
Isoleucine (Ile)	155.8	58.6	146.3	58.5	203.0	82.2	196.5	85.1
Leucine (Leu)	152.1	54.9	133.4	45.6	213.0	92.2	163.0	51.6
Lysine (Lys)	173.1	75.9	153.1	65.3	242.2	121.4	177.5	66.1
Methionine (Met) + Cysteine (Cys)	117.1	19.9	95.1	7.3	152.9	32.1	111.4	0.0
Phenylalanine (Phe) + Tyrosine (Tyr)	97.2	0.0	87.8	0.0	120.8	0.0	113.5	2.1
Threonine (Thr)	172.3	75.1	150.3	62.5	239.8	119.0	183.5	72.1
Valine (Val)	111.2	14.0	115.2	27.4	168.6	47.8	149.6	38.2
AASDC, %	45.6		38.6		39.6		42.6	
BV	54.4		61.4		60.4		57.4	

6. Discussion of results based on determining the effect of nanofiltration on the nutritional and biological value of whey

Our results confirm the positive effect of nanofiltration on improving the organoleptic characteristics of whey (Table 1). After treatment, an improvement in taste was observed, a decrease in the sharpness of the smell in sour whey, and an increase in the milky flavor in sweet whey.

According to data from [15], after membrane treatment, dairy products are characterized by a milder taste and a more pleasant consistency, which is confirmed by the studies. However, in [16] it is noted that intensive filtration could cause a decrease in the aroma of the product due to partial removal of aromatic molecules. In our study, these factors did not have a negative effect on the perception of the taste properties of whey.

The study of the physicochemical composition of whey before (samples A1, B1) and after (samples A2, B2) nanofiltration (Table 2) showed a slight increase in the value of active acidity (pH). In sample A2, by 3.2%, and in sample B2, by 8.9%, which indicates a shift in the acid-alkaline balance towards a decrease in acidity after nanofiltration. The density of the samples increased by 4.8% (sample A2) and 5.0% (sample B2), which indicates an increase in the concentration of dissolved components.

The most significant changes were noted in the dry matter content, which increased by 3.27 times (samples A2 and B2). Similarly, the protein content increased by 3.80 times (sample A2) and 3.87 times (sample B2), which indicates the effective concentration of protein fractions in the nanofiltration process. A significant increase in the lactose content (by 3.45 and 3.55 times in samples A2 and B2, respectively) confirms the general trend towards the concentration of low-molecular compounds. Thus, nanofiltration contributes to a significant concentration of the main nutritional and functional components of the dairy product, which has a positive effect on its nutritional value and technological properties.

Our results are consistent with those reported in study [4], namely with the justified prospects for using nanofiltration to improve the quality of dairy products through the selective removal of water and concentration of valuable substances.

A comprehensive study of changes in the organoleptic and physicochemical characteristics of whey provides the opportunity to assess not only individual components but also the overall nutritional value of the product after nanofiltration treatment. Analysis of protein, lactose, and dry matter content is an important tool for determining the efficiency of the technological process; it also reveals the potential of using whey as a basis for making functional food products.

Analysis of the amino acid composition (Fig. 1) revealed an increase in the concentration of both essential and non-essential amino acids after nanofiltration treatment. Thus, the content of lysine, leucine, threonine, and valine increased by 24–45% compared to untreated samples. Similar trends were observed in study [17], in which it was found that as a result of nanofiltration, the total level of amino acids in milk whey significantly increases due to the selective retention of peptides and free amino acids, which contributes to improving the nutritional value of the product.

Our results can be compared with the conclusions drawn in work [18]. The authors noted that nanofiltered protein concentrates retain bioactivity and have a high content of glutamic acid, isoleucine, and arginine. At the same time,

study [19] indicates the possibility of selective loss of small molecular peptides through the membrane, which may partially reduce biological activity. However, the correct choice of membrane porosity (within 100–300 Da) makes it possible to significantly minimize such losses.

The results of calculating the amino acid score of whey (Table 3) showed an increase in values by 7–69% after nanofiltration compared to untreated samples, which indicates an increase in the biological value of the protein. This especially applies to phenylalanine, tyrosine, methionine, cysteine – amino acids that are limiting in native whey samples. Similar results are reported in [20], which also show an increase in the amino acid score in whey protein concentrates after membrane technologies. This emphasizes the feasibility of using nanofiltration as a method for improving the qualitative composition of protein without significant structural changes. A comprehensive assessment of the biological value of whey after nanofiltration confirms its potential as a functional ingredient in food products, especially for children's, dietary, and sports nutrition.

Our results confirm that nanofiltration is an effective technology for increasing the nutritional and biological value of whey. With the correct selection of process modes, it is possible to achieve an improvement in organoleptic properties, concentration of protein components without significant loss of functionally active compounds. The increase in the biological value of whey when using nanofiltration is due to an improvement in the amino acid profile. This process is accompanied by an increase in the concentration of essential amino acids, in particular lysine, leucine, threonine, methionine, and cysteine. The increased content of these amino acids contributes to a more effective absorption of whey by the body. Thus, nanofiltration membranes retain larger molecules of protein components, while soluble non-protein compounds are removed. As a result, the concentration of amino acids in the concentrate increases and their composition approaches the optimal one for nutritional needs, which is confirmed by a decrease in the coefficient of divergence of the amino acid profile. Accordingly, nanofiltration is a highly effective method for increasing the nutritional and biological value of whey, which justifies the feasibility of its use in the production technologies of functional food products.

Our results are of practical importance since the ability of nanofiltration to selectively separate substances enables effective processing of whey while preserving its nutritional and biological properties. Whey treated by nanofiltration can be used in the production of functional food products (baby, diet, sports nutrition) and protein concentrates. Optimal process modes make it possible to preserve the amino acid profile and organoleptic properties, concentrating proteins and low-molecular compounds. The expected effect of using the results of the study involves the interest of manufacturers in integrating nanofiltration methods for whey processing. The use of this approach opens up opportunities for increasing the nutritional and biological value of whey, contributing to its more rational use when making functional food products. In turn, this can ensure a sustainable and cost-effective production process. Thus, our results confirm the feasibility of implementing nanofiltration as a promising method in the development of high-quality food components and expanding the possibilities of using whey in the food industry.

The main limitations of the study are the use of whey samples from a real enterprise, which applied standardized nanofiltration parameters (pressure, temperature, flow rate). Accordingly, options for optimizing these parameters to mini-

mize nutrient losses and maximize the preservation of biologically active components were not investigated. However, the technology of nanofiltration of whey could be applied to other enterprises.

The disadvantage of this study is the focus mainly on organoleptic, physicochemical parameters and amino acid profile of whey. Other important aspects, in particular, the effect of nanofiltration on enzymes, bioactive peptides, as well as changes in the composition of vitamins and minerals, were not taken into account. These data are of significant importance for a comprehensive assessment of the nutritional and biological value of the product. Possible areas for further research are the identification of bioactive peptides, as well as analysis of the content of vitamins and minerals in whey after nanofiltration.

7. Conclusions

1. Our comprehensive study of whey quality indicators before and after nanofiltration has demonstrated an improvement in organoleptic characteristics. The taste changes from sour-milk to sweet with pleasant whey notes, the smell softens, and the consistency becomes thicker and more viscous. Physicochemical indicators after nanofiltration demonstrate significant positive changes. The concentration of dry matter increased by 3.3 times, protein – by 3.8 times, minerals – by 2 times, and active acidity increased, which indicates changes in acid-alkaline balance.

2. The results of determining the amino acid profile of whey showed an increase in the concentration of essential amino acids (threonine +31.24%, leucine +31.13%, methionine +24.12%, lysine +28.65%) and non-essential amino acids (serine +69.08%, glycine +54.43%, alanine +67.36%, aspartic acid +21.46%). Comparison of samples before and after the application of nanofiltration showed that the process preserves and, in some cases, improves the amino acid profile, preserving important essential amino acids that are critical for the biological value of the product.

3. The analysis of the amino acid score, which characterizes the quality of protein from the point of view of amino acid composition, revealed that the indicators of most essential amino acids exceeded 110%. A decrease in the coefficient of variation was also recorded, indicating an improvement in the balance of the protein composition. Based on the obtained amino acid score values, the biological value of whey samples before and after nanofiltration was assessed. As a result, it was found that the biological value of whey after nanofiltration increased up to 60.4%. This demonstrates an increase in nutritional value and confirms the potential of the product as a functional ingredient for use in children's, dietary, and sports nutrition.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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