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PROTEIN COMPOSITION AND TECHNOLOGICAL PROPERTIES OF MILK WHEY CONCENTRATES

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Aim. To study the composition of commercial samples of whey protein concentrates obtained by the method of ultrafiltration from cheese whey and present on the Ukrainian market. **Methods.** Determination of nitrogen-containing fractions by the Kjeldahl method, protein composition – by electrophoresis in polyacrylamide gel, amino acid composition – on the amino acid analyzer, functional and technological properties of concentrates, characterizing their ability to participate in surface phenomena, – by conventional methods. **Results.** It was determined that the protein fractions of the investigated industrial samples are mainly β-globulin and α-lactalbumin, the total content of which is from 90 to 96 %. It was noted that the concentrates with a higher content of total protein have a smaller proportion of non-protein nitrogen and caseins. It was determined that the amino acid content of dry cheese whey is lower than that of the concentrates. It was established that the increase in protein content in concentrates up to 70–80 % leads to a significant increase in the foam-forming, water-retaining, fat-retaining and emulsifying capacities of concentrates in comparison with the milk whey. It was noted that the indicators characterizing the technological properties of the investigated commercial samples of concentrates with the same protein content may vary within 10–15 %. **Conclusions.** It was established that the investigated commercial samples of whey protein concentrates have an increased biological value and improved functional and technological properties in comparison with dry cheese whey, which allows using them in the formulations of other food products. To obtain final products with stable characteristics, it is necessary to evaluate the technological properties of concentrates entering the enterprise as part of implementing the activities of the product quality management system.

Keywords: ultrafiltration, whey protein concentrates, electrophoresis, non-protein nitrogen, essential amino acids, amino acid score, biological value, functional and technological properties.

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INTRODUCTION

Milk is a complicated polydisperse system, a relevant component of which is proteins. Casein constitutes the main part of milk protein substances (about 80 %). Whey proteins, mainly including β-lactoglobulin and α-lactalbumin, constitute about 20 % from the total amount of milk proteins [1].

The processing technology is the factor, determining the composition and properties of the obtained final products. For instance, the ultrafiltration of fat-free milk is used to obtain milk protein concentrate (MPC), containing both casein and whey proteins without any changes in their ratio, which is notable for milk. Whey protein concentrates (WPC) are obtained via methods of membrane filtration using milk whey, achieving from 25 to 80 % of protein content in the final product. It is possible to obtain isolates of whey proteins with the protein content over 90 % as well as their hydrolysates, using additional processing methods.

At present, scientific data on the impact of milk products on improving metabolic functions of people, suffering from metabolism disorders and cardio-vascular diseases, are of considerable interest. Some of the main factors of this impact are deemed to be such milk constituents as whey proteins, casein, minerals, and peptides [2, 3].

As stated above, milk proteins are a heterogeneous system, including almost 20 fractions. Cow milk casein contains α-s1, α-s2, β- and γ-fractions, whereas milk whey mostly contains globular proteins, like α-lactalbumin, β-lactoglobulin, lactoferrin, immunoglobulins, serum albumin, glycomacropeptides and enzymes. All these constituents may be capable of playing an important role in ensuring the connection between the decrease in the risk of metabolic diseases while increasing the consumption of milk products [4].

According to the common FAO/WHO evaluation of the ratio of amino acid score of proteins, caseins and whey proteins are referred to proteins with high biological value. Whey proteins contain more isoleucine and valine compared to casein, whereas casein contains more histidine, methionine, phenylalanine, glutamic acid, arginine, proline, serine, and tyrosine [5]. The differences in the physiological activity of whey proteins and caseins are referred to the differences in their amino acid composition [6]. There are data, testifying to faster consumption of whey proteins in human organism compared to casein [7].

Taking the abovementioned into consideration, the technologies of isolating and purifying whey proteins are used more extensively and implemented via commercial offers of different whey processing products. Whey protein concentrates are used in the production of other food products with the purpose of enhancing their nutritional and biological value. In addition, whey proteins are capable of foam- and gel-formation, thus, when they are added as fillers, it allows improving the technological properties of other food products, for instance, confectionery, bakery goods, meat products, *etc*.

MATERIALS AND METHODS

Four commercial samples of whey protein concentrates with the protein content of 70 % (WPC-70) and 80 % (WPC-80), submitted by different manufacturers, as well as dry cheese whey (control), obtained using a conventional technology, were used in the work.

The content of total nitrogen and protein in the experimental samples was determined according to DSTU ISO 8968-2:2005 (IDF 20-2:2001) [8], the content of casein nitrogen – according to DSTU ISO 17997-1/IDF 29-1:2009 [9], the content of non-protein nitrogen – according to DSTU ISO 8968-4:2005 (IDF 20-4:2001) [10]. The relative content of the main protein fractions of whey protein concentrates with different content of protein was determined via electrophoresis in polyacrylamide gel (12 %) in the presence of 6 M of urea and sodium dodecylsulfate [11]. The preparation of samples involved the extraction of lipids using hexane, drying and dissolving in tris-HCl buffer (pH 8.3). The quantitative evaluation of protein fractions was conducted spectrophotometrically. The obtained electrophoregrams were processed using Image Pro Gel Analyzer, Version 2.0 and Total Lab 1D programs. The amino acid composition of samples was determined via acid hydrolysis [12] at LC-2000 amino acid analyzer (Biotronik, Germany), the amino acid score – by calculations, the content of tryptophane – using the color reaction [13]. The biological value of the protein part was estimated via comparison against the amino acid composition of the protein, determined according to the recommendations of FAO/WHO expert committee [14].

The functional and technological properties of concentrates, characterizing their capability to participate in the surface phenomena while obtaining foam-forming and emulsion systems, including fat- and moisture-retaining properties, were estimated by conventional methods. The foam-forming capability of dry samples was determined by the relative increase in their solution volumes after shaking for 5 min at 800 rpm [15]. The emulsifying and fat-retaining capabilities of the investigated products were estimated using the emulsion solutions with refined oil according to the method [16]. The moisture-retaining capability of dry products was determined by the increase in the mass of wet precipitate after centrifugation at 8,000 rpm for 15 min [16].

The mathematical processing of the results was conducted by methods of statistical analysis and standard algorithms of Microsoft Excel programs. The experiments were conducted in three repeats. The results were deemed to be reliable at $P < 0.05$.

RESULTS AND DISCUSSION

The distribution of membrane technologies in the arsenal of industrial technological processes of milk-processing enterprises allowed enhancing the efficiency of processing raw milk materials and filling the market with competitive products. At present, the products of membrane processing of whey are used to enhance the nutritional and biological value of other food products, and improving their functional and technological properties. By organoleptic properties, whey protein concentrates are well connected with most food proteins of animal and plant origin, which expands the possibilities of using them in the composition of different products.

Taking the abovementioned into consideration, the study was conducted on determining the connection between the composition and technological properties of four kinds of dry whey protein concentrates. The characteristics of the investigated samples according to manufacturer's specifications are presented in Table 1.

As seen in the obtained experimental data (Table 2), the protein composition of experimental samples may be characterized as the combination of proteins and non-protein nitrogen-containing substances, the content of which is expressed via the indices of the content of total nitrogen, casein nitrogen, and nitrogen-containing non-protein compounds.

Non-protein nitrogen, which usually includes creatine, creatinine, uric acid, urea, purine compounds *etc.*, decreases nutrition, and as a result, biological value of the product. It should also be noted that with the increase in the degree of concentration for experimental samples, and the increase in total protein content in them, the share of casein protein decreases whereas the share of whey proteins increases.

Table 3 presents the relative content of the main protein fractions of the experimental samples. The electrophoresis data demonstrate that protein fractions of dry concentrates are mainly presented with β-lactoglobulin (β-Lg) and α-lactalbumin (α-La), the aggregate content of which is from 90 to 96 %. Other protein fractions are presented with immunoglobulins (Ig) and blood serum albumin (BSA). The traces of protein fractions of α-casein and β-casein are noted in all the electrophoregrams of experimental samples which is in agreement with the calculated data on the distribution of nitrogen (Table 2). Thus, the mass content of casein nitrogen in all the experimental samples of concentrates fluctuates within 0.235 g/100 g and 0.283 g/100 g. In whey, the relative content of casein and non-protein nitrogen ($N_{cas.}/N_{tot}$ and $N_{non\text{-}pr.}/N_{tot}$) is the highest, and the aggregate content of β-lactoglobulin and α-lactalbumin – the lowest (90.18 %).

The amino acid composition of the investigated concentrates and whey was determined. The estimated amino acid score is presented in Table 4.

Table 1. The characteristics of whey protein concentrates

Name of indices	Sample #1 dry cheese whey (Ukraine)	Sample #2 WPC-70 (Ukraine)	Sample #3 WPC-80 (the Netherlands)	Sample #4 WPC-80 (Belarus)
Mass content of dry substances, %	95.30	94.70	95.00	95.20
Mass content of total protein, %	14.2	72.3	81.1	83.3
Mass content of lactose, %	68.78	13.14	5.44	2.74
Mass content of fat, %	1.5	4.0	3.0	5.5
Mass content of ash, %	9.80	5.00	4.50	2.50
Organoleptic properties	Fine powder. Taste of milk with sour flavor. Color – from light yellow to cream.	Fine powder. Neutral taste with slightly sweet flavor. Color – from white to light yellow.		

Table 2. The content of nitrogen-containing fractions in the concentrates

Name of indices	Sample #1 dry cheese whey	Sample #2 WPC-70	Sample #3 WPC-80	Sample #4 WPC-80
Mass content of total protein, g/100 g	14.150 ± 0.002	72.300 ± 0.008	81.060 ± 0.006	83.300 ± 0.005
Mass content of total nitrogen, g/100 g	2.218 ± 0.005	11.332 ± 0.007	12.705 ± 0.004	13.056 ± 0.004
Mass content of casein nitrogen, g/100 g	0.062 ± 0.003	0.283 ± 0.002	0.267 ± 0.004	0.235 ± 0.001
Mass content of non-protein nitrogen, g/100g	0.037 ± 0.004	0.169 ± 0.005	0.165 ± 0.001	0.158 ± 0.002
$N_{cas.}/N_{tot.} \cdot 100$, %	2.8	2.5	2.1	1.8
$N_{non\text{-}pr.}/_{Ntot.} \cdot 100$, %	1.7	1.5	1.3	1.2

It was determined that amino acid score of essential amino acids is higher in the concentrates than in whey. For instance, the content of leucine in the concentrates is 1.2–1.4 times higher than its content in whey, and the content of lysine – 1.7–1.9 times higher respectively. Whey corresponds to FAO/WHO standard only in the content of threonine and valine. Methionine is a limiting acid both for whey and concentrates. Therefore, the experimental data demonstrate that all the industrial samples of whey concentrates are characterized by enhanced biological value compared to dry cheese whey, obtained using the conventional technology. The observed insignificant fluctuations in protein and amino acid composition of concentrates from different manufacturers are likely to be conditioned by differences in technologies and raw materials.

As stated above, the use of whey protein concentrates in food production allows both enhancing the nutritional and biological value of products and improving their consumption properties, expanding the assortment.

One of relevant properties of proteins is their capability to participate in surface phenomena. These properties are most widely used while obtaining products, based on foam-like and emulsion systems. A remarkable property of dry whey proteins is their capability of binding water.

The properties of dry protein concentrates are largely dependent on the ways and regimes of drying, the conditions of technological processes. To estimate functional-technological properties of the investigated whey concentrates, the foam-forming, moisture-retaining, fat-retaining and emulsifying capability of dry concentrates, produced by different manufacturers, were studied. The data obtained are presented in Table 5.

According to the presented data, the properties of concentrates, characterizing their capability to participate in surface phenomena, depend on the protein content in dry products. In particular, these are the capabilities of whey proteins of β-lactoglobulin and α-lactalbumin to emulsify and retain fats, and to bind water.

Thus, the highest indices of foam-forming capability, namely, 105.8 % and 117.5 %, are remarkable for concentrates with protein content of 80 %. A similar regularity was noted for moisture-retaining, fat-retaining and emulsifying capability of concentrates. Therefore,

Table 3. The relative content of the main protein fractions, g/100 of protein

Sample	α-casein	β-casein	β-Lg	α-La	BSA	(Ig)	∑ β-Lg and α-La
1	traces	traces	74.44	19.28	5.54	5.54	90.18
2	traces	traces	78.89	11.29	7.97	7.97	93.74
3	traces	traces	80.58	10.84	7.08	7.08	91.42
4	traces	traces	78.45	18.19	2.96	2.96	96.64

Table 4. Amino acid score of products under investigation

Essential amino acids	Scale FAO/WHO	Sample #1		Sample #2		Sample #3		Sample #4	
		g/100 g of protein	score, %	g/100 g of protein	score, %	g/100 g of protein	score, %	g/100 g of protein	score, %
Threonine	4.0	4.98 ± 0.03	124.50	5.03 ± 0.01	125.75	5.54 ± 0.06	138.50	6.22 ± 0.05	155.50
Valine	5.0	5.11 ± 0.06	102.20	5.17 ± 0.04	103.40	5.89 ± 0.03	117.80	5.19 ± 0.07	103.80
Methionine	2.2	0.33 ± 0.03	15.0	0.29 ± 0.07	13.18	0.30 ± 0.08	13.64	0.55 ± 0.06	25.00
Isoleucine	4.0	3.77 ± 0.01	94.25	5.83 ± 0.05	145.75	5.46 ± 0.05	136.50	4.71 ± 0.05	117.75
Leucine	7.0	5.80 ± 0.02	82.86	7.56 ± 0.03	108.00	7.24 ± 0.04	103.43	7.87 ± 0.01	112.43
Phenylalanine	2.8	2.15 ± 0.05	76.79	2.81 ± 0.02	100.36	2.80 ± 0.06	100.00	2.99 ± 0.09	106.79
Lysine	5.5	4.28 ± 0.05	77.82	7.39 ± 0.04	134.36	7.64 ± 0.02	138.91	8.41 ± 0.03	152.91
Tryptophane	1.0	0.87 ± 0.08	87.00	1.12 ± 0.09	112.00	1.35 ± 0.02	135.00	1.22 ± 0.08	122.00

Table 5. Functional and technological properties of concentrates

Sample number	Foam-forming ability, %	Moisture-retaining ability, %	Fat-retaining ability, %	Emulsifying ability, %
#1, dry whey	5.8 ± 0.6	12.2 ± 0.1	83.0 ± 0.6	27.0 ± 0.4
#2 WPC-70	64.1 ± 0.2	68.4 ± 0.5	154.9 ± 0.3	49.0 ± 0.4
#3 WPC-80	105.8 ± 0.2	74.1 ± 0.3	187.0 ± 0.2	57.0 ± 0.3
#4 WPC-80	117.5 ± 0.5	87.9 ± 0.2	195.0 ± 0.5	75.0 ± 0.2

the structure-forming functions of dry concentrates, related to their ability to emulsify and form foam, may be used in the production of structured milk products with the purpose of replacing stabilization systems. Noteworthy are high indices of moisture-retaining and fat-retaining ability of the investigated WPC. As seen in the presented data, the fat-retaining ability of WPC-80 is twice higher compared to milk whey, and the moisture-retaining one – almost seven times higher. However, there were some fluctuations in the values of investigated indices, noted for samples WPC-80 #3 and #4 from different manufacturers. These differences may be conditioned by the specificities of technological processes and drying regimes.

Thus, whey protein concentrates with protein content of 70–80 % may be used to improve fat and water absorption in different food products, the formulation of which includes dry milk whey or dry fat-free milk. It is evident that further use of dry concentrates in the production of other food products, for instance, meat products or bakery goods, are directly related to the properties, conditioned by the physical and chemical composition of concentrates.

RESULTS

It was determined that the protein fractions of the investigated industrial samples are mainly β-globulin and α-lactalbumin, the total content of which is from 90 to 96 %. It was noted that the concentrates with a higher content of total protein have a smaller proportion of non-protein nitrogen and caseins. It was determined that the amino acid content of dry cheese whey is lower than that of the concentrates. It was established that the increase in protein content in concentrates up to 70–80 % leads to a significant increase in the foam-forming, water-retaining, fat-retaining and emulsifying capacities of concentrates in comparison with the milk whey. It was noted that the indicators characterizing the technological properties of the investigated commercial samples of concentrates with the same protein content may vary within 10–15 %.

CONCLUSIONS

It was determined that whey protein concentrates have increased biological value compared to dry cheese whey. The investigated commercial samples are remarkable for improved functional and technological properties which allows using them in the formulations of other food products. To obtain final products with stable characteristics, it is necessary to evaluate the technological properties of concentrates entering the enterprise as part of implementing the activities of the product quality management system.

Білковий склад та технологічні властивості молочних сироваткових концентратів

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Мета. Дослідити склад комерційних зразків концентратів сироваткових білків, одержаних методом ультрафільтрації з підсирної молочної сироватки, та представлених на ринку України. **Методи.** Визначення азотовмісних фракцій за методом К'єльдаля, білковий склад методом електрофорезу у поліакриламідному гелі, амінокислотний склад на амінокислотному аналізаторі, функціонально-технологічні властивості концентратів, що характеризують їх здатність до участі в поверхневих явищах за загальноприйнятими методиками. **Результати.** Визначено, що білкові фракції досліджених промислових зразків представлені переважно β-глобуліном та α-лактоальбуміном, сумарний вміст яких становить від 90до 96 %. Відмічено, що у концентратах із більшим вмістом загального білка зменшується частка небілкового азоту та казеїнів. Визначено, що амінокислотний скор сироватки підсирної сухої нижчий у порівнянні з концентратами. Встановлено, що зі збільшенням вмісту білка у концентратах до 70–80 %, піноутворююча, вологоутримуюча, жироутримуюча та емульгуюча здатність концентратів у порівнянні із сироваткою молочною істотно зростає. Відмічено, що показники, які характеризують технологічні властивості досліджених комерційних зразків концентратів з однаковим вмістом білка, можуть коливатися в межах 10–15 %. **Висновки.** Встановлено, що досліджені комерційні зразки концентратів сироваткових білків мають підвищену біологічну цінність та покращені функціонально-технологічні властивості у порівнянні з сухою підсирною сироваткою, що дозволяє використовувати їх у рецептурах інших харчових продуктах. З метою одержання кінцевих продуктів зі стабільними характеристиками необхідно у рамках виконання заходів системи управління якістю продукції проводити оцінку технологічних властивостей концентратів, що надходять на підприємство.

Ключові слова: ультрафільтрація, концентрати сироваткових білків, електрофорез, небілковий азот, незамінні амінокислоти, амінокислотний скор, біологічна цінність, функціонально-технологічні властивості.

Белковый состав и технологические свойств молочных сывороточных концентратов

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Цель. Исследовать состав коммерческих образцов концентратов сывороточных белков, полученных методом ультрафильтрации из подсырной молочной сыворотки, и представленных на рынке Украины. **Методы.** Определение азотсодержащих фракций по методу Кьельдаля, белковый состав методом электрофореза в полиакриламидном геле, аминокислотный состав на аминокислотному анализаторе, функционально-технологические свойства концентратов, характеризующие их способность к участию в поверхностных явлениях по общепринятым методикам. **Результаты.** Определено, что белковые фракции исследованных промышленных образцов представлены преимущественно β-глобулином и α-лактоальбумином, суммарное содержание которых составляет от 90 до 96 %. Отмечено, что в концентратах с большим содержанием общего белка уменьшается доля небелкового азота и казеина. Определено, что аминокислотный скор сыворотки подсырной сухой ниже по сравнению с концентратами. Установлено, что с увеличением содержания белка в концентратах до 70–80 %, пенообразующая, влагоудерживающая, жироудерживающая и эмульгирующая способности концентратов по сравнению с сывороткой молочной существенно возрастает. Отмечено, что показатели, характеризующие технологические свойства исследованных коммерческих образцов концентратов с одинаковым содержанием белка, могут колебаться в пределах 10–15 %. **Выводы.** Установлено, что исследованные коммерческие образцы концентратов сывороточных белков имеют повышенную биологическую ценность и улучшенные функционально-технологические свойства по сравнению с сухой подсырной сывороткой, что позволяет использовать их в рецептурах других пищевых продуктов. С целью получения конечных продуктов со стабильными характеристиками необходимо в рамках выполнения мероприятий системы управления качеством продукции проводить оценку технологических свойств концентратов, поступающих на предприятие.

Ключевые слова: ультрафильтрация, концентраты сывороточных белков, электрофорез, небелковый азот, незаменимые аминокислоты, аминокислотный скор, биологическая ценность, функционально-технологические свойства.

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