engineering proceedings

MDPI

Proceeding Paper

Rational Use of Whey in Food Production †

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† Presented at the 2nd International Electronic Conference on Processes: Process Engineering—Current State and Future Trends (ECP 2023), 17–31 May 2023; Available online: https://ecp2023.sciforum.net/.

Abstract: This study describes the biotechnological significance of the by-product obtained in the production of milk products in the food industry.

Keywords: protein; milk; whey

1. Introduction

The processing of milk into milk-protein concentrates—cheese, cottage cheese, casein, is inevitably associated with the production of a by-product of sweet or sour whey. The global production of whey is estimated at about 180 to 190 tons/year. Of this amount, only 50% is recycled. In the Russian Federation, only 30–40% of the total amount of whey is processed, which is irrational since it can be used as a valuable nutrient. The composition of whey depends on several factors, including the type of processed cheese, the method of casein precipitation, heat treatment of milk, storage after milking, and others. Sour whey (pH about 5.0) is obtained in the production of fresh type cheeses, which includes acid coagulation of milk, cream or whey or a combination of acid and rennet or acid and heating, and it differs from sweet whey in the content of proteins, minerals, and lactose [1–3].

Based on literature sources, it is known that for 1 L of casein and acidic whey there are 65 g of solids, of which 6.0–6.2 g are true proteins [4]. For serum processing, thickening, drying, electro membrane (electrodialysis, electro activation) and bar membrane processes (ultrafiltration, nanofiltration, reverse osmosis) are used [5].

The purpose of this study was to evaluate the protein potential of whey and increase the yield of true proteins in protein concentrate.

2. Materials and Methods

To develop a method for isolating protein, concentrate from whey, the serum of the trademark "Tommoloko" was taken, produced in accordance with GOST 33957-2016 Milk whey and beverages based on it.

To standardize the methodology, as well as to compare the ratio of the amount of total protein in different volumes of serum, 200, 500 and 1000 mL of raw materials were taken alternately. 2 variants of obtaining protein from whey were investigated (before filtration and after filtration). all measurements were carried out in 5 repetitions in 3 parallels. Each volume of the tested liquid was heated in a heat-resistant glass on an electric stove to a temperature of 85–95 °C, having previously measured the pH of the medium for the initial values. When the temperature reached the desired value, 1 mL of 70% NaOH solution was gradually introduced into the heated serum with a dispenser at certain time intervals, bringing the pH of the medium to 6.5–7. After complete cooling of

Citation: Potapova, M.; Ivanova, S.; Lyakhovchenko, N.; Shaidorova, G.; Elena, K. Rational Use of Whey in Food Production. *Eng. Proc.* **2023**, *5*, x. https://doi.org/10.3390/xxxxx

Published: 17 May 2023

the mixture, the resulting precipitate was filtered using vacuum filtration (pore diameter 3 microns), then the filtered protein concentrate was poured into Petri dishes and frozen at a temperature of 40 °C for lyophilization for 24 h. After that, the samples were dried on the AK5-4.0 brand freeze-drying under conditions. To compare the protein content in 1 L of whey, the technology was repeated, and then the data obtained after drying the filtered concentrate and the mixed concentrate were compared.

Confidence intervals (Δ) were calculated using the formula [6]:

$$\Delta = t_{st} \times m, \quad (1)$$

where t_{st}—is the standard value of the Student's confidence criterion, m is the error of representativeness of the arithmetic mean and is calculated by the formula [6]:

$$m = \frac{\sigma}{\sqrt{n}}, \quad (2)$$

where σ—is the standard deviation and is calculated as, n—is the number of measurements [5]**:**

$$\sigma = \sqrt{\frac{\sum(V-M)^2}{n-1}}, \quad (3)$$

where V—is the date, M—is the arithmetic mean of the dried protein mass, n is the sample size. The relative measurement error was calculated using the formula [6]:

$$\varepsilon = \frac{m}{M} \cdot 100\%, \quad (4)$$

where M—is the arithmetic mean and m is the error of representativeness of the arithmetic mean.

The reliability of the difference in the averaged values was calculated statistically by the difference method [6].

The resulting protein concentrate was tested using standard protein determination methods [7]. One of the qualitative methods was determined by the ninhydrin reaction, which allows to determine the presence of an α-amino group: 5 drops of 0.1% of the product under study and 2 drops of 0.1% ninhydrin solution in 95% acetone solution were added to the test tube. The contents of the test tube are thoroughly mixed in a water bath at a temperature of 70 °C for 5 min. The presence of the α-amino group is seen by the presence of a blue-violet color.

The presence of aromatic amino acids in the protein mixture was determined using a xanthoprotein reaction. To do this, 3 mL of 0.01% solution of the test concentrate and 1 mL of concentrated nitric acid were added to the test tube. The mixture was carefully heated until a yellow color appeared. After cooling, a solution of 10% sodium hydroxide was added to the test tube until an orange color appeared.

Quantitative determination of pure protein in protein concentrate was used and determined by biuretic method [8]. For this purpose, the following solutions were prepared: a solution of a biuretic reagent (the following substances were sequentially dissolved in 400 mL of distilled water: 9.0 g sodium-potassium tartrate ($C_4H_4KNa0_6 \bullet 4H_20$) 3.0 g copper sulfate ($CuS0_4 \bullet 5H_20$) 8.0 g sodium hydroxide (NaOH) 5.0 g potassium iodide (KI,)); a solution of 3 M trichloroacetic acid (TCU, $C_2H_{13}\ 0_2$) 49 g was brought to 100 mL with water). As a control, a ready-made protein solution with an exact concentration of 70 g/l was used. Various amounts of the protein sample (0.01–0.1 mL) containing from 10 to 50 mg of protein/mL were brought to a volume of 1 mL with water. 1 mL of water was used as a comparison solution. Then 0.15 mL of 3 M TCU was added to the samples and centrifuged for 3 min at 5000 rpm. The supernatant was drained, and the precipitate was dissolved in 1 mL of biuretic reagent. The mixture was left for 30 min at room temperature, and then the absorption was measured at 546 nm relative to the reference solution in small volume cuvettes.

3. Results

We have developed a new method for obtaining protein from whey. as a result, with this method, the yield of pure protein is 98%, which is a high indicator compared to previously known methods. According to the results of standardization of the method for isolating protein from whey, it was revealed that for 1 L of whey from the manufacturer "Tommoloko" there are from 19 to 21 g of protein.

The study showed that when using the first variant of obtaining protein from 1 L of whey, 20.7 ± 1.4 g /l (dry matter weight) was obtained, whereas as a result of the second variant—18.3 ± 1.43 g /l, the data obtained can be considered reliable, since the relative measurement error for each of the cases it was below 3% (1.6% for option 1 and 1.8% for option 2).

It was revealed that the difference between the obtained result for the dry matter of the two variants is statistically unreliable since the calculated Student reliability criterion turned out to be lower than the tabular value at an error level of $p < 0.05$.

Table 1. Arithmetic averages of dry matter as an indicator of the efficiency of precipitation of protein from whey using different methods.

General Parameters	Variants	
	Method of Obtaining Protein 1	**Method of Obtaining Protein 2**
Arithmetic averages, M ± Δ	20.7 ± 1.4	18.3 ± 1.43
Mean square deviation, σ	0.6	0.6
Relative measurement error, ε, %	1.6	1.8

According to the results of the experiment, it was also revealed that filtration of the protein is necessary before lyophilization, since the mixture contains a significant amount of lactose, which changes the physical properties of the protein concentrate during long-term storage.

The resulting dry sediment is a white, crumbly mass with a slightly milky smell. After filtration, the protein product is perfectly stored at a temperature of 4 °C for more than six months without changing its physico-chemical properties.

The results of qualitative analysis showed that the composition of the protein mixture includes α-amino groups, as well as aromatic amino acids (blue-purple staining was observed during the ninhydrin reaction and orange staining of the mixture after the xanthoprotein reaction).

The quantitative analysis by the biuretic method showed the results presented in Table 2.

Table 2. Quantitative determination of protein content in protein concentrate.

Optical Density (A); Calibrator Protein Concentration, mg/mL	Weight of the SUSPENSION of Protein Concentrate, g	Optical Density (A) of Protein Concentrate Solution	Estimated Protein Concentration in the Sample, mg/ml	True Protein Concentration in the Sample, mg/ml	Protein Content, %
0.427 ± 0.05 70 mg/ml	0.02 ± 0.001	0.120 ± 0.03	20 ± 0.03	19.72 ± 0.09	98.6 ± 0.05
0.427 ± 0.05 70 mg/ml	0.02 ± 0.001	0.115 ± 0.06	20 ± 0.1	18.8 ± 0.09	96.5 ± 0.07

4. Discussion

In the 2 variants developed by us for obtaining whey concentrate from whey: option 1 is a concentrate before filtration (20 g per 1 L), and option 2 is a concentrate after filtration of 19.72 g per liter), the yield of pure protein was 98.6% and 96.5%, respectively. While in the previously known method of protein production, the essence of which is to use chitosan as a complexing agent, the yield was 95% [9]. There is also a known method of effective sorption of whey protein by macroporous and macro reticular ionites in pH ranges one to three units away from the isoelectric point. With this method, the protein yield also does not exceed 95% [10]. With another method of production, it occurs by introducing water-soluble polymers into the whey—the initial high-anionic polyacrylamide (PAA) or the same polyelectrolyte modified with glycine, followed by mixing the mixture and separating the resulting precipitate with a protein yield of no more than 95% [11].

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