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Professional paper

EXAMINATION OF THE COMPOSITION OF WHEY FROM MIXED CHEESE

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Abstract

In recent years, the ingredients of milk represent a functional food, because their use has a great effect on health. Whey is a byproduct of cheese production, which was previously considered waste. Whey proteins strengthen the immune system by helping the body produce the antioxidant glutathione, which protects against free radicals, pollution, toxins and infections. In this research we examine the composition of whey (minerals: Ca, K, Fe and P, total proteins and albumins) from mixed cheese from the "Ideal Shipka" dairy factory - Bitola. All parameters were determined spectrophotometrically, spectrophotometric, photometric - colorimetric and nephelometric methods were applied to prove the amount of certain components present in the whey composition. From the obtained results we can see that whey has the highest amount of total proteins, it contains 17.6 g/L, from which amount of albumen is 1,99 g/L. Of the minerals, the highest value was obtained for potassium 26,6 mmol/l, followed by calcium 9,47 mmol/l, amount of phosphorus was 9,39 mmol/l, while iron is found in a very small percentage 11,74 μmol/l. From the research we can conclude that whey contains a large percentage of total proteins, and of the minerals potassium. Thus, whey can be used as a dietary supplement for protein and potassium and it is attractive product for both the food and pharmaceutical industries and consumers.

Keywords: whey, protein, minerals, spectrophotometry.

1. Introduction

Whey is a byproduct of cheese production, which was previously considered waste. Whey is a clear yellowish liquid that separates when cheese is made. It represents 90% of the original weight of the milk used to make cheese. It contains between 6-6.4% solids, which is half of the total content in milk. It contains β-lactoglobulin, α-lactoalbumin, serum albumin, lactoferrin, immunoglobulins, lactoperoxidase, glycomacropeptides, lactose and minerals. Whey proteins strengthen the immune system by helping the body produce the antioxidant glutathione (Marshall, 2004). Glutathione protects against free radicals, pollution, toxins and infections. Adding whey protein to the diet can improve the health of people of all ages. Due to the wide spectrum of essential and non-essential amino acids, minerals, fats and biologically active proteins, whey is widely used in the treatment of various diseases.

Whey is still an enigma and underutilized even in the 21st century and is interesting for researchers, for production and marketing. The emphasis is on the fact that it is a medicinal liquid that contains 16 types of proteins, 8 minerals, 7 vitamins, up to 23 amino acids, up to 11 enzymes and many other substances for the development of the animal world. Whey is rich in minerals: calcium, which is in a good ratio with phosphorus, as well as a good ratio of potassium with sodium, contains Cl, Cu, Zn, Fe, Mn and Mo in traces that maintain cell tone, prevent high blood pressure, stroke, heart attack.

The separation of whey depends on the production technology of the basic product, as well as on the quality of the milk used.

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Whey proteins

Whey is composed of several proteins including beta-lactoglobulin, alpha-lactalbumin, serum albumin (BSA), and glucomacropeptide (GMP). Whey protein contains all the essential amino acids and in higher concentrations compared to some vegetable protein sources such as soy, corn and wheat (Walzem RL, Dillard CJ, German JB, 2002).
Proteins in milk have many roles that make dairy products and other products containing these components so valuable.
These proteins are not sensitive to the action of acids or enzymes, so they remain unchanged during coagulation and after removing the casein curd, they pass into the whey. That is why the amount of protein in sweet and sour whey is similar (Presilski, 2004).

Whey as a functional food. Therapeutic application of whey

Whey proteins are high quality proteins and with their high percentage of branched chain amino acids (BCAA- branched chain amino acids), they have been popular in the fitness industry for a long time as muscle building supplements. But research shows that they may have much wider applications as functional foods for cancer, hepatitis B, cardiovascular disease, osteoporosis and chronic stress (Bounous, 2000).

2. Material and methods

In this research we examine the composition of whey (minerals: Ca, K, Fe and P, total proteins and albumins) from mixed cheese from the "Ideal Shipka" dairy factory - Bitola. All parameters were determined spectrophotometrically, on a Screen Master spectrophotometer.

Determination of iron - photometric colorimetric iron test with fat clearance factor (LCF)

Iron (III) reacts with chromazurol B (CAB) and cetyltrimethylammonium bromide (CTMA) and a colored complex is formed with an absorbance maximum at 623 nm. The intensity of the color is directly proportional to the concentration of iron in the sample (Garcic, 1979)
Care should be taken to avoid reagent contamination. Fatty samples cause falsely high results, so this reagent has a fat clearance factor (LCF).
Mix 500 µl of reagent with 25 µl of the sample and incubate for 15 minutes at room temperature. The absorbance of the sample is measured within 60 minutes against a blank for the entire run and measured at a wavelength of 623 nm.
This test is linear up to 500 µg/dl or 89.5 µmol/l. Great care should be taken when performing this test because the test is very sensitive, disposable materials are used and the distilled water must not contain iron.

Determination of calcium - photometric method

Calcium ions react with o-cresolphthalein-complex in an alkaline environment and a violet-colored complex is formed. The absorbance of this complex is proportional to the concentration of calcium in the sample (Gitelman, 1967).
The reagent consists of two parts, from which the working reagent is prepared in a ratio of 1:1 and stands for 30 minutes at room temperature before use. The absorbance is measured at a wavelength of 570 nm (Hg 578 nm) and 546 nm, the optical path is 1 cm, the temperature should

be 20-25 oC. The measurement is performed against a blank. 600 µl working reagent and 12 µl of the sample are mixed, after 5-30 minutes they are read.

Determination of phosphor - photometric UV test

Phosphorus reacts with molybdate in a strongly acidic environment and a complex is formed. The absorbance of this complex is directly proportional to the concentration of phosphorus (Gamst O., Try, K., 1980).
$7\ H3PO4 + 12\ (MO7O24)6- + 51\ H+ \rightarrow 7\ [P(MO12O40)]3- + 36\ H2O$
The reagent is stable but contamination must be avoided. Absorbance is measured at a wavelength of 340 nm or 343 nm. Mix 600 µl of the reagent with 6 µl of the sample, incubate for at least 1 minute and within 60 minutes measure against the blank.

Determination of potassium - nephelometric method (endpoint)

Potassium ions in a protein-free alkaline medium react with sodium tetraphenylboron (TPB-Na) to produce a dispersed colloidal suspension of potassium tetraphenylboron. The resulting turbidity is proportional to the concentration of potassium in the sample (Terri, A. E., Sesin, P.G, 1958).
The reagent consists of three parts. Reagent 1 is precipitate (trichloroacetic acid), reagent 2 is TPB-Na and reagent 3 is NaOH. The working reagent is prepared by mixing R2 and R3 in a 1:1 ratio. After it is prepared, it is preferable to let it stand for 15-30 minutes.
The first part of the procedure is precipitation, that is, it is necessary to precipitate the proteins, therefore, mix 600 µl of reagent 1 with 60 µl of the sample, centrifuge for 10 minutes at 4000 rpm and then separate the clean supernatant.
The second part of the procedure is colorimetry. Mix 600 µl of the working solutionreagent with 60 µl of the supernatant, incubate for at least 5 minutes and measure the absorbance at a wavelength of 578 nm against a blank sample.

Determination of albumen - photometric colorimetric test for albumen with BCG-method

Bromocresol green in citrate buffer forms a colored complex with albumin. The absorbance of this complex is proportional to the concentration of albumen in the sample (Rodkey, 1965, Doumas, B., et al, 1971).
The absorbance is measured at a wavelength of 578 nm, the measurement is performed against a blank sample. Mix 600 µl of reagent with 6 µl of the sample and incubate for 5 minutes.

Determination of total proteins - colorimetric photometric method (biuret method)

Copper ions from proteins and peptides with an alkaline environment form a violet colored complex. The absorbance of this complex is proportional to the concentration of proteins in the sample (Weichsebaum, 1946, Josephson, B., Gyllenswärd, C., 1957).
Absorbance is measured at a wavelength of 520-580 nm and the measurement is performed against a blank. Mix 600 µl of reagent with 12 µl of the sample and incubate for 10 minutes.

Statistical analysis of the results by analysis of variance-ANOVA

In scientific-research work in agriculture, animal husbandry, veterinary medicine and more widely in biological sciences, comparisons of multiple modalities of one factor are carried out. The factor under investigation has at least three modalities (variants). This is the case in our research where the factor is whey and we have three modalities, that is, three groups of piglets. The null hypothesis (H0) is that $\bar{x}1= \bar{x}2$; $\bar{x}1= \bar{x}3$; $\bar{x}2= \bar{x}3$ (Ott, R. L., Longnecker, M., 2001). If the null hypothesis is rejected, in order to confirm exactly which groups have a significant difference, further analysis is performed, in our case Tukey's HSD Post-hoc test. HSD is calculated using the formula:
HSD= q*
q-table value
n the number of values used in the calculations
MSwithin- or variance, represents the square of the standard deviation, i.e. the average of the squared deviations of the variances from the mean value.

3. Results and discussion

Analysis of the constituent components of whey

The composition of the whey was examined, namely the mineral composition (Ca, P, K and Fe) and total proteins and albumen.

Table 1. Amount of Ca in whey

Number of sample Ca/ (mmol/l)	**C_1**	**C_2**	**C_3**	**C_4**	**C_5**
1	7,58	6,12	8,91	6,22	7,31
2	8,22	6,89	8,93	6,14	7,22
3	8,28	6,12	9,88	6,28	7,15
4	7,53	4,78	8,78	6,13	7,11
5	7,58	6,19	9,15	6,2	7,11
6	7,49	5,42	9,54	6,42	7,11
7	8,3	5,78	8,77	6,16	7,19
8	7,59	5,56	12,5	6,14	7,13
9	8,12	5,75	9,24	6,14	7,2
10	8,01	5,81	8,99	6,22	7,25
$\bar{x}$	7,87	5,842	9,469	6,205	7,178
SD±	0,34	0,55	1,12	0,08	0,06

The third whey is the richest in Ca, around 9.5 mmol/l, while the second one contains at least 5.8 mmol/l Ca (Table 1). The second whey contains the most P, 9.4 mmol/l, and the others have approximately the same amount (Table 2). All 5 types of whey are rich in K and there is not much difference between them, the highest level of K has C4 26.6 mmol/l (Table 3). Fe is present in very small amounts compared to other minerals, the first whey contains the most 11.7 µmol/l, while the others have about 9 µmol/l (Table 4). It is important to note that the first two samples of whey are obtained from cheese, while the rest are from mixed cheese.

Table 2. Amount of P in whey

Number of P/ (mmol/l)	**C 1**	**C 2**	**C 3**	**C 4**	**C 5**
1	7,16	8,12	6,28	7,75	8,21
2	7,78	11,09	10,99	7,85	8,22
3	6,76	4,89	7,25	7,93	8,2
4	7,15	9,73	8,91	7,65	8,23
5	6,84	8,79	7,33	7,83	8,25
6	6,95	11,5	7,58	7,8	8,2
7	7,04	11,11	7,62	7,62	8,2
8	7,23	9,02	7,49	7,71	8,15
9	6,75	9,49	8,05	7,8	8
10	6,9	10,21	9,15	7,75	8,2
$\bar{x}$	7,056	9,395	8,065	7,769	8,186
SD±	0,30	1,92	1,31	0,09	0,07

From Table 1, it can be seen that in C5 the deviation from the mean value of Ca is the smallest (SD±=0.06), and the largest is in C3.

Table 3. Amount of K in whey

Number of sample K/ (mmol/l)	**C1**	**C 2**	**C3**	**C4**	**C 5**
1	26,2	27	24,75	27	25
2	22,5	26,3	28,5	24,9	25,45
3	21,75	26,3	26	26,8	25,66
4	24,5	24,8	25,9	27,7	25,75
5	23,5	26	23,8	26,7	26,05
6	26,19	26,1	25,3	26,3	26,15
7	22,49	22,3	26,8	27,3	24,9
8	23,83	24,8	25,4	26,9	26,05
9	25,31	25,3	26,3	25,4	26,1
10	24,15	24,5	27	27	25,8
$\bar{x}$	24,042	25,34	25,975	26,6	25,691
SD±	1,54	1,33	1,30	0,85	0,44

Whey is rich in proteins, it can be seen from table 5 and 6. The highest content of total proteins is the first whey obtained during the production of cheese, it contains 17.6 g/L, and the least contains C4 9.5 g/L obtained during the production of mixed cheese. Normally, C2 has the highest albumen content, 2.36 g/L, and C4 has the lowest content, 1.34 g/L.

Table 4. Amount of Fe in whey

ordinal number of trial Fe/ (µmol/l)	C_1	C_2	C_3	C_4	C_5
1	10,2	10,12	10,4	12	10,1
2	11,3	10,1	10,3	9	10,4
3	11,4	9,3	10,2	11,3	10,1
4	12,5	8,4	9,9	9,5	10,15
5	12,6	5,1	9,9	9,4	10,2
6	13	10,2	10,1	9,4	10,1
7	11,2	10,3	9,8	9,5	9,41
8	10,9	9,5	9,8	9,9	9,43
9	12,5	9,5	9,7	10,2	9,9
10	11,8	9,5	9,8	10,3	10,2
$\bar{x}$	11,74	9,202	9,99	10,05	9,999
SD±	0,89	1,54	0,24	0,94	0,32

From Table 5, it can be seen that the smallest deviation from the mean value for total proteins is in C 4 (SD± =1.17), and the largest is in C1 (SD±=3.94).

Table 5. Amount of total protein in whey

ordinal number of trial TP/ (g/L)	C_1	C_2	C_3	C_4	C_5
1	14	12	12	11	13
2	18	13	13	11	12
3	17	12	15	8	14
4	27	15	12	8	12
5	19	12	10	9	12
6	18	18	9	9	14
7	18	16	10	9	10
8	12	12	10	10	11
9	15	15	9	11	13
10	18	12	10	9	13
$\bar{x}$	17,6	13,7	11	9,5	12,4
SD±	3,97	2,16	1,94	1,17	1,26

Table 6. Amount of albumen in whey

ordinal number of trial Alb/ (g/L)	C 1	C 2	C 3	C4	C 5
1	2	1,5	1,4	2	1,9
2	2,2	2	1,5	1,3	1,8
3	4,7	1,3	1,6	1,1	2
4	3	2,1	2	1,3	2,1
5	2,5	1,4	2,2	1,5	2,3
6	2,1	2,3	1,3	1,1	2
7	1,1	2,5	1,1	1,2	2,1
8	2	1,1	1,3	1,1	1,9
9	1,8	1,4	1,5	1,3	1,8
10	2,2	2	1,4	1,5	2
$\bar{x}$	2,36	1,76	1,53	1,34	1,99
SD±	0,95	0,47	0,33	0,27	0,15

If all the average values from the examined parameters are converted into mg/dL, the representation of each of them in the whey can best be seen (Chart 1). Total proteins has the highest amount, K is the most concentrated of the minerals, and Fe has the lowest content.

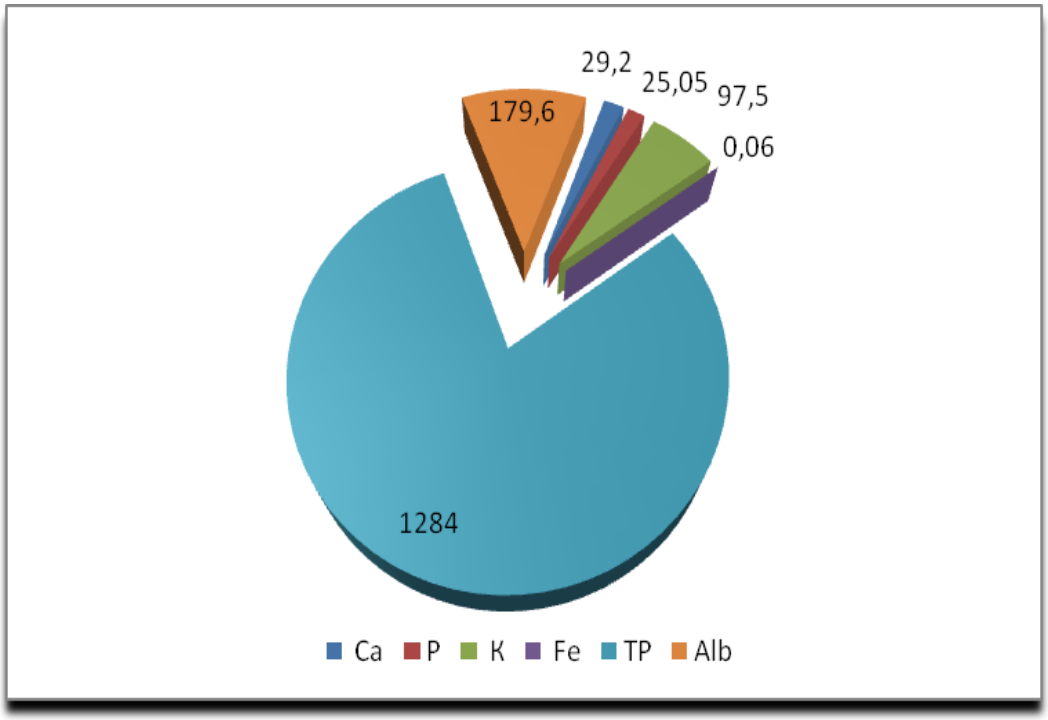

Chart 1. Composition of the applied whey

In the study by González-Weller et al (Dailos Gonzalez-Weller, Soraya Paz-Montelongo, Elena Bathencourt-Barbuzano, 2023 June) the content of Ca is 3811mg/kg, K is 4689 mg/kg and Fe 25,74 mg/kg.

It should be noted that the variability in the results is high for some minerals. However, this is considered normal, as the content of minerals in foods depends on a variety of factors, ranging

from production and processing methods to the environmental conditions of the whey origin (Sánchez O, 2013)

4. Conclusion

From the obtained results we can see that whey has the highest amount of total proteins, it contains 17.6 g/L, from which amount of albumen is 1,99 g/L. Of the minerals, the highest value was obtained for potassium 26,6 mmol/l, followed by calcium 9,47 mmol/l, amount of phosphorus was 9,39 mmol/l, while iron is found in a very small percentage 11,74 µmol/l.
From the research we can conclude that whey contains a large percentage of total proteins, and of the minerals potassium. Whey is not only rich in proteins but is also a source of minerals, that's why whey protein supplements should be considered as relevant dietary sources of minerals. Thus, whey can be used as a dietary supplement for protein and potassium and it is attractive product for both the food and pharmaceutical industries and consumers.

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