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BIOAVAILABILITY OF SOME MACROELEMENTS FROM POST-ULTRAFILTRATION PERMEATES AND WHEY

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The bioavailability of calcium, phosphorus and magnesium from post-ultrafiltration permeates and whey obtained during curd cheese production was determined. The bioavailability of mineral components was expressed with the co-efficients of apparent absorption (A) and retention (R). Dietary studies on rats indicated a high bioavailability of calcium and phosphorus from post-ultrafiltration permeates and whey. Diets containing acid permeate showed significantly higher calcium and phosphorus absorption and retention values. Acid-rennet (ARP) and acid (AP) permeates and acid-rennet whey (ARW) are considered poor sources of bioavailable magnesium. Among the analysed by-products, the acid-rennet whey (ARW) exhibited the most advantageous magnesium apparent absorption and retention values. Acid-rennet whey (ARW), as well as acid-rennet (ARP) and acid (AP) permeates can be used as a valuable component of many food products.

INTRODUCTION

In the dairy industry, as in other branches of the food industry, in addition to the final primary product, a range of by-products is obtained. By-products can often contain valuable nutrients. Whey and post-ultrafiltration permeate are by-products obtained in the production of rennet cheese, curd and protein preparations. They contain considerable amounts of lactose, nitrogen compounds, minerals and vitamins. Direct discharge of whey and permeate to sewage poses a serious ecological threat and does not comply with economic utilisation of all milk components in the food processing.

Milk and dairy products are a valuable source of calcium and phosphorus, in particular. Due to their high assimilability, these elements are required in the human diet. Almost 90% of the calcium, phosphorus and magnesium contained in milk is transferred into whey or permeate after protein coagulation in the production of curd. Thus, the problem of efficient utilisation of the valuable components of whey and permeate obtained, particularly in the production of cottage cheese, is still the focus. The production technology of cottage cheese not only determines the amount of milk components which are transferred into whey and permeate but also determines their nutritive value.

Bioavailability is a key criterion of assimilation of mineral components in the human body. Therefore the aim of this study was to asses the bioavailability of calcium, phosphorus, magnesium from diets with post-ultrafitration permeates and whey.

MATERIAL AND METHODS

The scope of the study included the determination of the content of dry matter, calcium, phosphorus and magnesium in lyophilised permeates (drip) and whey as well as a dietary experiment. The whey used in the study was obtained in the acid-rennet coagulation of protein and the separation of curd in a centrifuge (**SKP**). The experimental permeate was obtained in the acid-rennet coagulation of protein and the separation of curd by ultrafiltration (**PKP**) or acid coagulation of protein and the separation of curd by ultrafiltration (**PK**).

The permeate and whey were dried using the lyophilisation technique in Edward Kniese equipment with a shelf area of 2.77 m^2 and a condenser capacity of 60 kg ice per day. The lyophilisation was carried out in three stages: freezing (at -50°C for 2–3 hours); main drying (at temperature ranges from -50°C to +30°C, vacuum of 0.4–0.5 mbar, for 22–24 hours); further drying (at -0.5–0.6 mbar, at 25°C, for 4–5 hours). The samples were lyophilised to reach 4.5–5% of humidity in the dried material and were stored in tight glass containers at 4–5°C.

A 10-day dietary experiment was carried out (5-day initial period and 5-day proper period). The experiment was based on the balance of a mineral component in an animal alimentary tract and body. The dietary experiment involved 54 standardised Wistar rats (males) with an initial body weight from 54.0 to 58.0 g. The animals were divided into experimental groups (6 individuals per group) so that the individuals from one litter were not represented in the same group.

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During the experiment, the rats were fed *ad libitum* with diets of the following basic composition: protein (fat-free casein) – 13.2% (N×6.38), fat (soybean oil) – 8.0%, vitamin mix – 1.0% [AOAC, 1975], modified mix of mineral salts – 4% [NRC, 1976], potato starch – 5.0%, maize starch as a supplementary component to obtain 100 g of diet dry matter. Insignificant amounts of nitrogen compounds present in the permeates were neglected when balancing protein. The diets were composed in such a way that permeates and whey constituted the sole source of a given mineral in a diet (Tables 1, 2, 3, 4). The dietary components were thoroughly mixed, first manually and then using an electrical mill. In order to determine the bioavailability of calcium, magnesium and phosphorus, the left overs, faeces and urine were collected every day during the 5 days of the experiment. After the end of the experimental period, the faeces were collected, dried, cleaned, comminuted and analysed. The bioavailability of mineral elements was expressed as coefficients of apparent absorption (A) and retention (R), which were determined based on their consumption and excretion with faeces and urine according to the following formulae:

TABLE 1. Content of calcium, phosphorus and magnesium in the lyophilisates used in dietary studies (mg/g d.m.).

Specification	Ca	P	Mg
Acid-rennet whey	14.54	11.58	1.52
Acid-rennet permeate	15.51	12.95	1.61
Acid permeate	15.06	12.83	1.59

TABLE 2. Composition of experimental diets used in a calcium bioavailability study (g/100 g d.m.diet)

Component	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Casein	14.04	14.04	14.04
Test	38.51	36.10	37.18
Soybean oil	8.00	8.00	8.00
Vitamin mix	1.00	1.00	1.00
Mineral component mix	4.00*	4.00*	4.00*
Potato starch	5.00	5.00	5.00
Maize starch	29.45	31.86	30.78

* calcium-free mineral salt mix (g/100 g) [NRC USA, 1976]: K_2HPO_4 – 8.1, K_2SO_4 – 6.8, NaCl – 3.06, Na_2HPO_4 – 2.14, MgO – 2.5, microelements – 1.8, saccharose – 75.6

TABLE 3. Composition of experimental diets used in a phosphorus bioavailability study (g/100 g d.m. diet).

Component	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Casein	14.04	14.04	14.04
Test	38.00	33.98	34.29
Soybean oil	8.00	8.00	8.00
Vitamin mix	1.00	1.00	1.00
Mineral component mix	4.00**	4.00**	4.00**
potato starch	5.00	5.00	5.00
Maize starch	29.96	33.98	33.67

** phosphorus-free mineral salt mix (g/100 g) [NRC USA, 1976]: K_2SO_4 – 6.8, NaCl – 3.06, $CaCO_3$ – 2.1, MgO – 2.5, microelements – 1.8, saccharose – 83.74

TABLE 4. Composition of experimental diets used in a magnesium bioavailability study (g/100 g d.m. diet).

Component	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Casein	14.04	14.04	14.04
Test	26.32	24.84	25.16
Soybean oil	8.00	8.00	8.00
Vitamin mix	1.00	1.00	1.00
Mineral component mix	4.00***	4.00***	4.00***
Potato starch	5.00	5.00	5.00
Maize starch	41.64	43.12	42.80

***magnesium-free mineral salt mix (g/100 g) [NRC USA, 1976]: $CaHPO_4$ – 73.5 , K_2HPO_4 – 8.1, K_2SO_4 – 6.8, NaCl – 3.06, $CaCO_3$ – 2.1, Na_2HPO_4 – 2.14, microelements – 1.8, saccharose – 2.5

$$A\ (\text{mg/5 days}) = S_{spoż} - S_k$$
$$A\ (\%) = (S_{spoż} - S_k)/\ S_{spoż} \times 100$$
$$R\ (\text{mg/5 days}) = S_{spoż} - (S_k + S_m)$$
$$R\ (\%) = [S_{spoż} - (S_k + S_m)]/\ S_{spoż} \times 100$$

where: A – apparent absorption, R – apparent retention, $S_{spoż}$ – amount of mineral component consumed, S_k – amount of mineral component excreted with faeces, S_m – amount of mineral component excreted with urine.

The water content was determined according to AOAC [1984]. Moreover, the content of calcium and magnesium was determined in the lyophilisates, diets, faeces and urine after wet mineralisation using the AAS method (flame: acetylene-air) [Whiteside, 1976]. When analysing the total Ca, to eliminate the interference of phosphorus, all samples and standards were added a lanthanum chloride solution to reach 1% concentration of La^{3+} in the solutions analysed. An ASA spectrophotometer, a Unicam 939 Solar equipped with an ADAX data station and background correction combined with cathode lamps were used. The content of phosphorus was determined with the molybdenum technique with a quinol and sodium sulphite [Whiteside, 1976]. Properly diluted 0.1 mol/L solutions of HNO_3 (Suprapur – Merc) standards of BDH with the concentration of 1 mg/mL were used as the experimental standards. The obtained results were analysed statistically using Statistica v.6.0 software.

RESULTS AND DISCUSSION

Meeting the body demand for minerals depends on both their content in the diet and possibilities of their utilisation by an organism – their bioavailability [Brzozowska & Roszkowski, 1998; Kłobukowski, 1999]. Bioavailability of mineral components is defined as a degree in which the consumed nutrient after release from bonds occurring in food may be assimilated in an alimentary tract and utilised in the system [Brzozowska, 1996, 2000].

A somewhat higher calcium consumption was found (Table 5) in the animal group fed a diet supplemented with acid permeate (AP) compared to the groups of rats fed diets supplemented with acid-rennet permeate (ARP) or acid-rennet whey (ARW) (α=0.05). An increase in the consumption of phosphorus (Table 6) accompanied by a decrease in the consumption of magnesium (Table 7) was found in animals fed diets supplemented with acid-rennet whey or acid-rennet permeate. It could be concluded that the higher the phosphorus consumption, the higher its excretion with faeces. The amount of a component excreted with faeces comprises the amount excreted with digestive juices and the amount that is not assimilated from a diet due to exceeding the mucosa absorption capacity, homeostasis mechanisms, and the presence of diet components hindering or facilitating absorption [Brzozowska, 2000].

The total amount of the mineral components analysed was determined in faeces which allowed for calculating the apparent absorption, understood as a component balance within an alimentary tract. The calcium apparent absorption (expressed per rat during 5 days) was the highest (235.9 mg) for the diet supplemented with AC. It was 216.3 mg for the diet with ARP and 214.9 mg for the diet with ARW (Table 5). The value of the calcium apparent absorption coefficient reached similar levels for diets supplemented with acid-rennet whey or acid-rennet permeate (64.2% and 65.3%, respectively). Statistically significantly (α=0.05) higher calcium apparent absorption was recorded in animals fed diets supplemented with acid permeate (68.2%). Rats fed diets supplemented with acid permeate retain greater amounts of calcium in the body in comparison with the group fed diets with acid-rennet permeate (Table 5).

No statistically significant (α=0.05) differences were found in the values of phosphorus apparent absorption and retention expressed in mg/5 days between animal groups fed diets with acid-rennet whey and acid-rennet permeate (Table 6). Significantly lower (α=0.05) values of both coefficients expressed in mg/5 days were observed for the animal groups fed diets with acid permeate. The phosphorus apparent absorption coefficient (expressed in percent) reached similar levels for diets supplemented with acid-rennet whey and acid-rennet permeate (70.4% and 71.2%, respectively). Statistically significantly (α=0.05) higher apparent absorption of phosphorus was observed in animals

TABLE 5. Mean values of dietary indices for calcium obtained in the experiment (n=6).

Specification	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Diet consumption (g/5 days)	60.1±1.4^{A}	58.4±2.8^{A}	61.5±1.6^{A}
Mineral component consumption (mg/5 days)	334.4±7.9^{A}	331.4±15.9^{B}	345.7±9.0^{C}
Amount of the component excreted with faeces (mg/5 days)	119.5±7.5^{A}	115.1±8.9^{B}	109.8±6.9^{C}
Amount of the component excreted with urine (mg/5 days)	19.5±2.0^{A}	17.5±2.3^{A}	17.2±0.9^{A}
Apparent absorption (mg/5 days)	214.9±12.1^{A}	216.3±12.3^{A}	235.9±15.2^{B}
Absorption (%)	64.2±2.6^{A}	65.3±2.0^{A}	68.2±2.7^{B}
Apparent retention (mg/5 days)	195.3±12.6^{A}	198.8±13.7^{B}	218.7±16.0^{C}
Retention (%)	58.4±2.7^{A}	60.0±2.0^{B}	63.2±3.0^{B}

A, B, C – values in the same line denoted with different capital letters are statistically significantly different (α=0.05); A, A or B, B – values in the same line denoted with the same capital letter are not statistically significantly different (α=0.05)

TABLE 6. Mean values of dietary indices for phosphorus obtained in the experiment (n=6).

Specification	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Diet consumption (g/5 days)	59.3±0.6^{A}	57.5±3.1^{A}	53.2±1.3^{A}
Mineral component consumption (mg/5 days)	285.8±2.8^{A}	287.7±15.7^{A}	258.6±6.5^{B}
Amount of the component excreted with faeces (mg/5 days)	84.6±6.3^{A}	83.0±13.4^{A}	63.3±5.1^{B}
Amount of the component excreted with urine (mg/5 days)	52.0±1.0^{A}	53.0±5.7^{A}	55.8±5.5^{A}
Apparent absorption (mg/5 days)	201.2±7.1^{A}	204.7±10.2^{A}	195.3±10.3^{B}
Absorption (%)	70.4±2.2 ^{A}	71.2±3.6^{A}	75.5±2.4^{B}
Apparent retention (mg/5 days)	149.2±7.5^{A}	151.7±13.8^{A}	139.5±5.9^{B}
Retention (%)	52.2±2.5^{A}	52.7±3.6^{A}	53.9±1.6^{A}

A, B, C – values in the same line denoted with different capital letters are statistically significantly different (α=0.05); A, A or B, B – values in the same line denoted with the same capital letter are not statistically significantly different (α=0.05)

TABLE 7. Mean values of dietary indices for magnesium obtained in the experiment (n=6).

Specification	Acid-rennet whey	Acid-rennet permeate	Acid permeate
Diet consumption (g/5 days)	56.3±1.2^{A}	54.7±1.6^{A}	59.7±3.3^{A}
Mineral component consumption (mg/5 days)	15.8±0.4^{A}	14.6±0.4^{B}	17.5±1.0^{C}
Amount of the component excreted with faeces (mg/5 days)	8.7±0.6^{A}	8.5±0.1^{B}	11.3±0.8^{C}
Amount of the component excreted with urine (mg/5 days)	2.9±0.1^{A}	2.9±0.2^{A}	3.4±0.2^{B}
Apparent absorption (mg/5 days)	7.2±0.4^{A}	6.1±0.5^{B}	6.2±0.3^{B}
Absorption (%)	45.3±2.9^{A}	41.9±2.4^{B}	35.6±1.7^{C}
Apparent retention (mg/5 days)	4.2±0.4^{A}	3.2±0.3^{B}	2.8±0.4^{C}
Retention (%)	26.7±2.9^{A}	22.1±1.4^{B}	16.1±2.2^{C}

A, B, C – values in the same line denoted with different capital letters are statistically significantly different (α=0.05); A, A or B, B – values in the same line denoted with the same capital letter are not statistically significantly different (α=0.05) fed diets supplemented with acid-permeate (75.5%). No significant differences were found in the value of the retention coefficient (expressed in percentage) among all the studied groups of rats. It ranged from 52.2% for diet with acid-rennet whey to 53.9% for diets with acid permeate.

The values of apparent absorption and retention of magnesium (expressed both in mg/5 days and in percentage) were the lowest for animal groups fed diets supplemented with acid permeate (Table 7). It could be stated that the higher consumption of magnesium caused a higher excretion of this element with faeces and urine, thus a decrease in its absorption and retention.

The amount of mineral components consumed with a diet is a very important factor affecting component bioavailability. Based on studies completed by other authors, calcium content of a diet and the volume of its consumption or an interaction of these two factors had a significant effect on the apparent absorption of this element in rats [Schaafsma, 1997; Buchowski & Miller, 1991]. It was shown that a 10-fold increase in the amount of calcium in a diet caused a 2–3-fold decrease in the percentage values o apparent absorption coefficients of this mineral. The differences between the values of calcium absorption in the physiological conditions result, to a greater extent, from differences in the volume of consumption of this element than from the type of its source. The physicochemical form in which this element is introduced into the human or animal body significantly determines the utilisation of calcium in an organism. Soluble and ionised forms are considered to be the most available. Better assimilation of calcium has been observed from a mix of organic salts [Miller *et al*., 1988]. On the other hand, other authors did not find any differences in the intestinal assimilation of calcium salts with different solubility in humans and rats [Goddart *et al*., 1996; Greger, 1987]. Mean calcium absorption from a diet ranges from 30 to 40% and increases to 75% during intensive growth, pregnancy, lactation and with deficiencies in a body, while it decreases in the elderly especially with an insufficient supply of vitamin D in the system [Pasternak, 1998; Pisarek-Miedzińska, 1998].

An increase in milk acidity (decrease in pH) causes the transformation of calcium colloidal forms into soluble compounds occurring mainly in an ionic form. With an active acidity of pH 5, almost all calcium contained in milk is transformed into soluble compounds [Pijanowski & Zmarlicki, 1980; Ziajka *et al*., 1997]. It could be stated that the lower the permeate pH, the higher the degree of calcium ionisation, and its bioavailability is likely to be higher. Calcium assimilability is also determined by the content of other salts in a diet. The consumption of sodium and potassium salts as well as sulphates, carbonates and hydrocarbons of these elements was found to significantly effect an increase in calcium excretion with urine [Kaup & Greger, 1990]. Lutz [1984] showed that the consumption of sodium carbonate with a high content of protein in a diet decreases calcium losses in urine by 32%.

Milk components have a considerable effect on the level of apparent absorption of calcium and magnesium. Based on the studies, the content of milk components has a greater effect on calcium absorption than the type of food product it originates from [Toba *et al*., 1999a, b]. The examined permeates supplemented to the experimental diets contained considerable amounts of lactose, which could have a significant effect on the values of bioavailability coefficients of calcium, magnesium and phosphorus. Other authors [Buchowski & Miller, 1991; Schaafsma, 1988, 1997; Toba *et al*., 1999a, b] showed an increase in the calcium assimilability with the presence of lactose. Lactose interacts with chorion and enhances the permeability of the intestinal mucous membrane by modifying the polarity [Miller *et al*., 1988; Schaafsma, 1991]. Greger *et al*. [1989] in their studies on the effect of lactose on the bioavailability of elements obtained 72% of the value of calcium apparent absorption from milk, 83% from a casein diet supplemented with $CaHPO_4$ and lactose and 69% from a casein diet supplemented with dolomite and lactose. In the same studies, the values of magnesium apparent absorption ranged from 81 to 84% with a significantly lower magnesium absorption (74%) for diets supplemented with hydrolysed lactose. The present study into the bioavailability of mineral elements from acid whey and permeates does not allow for the determination of the lactose effect on their assimilability because in all the products examined, this sugar occurred in similar concentrations. Moreover, the effect of lactose-free control diet was not included in the analyses.

High calcium consumption with diets can also decrease the intestinal assimilability of magnesium. According to the National Research Council (NRC) [1976], calcium demand of young, growing rats is approximately 12-fold higher than the demand for magnesium. This factor could have an effect on the values of magnesium apparent absorption obtained in this study, because rats consumed approx. 21–25-fold more calcium than magnesium (Tables 5, 7).

According to Greger [1989], a higher consumption of protein has a beneficial effect on calcium assimilation due to the formation of easily soluble bonds between calcium and peptides and amino acids in the intestinal lumen. The effect of lysine and arginine is particularly significant. These amino acids stimulate intestinal assimilation of calcium, whereas tryptophan, leucine and aspargic acid affect calcium assimilation to a smaller degree.

Phosphorus assimilation from diets increases along with an increase in its contents in a diet and can reach 3 g of phosphorus assimilated per day. Phosphorus assimilation from a diet is determined, to a large degree, by bonds of this element with other compounds. It was found that assimilation of phosphorus associated with phytates is very low due to a low degree of hydrolysis of this complex in the alimentary tract. On the other hand, inorganic phosphates occurring in food are easily hydrolysed in the alimentary tract. Parathormon and vitamin D have an effect on the assimilation and excretion of phosphorus similar to their effect in calcium regulation in the system [Brzozowska, 2000; Kaup & Greger, 1990].

To a considerable degree, phosphorus decreases calcium excretion with urine. Due to the association of phosphorus and calcium metabolism, it is necessary that diets comply with the relevant content ratio of these elements. In milk, the Ca:P ratio is 1.2:1 and is highly desirable for humans [Pijanowski & Zmarlicki, 1980; Rutkowska & Kunachowicz, 1994; Starzyński & Masłowska, 1995]. The examined permeates and whey were characterised by similar proportions of these elements to those contained in milk.

CONCLUSIONS

1. Dietary studies on rats showed a high bioavailability of calcium and phosphorus from post-ultrafiltration permeates and whey. Significantly higher values of apparent absorption and retention coefficients of calcium and phosphorus were recorded for diets supplemented with acid permeate.

2. Acid-rennet (ARP) and acid (AP) permeate and acid--rennet whey (ARW) are not a rich source of bioavailable magnesium. The most desirable values of the apparent absorption and retention coefficients of this element were exhibited by acid-rennet whey (ARW).

3. Acid-rennet whey (ARW) as acid-rennet (ARP) and acid permeate can be used as a valuable component of many food products such as juices and jellies.

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BIODOSTĘPNOŚĆ WYBRANYCH MAKROELEMENTÓW Z PERMEATÓW POULTRAFILTRACYJNYCH I SERWATKI

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Celem podjętych badań było określenie biodostępności wapnia, fosforu, magnezu z permeatów poultrafiltracyjnych oraz serwatki otrzymanych podczas produkcji serów twarogowych. Biodostępność składników mineralnych oznaczono metodą bilansową z wykorzystaniem szczurów i wyrażono za pomocą współczynników absorpcji (A) i retencji pozornej (R). Na podstawie przeprowadzonego badania żywieniowego z udziałem zwierząt, stwierdzono, że wartość współczynnika absorpcji pozornej wapnia kształtowała się na zbliżonym poziomie dla diet z dodatkiem serwatki kwasowo-podpuszczkowej (SKP) i permeatu kwasowo-podpuszczkowego (PKP, odpowiednio 64,2% i 65,3%) – tab. 5. Zanotowano natomiast istotnie wyższą absorpcję wapnia u szczurów żywionych dietą zawierającą permeat kwasowy (PK) – 68,2% (tab. 5). Podobne zależności zaobserwowano w przypadku współczynnika retencji pozornej wapnia (tab. 5). Stwierdzono istotnie wyższą absorpcję pozorną fosforu u zwierząt żywionych dietą z dodatkiem permeatu kwasowego – 75,5%, w porównaniu z dietami zawierającymi serwatkę kwasowo-podpuszczkową (70,4%) i permeat kwasowo-podpuszczkowy (71,2%) – tab. 6. Zaobserwowano, że wartości absorpcji oraz retencji pozornej magnezu, wyrażone zarówno w mg/5 dni, jak i w jednostkach procentowych były najniższe w przypadku grup zwierząt spożywających dietę z dodatkiem permeatu kwasowego – tab. 7.

Badania żywieniowe na szczurach wykazały wysoką biodostępność wapnia, fosforu z permeatów poultrafiltracyjnych oraz serwatki. Istotnie wyższą wartość absorpcji i retencji wapnia oraz fosforu charakteryzowały się diety z udziałem permeatu kwasowego. Permeaty kwasowo-podpuszczkowy (PKP) i kwasowy (PK) oraz serwatka kwasowo-podpuszczkowa (SKP) są niezbyt bogatym źródłem biodostępnego magnezu. Najkorzystniejsze wartości współczynników absorpcji i retencji pozornej tego pierwiastka wykazywała serwatka SKP. Serwatka kwasowo-podpuszczkowa (SKP), jak i permeaty kwasowo-podpuszczkowy (PKP) oraz kwasowy (PK) mogą być wykorzystywane jako wartościowy składnik wielu produktów spożywczych np. soków lub galaretek.