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MINERAL COMPOSITION AND BIOAVAILABILITY OF CALCIUM AND PHOSPHORUS FROM ACID WHEY CONCENTRATED BY VARIOUS MEMBRANE PROCESSES*

Maria Soral-Śmietana[1], Zenon Zduńczyk[1], Małgorzata Wronkowska[1], Jerzy Juśkiewicz[1], Lidia Zander[2]

[1]Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences
[2]University of Warmia and Mazury in Olsztyn

Abstract

This study has been undertaken to investigate the effect of different membrane separation processes (nanofiltration, nanofiltration with diafiltration, and ultrafiltration) on the content of macro- and microelements in acid whey from tvarog cheese (white cheese) production and on bioavailability of calcium and phosphorus in rats' diets with 20 or 40% content of spray dried whey. The use of nanofiltration and ultrafiltration processes in whey concentration did not cause differences in the content of Ca, Mg, P, Fe, nor Zn in the end product. Compared to nanofiltration and ultrafiltration, the introduction of diafiltration to the nanofiltration process was observed to reduce the content of Ca from over 14 to less than 10 mg g^{-1}, and that of phosphorus from 8.3 and 7.4 to 5.9 mg g^{-1}, although the biggest reduction was noted in the content of monovalent Na and K. The changes in the mineral composition of whey did not affect coefficients of Ca bioavailability in diets for rats. By substituting Ca in a standard mineral mixture with a 20% addition of whey concentrate, coefficients of the apparent absorption of this element were increased from 36.4% to 42.8-44.6%, and coefficients of its apparent retention from 33% to 38.5-41.7%. The 40% addition of whey concentrates lowered Ca bioavailability coefficients compared to the diets with the 20% whey concentrate content, but not in respect of the control diet. The application of nanofiltration with diafiltration for whey concentration deteriorated phosphorus ab-

prof. dr hab. Maria Soral-Śmietana, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, 10 Tuwima Str., 10-747 Olsztyn, Poland

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sorption from the diet with 40% of whey, which was indicated by decreased values of the apparent retention coefficient and absolute retention of P in the body of rats within 5 days, i.e. from 35.2 to 18.5% and from 98.8 to 64.5 mg, respectively, compared to the control diet.

Key words: acid whey, calcium, phosphorus, bioavailability.

SKŁAD MINERALNY I BIODOSTĘPNOŚĆ WAPNIA I FOSFORU Z SERWATKI KWASOWEJ KONCENTROWANEJ PRZEZ SEPARACJĘ MEMBRANOWĄ

Abstrakt

W pracy analizowano wpływ różnych procesów separacji membranowej (nanofiltracji, nanofiltracji z diafiltracją i ultrafiltracji) na zawartość makro- i mikroelementów w kwasowej serwatce potwarogowej oraz biodostępność wapnia i fosforu w dietach szczurów z 20 lub 40% zawartością suszonej serwatki. Zastosowanie nanoflitracji i ultrafiltracji w procesie zagęszczania serwatki nie zróżnicowało zawartości Ca, Mg, P, Fe i Zn w produkcie końcowym. W porównaniu z nanofiltracją i ultrafiltracją, zastosowanie diafiltracji przy nanofiltracji zredukowało zawartość Ca z ponad 14 do poniżej 10 mg g^{-1}, a fosforu z 8.3 i 7.4 do 5.9 mg g^{-1}, a najbardziej zmalała zawartość jednowartościowych Na i K. Odnotowane zmiany w składzie mineralnym serwatki nie wpłynęły na wskaźniki biodostępności Ca w dietach szczurów. Zastąpienie Ca ze standardowej mieszanki mineralnej 20% dodatkiem koncentratu serwatki kwasowej wpłynęło na zwiększenie współczynników absorpcji pozornej tego pierwiastka z 36.4% do 42.8-44.6%, a współczynnika retencji pozornej z 33% do 38.5--41.7%. Dodatek 40% pogorszył wskaźniki biodostępności Ca w stosunku do diet z 20% udziałem koncentratu serwatki, jednakże nie w stosunku do diety kontrolnej. Zastosowanie nanoflitracji z diafiltracją do zagęszczania serwatki pogorszyło wykorzystanie fosforu z diety zawierającej 40% serwatki, skutkujące obniżeniem współczynnika retencji pozornej z 35.2 do 18.5% oraz bezwzględnej retencji P w ciele szczurów z 98.8 do 64.5 mg w ciągu 5 dni, w stosunku do diety kontrolnej.

Słowa kluczowe: serwatka kwasowa, wapń, fosfor, biodostępność.

INTRODUCTION

Processed acid whey subjected may become a food component providing low-molecular milk proteins as well as macro- and microelements to diet. Concentration of whey through membrane filtration and dehydration facilitates the use of its valuable ingredients, thus improving nutritive and sensory properties of food products, including bread. Supplementation of wheat and wheat-rye bread with a dry whey commercial concentrate increases concentrations of minerals as well as the content and biological value of protein (WRONKOWSKA et al. 2012). Enrichment of food products with calcium is especially desirable, although the bioavailability of this element from dairy products is very high (GREGER et al. 1987).

Recently, valuable components have been separated from highly hydrated materials with the use of membrane techniques. The concentration process affects the content and ratios between mineral components. The biggest changes in the composition of whey solids occur after separation processes which employ ultra- and nanofiltration membranes. Ultrafiltration, usually performed at 20-25 kDa cut-off value, is used in order to increase the concentration of whey proteins while simultaneously reducing lactose and the content of mineral compounds (YORGUN et al. 2008, DUSHKOVA, DINKOV 2009). Therefore, ultrafiltrated whey concentrates contain an increased amount of proteins of high nutritive value (SMITHERS 2008). Nanofiltration enables partial demineralization of whey and removal of both salt and lactic acid from acid whey (NGUYEN et al. 2003, SUÁREZ et al. 2006). This process improves the composition of whey concentrate by partial removal of monovalent ions responsible for salty taste and low nutritive value. Depending on the molecular cut-off value of a membrane, the pH of whey as well as its processing characteristics, the total content of minerals in whey solids can be reduced by 30-70% (RICE et al. 2005, SUÁREZ et al. 2006). Nanofiltrated whey concentrate contains an increased amount of Ca^{2+}, Mg^{2+} and phosphorus, whereas the concentration of monovalent ions like in NaCl, which negatively affect the quality of food products, is reduced (NGUYEN et al. 2003). This effect is enhanced if nanofiltration is combined with diafiltration, but in this case some lactose is lost due to dilution (KELLY, KELLY 1995, SUÁREZ et al. 2006). Nonetheless, little information is available on effects of particular membrane techniques on the mineral composition of whey and bioavailability of the most important components, i.e. bioelements.

In view of the above, the objective of this study has been to determine the effect of different processes of membrane separation (nanofiltration, nanofiltration with diafiltration, and ultrafiltration) on the content of macro- and microelements in experimental concentrates of acid whey from tvarog cheese (white cheese) production and on the bioavailability of elements vital for health, including calcium and phosphorus.

MATERIAL AND METHODS

Material

Whey after lactic fermentation and acidic coagulation of milk proteins during tvarog manufacture was used as raw material for production of liquid concentrate with a high content of non-denaturized proteins. Physical membrane separation methods were used: nanofiltration (N), nanofiltration with diafiltration (ND), ultrafiltration (U). The retentates were dehydrated by spray drying (A/S Niro Atomizer Type P-6.3, Denmark) at the inlet/outlet temp. of 190/86°C. Powdered dried concentrates were used in this study.

Membrane separation processes

Acid whey (pH 4.4) was cooled to 5°C immediately after production. Membrane separation processes were performed using a pilot scale system with 18 tubular membranes in series forming a separation area of 0.85 m^2. The flow rate through the module was maintained from 26.1 L min^{-1} to 34.8 L min^{-1} depending on the membrane type and pressure values. An AFC 30 (PCI) membrane capable to retain 75% $CaCl_2$ was used in the nanofiltration (N) and nanofiltration with diafiltration (ND) experiments. Each process was carried out in a batch mode under the pressure of 1.7-3.2 MPa, achieving the concentration of total solids in the retentate of approximately 18-20%. In the ND separations, the retentate from regular N concentration was diluted with demineralized water added in the amount equal to the volume of extracted permeate. The diluted solution was then concentrated in a replicated N process. A EM006 type membrane (6 kDa cut-off) was applied in ultrafiltration (U) experiments. The process was carried out at the pressure of 1.21-1.32 MPa. The resulting retentates were used as the material for further experiments.

Analysis of the content of elements

The content of elements was determined using the atomic absorption spectroscopy (AAS) method. Samples were wet-mineralized in a mixture of nitric and perchloric acids (3:1). Potassium was assayed with the photometric flame method and phosphorus was investigated with the colorimetric molybdate method with hydroquinone and sodium sulphate (IV). For the validation of calcium determinations, solution of lanthanum chloride was added to all samples in the amounts providing 0.5% concentration of La^{3+}.

Evaluation of Ca and P bioavailability

The bioavailability of Ca and P, indicated by coefficients of apparent absorption and retention, was analyzed using 48 Wistar rats from a 4-week experiment that evaluated physiological effects of diets with 20% or 40% content of acid whey concentrates. In a balance experiment, each group included 8 rats kept in individual cages. The body weight of the rats reached 320 g. The diet for rats was a modified standard AIN-93 diet (Reeves 1997) (Table 1). In order to balance the Ca content in diets in respect of the Ca content in whey concentrates, the quantity of this element was regulated by adjusting the content of the mineral mix. In the diets with 20% of whey concentrates, the content of the mineral mix was ca 5 g kg, and in those containing 40% of whey concentrates, the added mix dose was higher (Table 1). The content of P in the control diet was 2 g kg^{-1}, while in the experimental diets it was increased through addition of whey concentrates.

In the fourth week of the experiment, a daily collection of faeces and urine was continued for 5 days from rats kept in balance cages. The collect-

Table 1

Composition of experimental diets containing spray dried acid whey concentrates after nanofiltration (N), nanofiltration with diafiltration (ND) and ultrafiltration (U)

Component (%)	Diet						
	C	N_{20}	ND_{20}	U_{20}	N_{40}	ND_{40}	U_{40}
Casein	20.0	17.6	17.4	15.8	15.2	14.8	11.6
DL-methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Saccharose	10.0	-	-	-	-	-	-
Acid whey	-	20	20	20	40	40	40
Soybean oil	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Mineral mix	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Maize starch	52.2	44.6	44.8	46.4	27.0	27.4	30.6
Ca source (g k^{-1})							
Mineral mix	5	2.94	1.97	2.98	5.87	3.93	5.77
Acid whey	-	2	3	2	-	1.0	-
Total	5.0	4.94	4.97	4.98	5.87	4. 93	5.77

ed samples were averaged and determined for the content of Ca and P by calculating quantities of these elements in diets and in excreted faeces for 5 days. The coefficient of apparent absorption was calculated as the difference between intake of minerals and their quantity excreted with faeces. The coefficient of apparent retention was calculated as the difference between the quantity of minerals absorbed (digested) with a diet and excreted with urine and expressed in absolute (as mg/5 days^{-1}) or relative (%) values.

Statistical analysis

Results of the physiological response of the treated animals are expressed as means and pooled standard error (SEM). Statistical comparisons were done transversely among different dietary groups. Data were analyzed by one-way ANOVA with one factor (diet) or by two-way ANOVA with two factors (diet and dose). If significance was observed ($P<0.05$), Duncan's multiple range test was used to identify differences in the effect of individual diets. Calculations were made with Statistica 6.0 software (StatSoft Corporation, Kraków, Poland).

RESULTS AND DISCUSSION

The mineral composition of the acid whey concentrates produced experimentally differed depending on the applied method of membrane filtration applied prior to the dehydration of the product (Table 2). Similar results of the analyzed micro- and macroelements were achieved using nanofiltration and ultrafiltration. Significantly lower concentrations of most of the elements were noticed in the acid whey concentrate obtained by the coupled process of nanofiltration and diafiltration. At a lower content of Ca and P in this product (ND), the quantitative ratio between these elements was 1.7:1. In turn, in the concentrate after nanofiltration (N), this ration was 1.8:1, whereas in the concentrate after ultrafiltration (U) it was close to 2.0:1 (Table 2). The coupling of diafiltration with nanofiltration caused a decrease in the content of monovalent elements (Na, K), and in that of the microelement Zn. Such an effect of diafiltration used for acid whey concentration was also reported by ROMAN et al. (2009, 2012).

Table 2

Mineral composition of the spray dried acid whey

	Type of spray dried acid whey		
	N	ND	U
Ca (mg g^{-1})	14.68	9.83	14.42
P (mg g^{-1})	8.25	5.86	7.38
Na (mg g^{-1})	2.36	0.64	3.06
K (mg g^{-1})	8.16	2.48	9.17
Mg (mg g^{-1})	1.58	1.16	1.42
Zn (μg g^{-1})	51.2	40.9	47.8
Fe (μg g^{-1})	23.2	25.7	20.9

Explanations see Table 1

Bioavailability of dietary supplements, including minerals, can be defined as the proportion of the administered substance capable of being absorbed and available for use or storage (SRINIVASAN 2001). The bioavailability of calcium from foods, including dairy products, is influenced by many factors, including its intake and content of other dietary components, which either facilitate or inhibit transport through intestinal walls (GUEGUEN, POINTILLART 2000).

During the balance test, an unexpected decrease was observed in the intake of some diets (U_{20} and ND_{40}), which resulted in a significantly lower Ca intake when compared to the control group (Table 3). Simultaneously, differences were noted in Ca excretion with faeces proportionally to its in-

Table 3

Calcium balance in an in vivo experiment

Group	Ca intake (mg/5 days)	Ca fecal (mg/5 days)	Ca urinal (mg/5 days)	Ca absorption		Ca retention	
				(mg)	(%)	(mg)	(%)
Control	438.5	278.9	14.83	159.6	36.4	144.8	33.0
2-way Anova							
N20	429.8^{ab}	243.0	13.23	186.8*	43.9*	171.6*	40.9*
ND20	417.0^{ab}	238.5*	17.85	178.5*	42.8*	160.6*	38.5*
U20	378.3^{b}*	209.8*	11.17	168.5*	44.6*	157.3*	41.7*
N40	463.4^{a}	259.4	21.99*	204.0*	44.3*	180.0*	39.5*
ND40	381.7^{b}*	215.3*	29.60*	166.4*	43.4*	136.8*	35.7
U40	442.6^{a}	260.6	22.18*	182.0*	40.8	159.8*	35.8
SEM	8.817	6.446	1.568	4.626	0.797	4.602	0.890
Acid whey (W)							
N	446.6^{a}	251.2	17.61^{b}	195.4	44.1	177.8^{a}	40.2
ND	399.4^{b}	226.9	23.72^{a}	172.5	43.1	148.8^{b}	37.1
U	410.5_{b}	235.2	16.68^{b}	175.3	42.7	158.6^{ab}	38.8
P values	0.039	0.245	0.046	0.089	0.780	0.028	0.343
Dosage (D)							
20	408.4	230.4	14.08^{b}	178.0	43.8	163.9	40.4
40	429.2	245.1	24.59^{a}	184.1	42.8	159.5	37.0
P values	0.170	0.222	<0.001	0.488	0.591	0.609	0.061
P (W × D)	0.032	0.052	0.865	0.347	0.483	0.258	0.566

SEM – Standard error of the mean (SD for all rats divided by square root of rat number, $n = 48$);
^{a,b} Data with different superscripts in the same column differ significantly at $P<0.05$ (two-way ANOVA followed by Duncan's multiple range test);
*Data significantly different from the control group at $P<0.05$ (*t*-test procedure).

take, but excretion with urine was different. In all the groups of rats receiving diets with whey concentrates, the absorption of Ca from the gastrointestinal tract was higher than in the control group. The difference appeared in the quantity of absorbed Ca and/or value of apparent absorption coefficient. The two-factor analysis demonstrated alike coefficients of Ca absorption from the diet, irrespective of the dose/type of the applied whey concentrate. The inclusion of whey concentrates to diets affected a significant increase in coefficients of Ca retention in rat bodies against the control, especially at dose 20%. The two-factor statistical analysis demonstrated that the application of the studied whey concentrates in the diet had a similar effect on Ca retention coefficients. Additionally, a higher content of Ca in diets (40%), reduced apparent retention to an extent close to statistical significance (p=0.061). This indicates that the 40% dose of the analyzed concentrates does not yield a beneficial effect in Ca bioavailability.

The present experimental results corresponded to findings of other authors, who demonstrated that calcium bioavailability from organic sources, milk in particular, was superior to that of mineral calcium (RANHOTRA et al. 1997, TOBA et al. 1999). Some reports also showed that an increasing content of Ca in a diet was accompanied by its reduced retention (BUCHOWSKI, MILLER 1991, SCHAAFSMA 1997). Such a tendency occurred in our experiment when the rats were fed a diet with a 40% content of N and U concentrates, and the content of calcium exceeded the level of 5 g kg^{-1} recommended in standard diets for rats (REEVES 1997). In the reported experiment, coefficients of apparent absorption and apparent retention of Ca accounting for 40.8--44.6% and 35.7-41.7%, respectively, were high. Their values corresponded with results of other experiments with similar content and sources of Ca in diets (HOWE, BEECHER 1981, CAMPOS et al. 1998, KŁOBUKOWSKI et al. 2006).

The intake of phosphorus was significantly higher in the groups of animals fed the diets containing whey concentrates than in the control group (Table 4). This was due to an additional source of P in respect of the P level in the standard mineral mix (Table 1). Simultaneously, it was demonstrated that P excretion with faeces was significantly higher and, to a large extent, proportional to its content in the diet. The increased content of P in diets as a result of whey concentrate supplementation was the reason for a significant increase in phosphorus absorption from the gastrointestinal tract and in coefficients of apparent phosphorus digestibility. The significance of differences in absorption coefficients versus the control group was not confirmed for all the treatments except 40% whey supplementation in the ND and U groups. Apart from the ND_{40} group, absolute increase was noted in the content of phosphorus retained in the rat's body, yet the coefficient of its apparent retention was observed to decrease. Results of the two-factor statistical analysis demonstrated that the coefficients of apparent digestibility of P were alike, irrespective of differences in its intake and the applied membrane separation method. No statistically significant differences were noted in the value of phosphorus apparent retention coefficient as affected by the method of whey concentrate production.

Dairy products are claimed to be a rich source of easily available phosphorus and to assure an optimal ratio between calcium and phosphorus, taking into account demands of human and animal bodies (TSUCHITA et al. 1995). Hence, in the present study, despite a higher level of phosphorus in diets owing to added whey concentrate, the coefficients of apparent digestibility of P were high and ranged from 57 to 60%, compared to the value of 54% achieved in the control diet. In contrast, significantly lower values were noted for coefficients of apparent retention of P from diets containing whey

Table 4

Phosphorus balance in an *in vivo* experiment

Group	P intake (mg/5 days)	P fecal (mg/5 days)	P urinal (mg/5 days)	P absorption		P retention	
				(mg)	(%)	(mg)	(%)
Control	227.6	105.5	42.29	122.1	53.7	79.8	35.2
2-way Anova							
N20	347.1*	139.6*	118.5*	207.5*	60.1*	89.0*	25.9*
ND20	312.8*	124.8*	103.2*	188.0*	59.9*	84.4*	27.0
U20	296.7*	117.9	94.25*	178.8	60.3*	84.5*	28.5
N40	439.8*	176.9*	162.2*	262.9*	60.0*	100.7*	23.0*
ND40	344.3*	148.7*	131.1*	195.6*	56.6	64.5*	18.5*
U40	375.1*	159.3*	127.8*	215.8*	57.3	88.0*	23.2*
SEM	10.250	5.206	5.001	6.733	0.827	4.289	1.181
Acid whey (W)							
N	393.5^{a}	158.2	140.4^{a}	235.3^{a}	60.1	96.9	24.5
ND	328.5^{b}	136.7	117.2^{b}	191.8^{b}	58.3	74.6	22.7
U	335.9^{b}	138.6	111.0^{b}	197.3^{b}	58.8	86.3	25.8
P values	<0.001	0.072	0.003	0.002	0.682	0.16	0.528
Dosage (D)							
20	318.9^{b}	127.4^{b}	105.3^{b}	191.5^{b}	60.1	86.2	27.1a
40	386.4^{a}	161.6*a*	140.4^{a}	224.8^{a}	58.0	84.4	21.6b
P values	0.000	0.000	0.000	0.002	0.221	0.836	0.019
P (W × D)	0.122	0.651	0.602	0.252	0.700	0.287	0.603

SEM – Standard error of the mean (SD for all rats divided by square root of rat number, n = 48);
^{a,b} Data with different superscripts in the same column differ significantly at $P<0.05$ (two-way ANOVA followed by Duncan's multiple range test);
*Data significantly different from the control group at $P<0.05$ (*t*-test procedure).

concentrates (18.5-28.5 vs 35.2%), which resulted from a higher supply of P with these diets. A similar tendency was observed by Campos et al. (1998), who reported that despite a larger uptake of phosphorus at the intestinal level, the phosphorus balance decreased as a result of increased urinary excretion. The same tendency was signalled in the case of calcium, showing that an increasing Ca content in a diet resulted in its diminished uptake (Buchowski, Miller 1991, Schaafsma 1997). The absolute retention of P from the diets containing whey concentrates, except for ND_{40} group, was comparable or even higher than in the control group. The inferior phosphorus retention from the concentrate after nanofiltration coupled with diafiltration was probably due to the diminishing amount of monovalent elements, sodium and potassium, in the end product. Elevated intake of both of these elements in a diet may increase urinal excretion of other elements, in particular calcium (Kaup, Gregor 1990). In the present study, the decrease in the Na and K content had no beneficial effect on the bioavailability of the analyzed elements. This could have resulted from the fact that the content of P in the diets containing whey concentrates exceeded the value of 3 g kg^{-1}, which is an accepted standard value of nutritional demands of young rats (Reeves 1997).

CONCLUSION

1. The application of nanofiltration and ultrafiltration processes did not cause differences in the mineral composition of acid whey concentrates, whereas the application of nanofiltration with diafiltration reduced contents of Ca and as well as these of Na and K in the finished product, compared to the concentrate after nanofiltration and ultrafiltration.

2. Differences in the chemical composition of acid whey concentrates had no effect on the coefficients of calcium bioavailability in diets for rats, but lowered phosphorus absorption from the acid whey concentrate produced by nanofiltration with diafiltration.

3. Supplementation of rats' diets with dry acid whey concentrates at a level of 40% led to inferior coefficients of the Ca and P bioavailability, mostly due to the increased intake of these elements in excess of the nutritional demands of the rats.

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