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CALCIUM BIOAVAILABILITY FROM DAIRY PRODUCTS AND ITS RELEASE FROM FOOD BY *IN VITRO* DIGESTION

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Abstract

Food products are an exclusive source of nutrients necessary for the development and proper functioning of human organism. The key issue in nutrition is the bioavailability of nutrients rather than the supply of their adequate amounts in a diet. Calcium is the basic structural element of bones and teeth. It is also an element with a wide range of physiological functions, e.g. it is responsible for the right excitability of muscular and nervous system, normal heart function, hormonal secretion, blood clotting, cell membrane permeability and activation of various enzymes. It is universally acknowledged that the main source of calcium for a human organism is milk and dairy products, owing to their rich content and high availability of calcium. However, certain stages in food processing technologies may depress the high bioavailability of dietary nutrients.

In this paper, long-term research findings about the bioavailability of calcium in milk products have been summarized, in addition to the information about calcium uptake from bread or other popular breakfast meals composed of milk products in combination with cereal component. The popular opinion that milk and other dairy products are the best and most valuable source of highly available calcium has indeed been supported. However, an *in vitro* study demonstrates that an addition of cereal to milk or yoghurt significantly diminishes the quantity of calcium released during digestion, which could contribute to the development of calcium insufficiency or augmentation of its symptoms.

Keywords: milk, dairy products; bread, cereals, calcium deficiency.

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BIODOSTĘPNOŚĆ WAPNIA Z PRODUKTÓW MLECZNYCH I JEGO UWALNIANIE Z ŻYWNOŚCI PODCZAS TRAWIENIA IN VITRO

Abstrakt

Pożywienie jest jedynym źródłem składników odżywczych niezbędnych do rozwoju i prawidłowego funkcjonowania organizmu człowieka. Kluczowym zagadnieniem w żywieniu jest dostarczenie odpowiedniej ilości składnika odżywczego, w związku z czym duże znaczenie ma biodostępność z diety. Wapń to podstawowy składnik budulcowy kości i zębów, również pierwiastek o bardzo szerokim znaczeniu fizjologicznym, odpowiada bowiem za prawidłową pobudliwość układu mięśniowo-nerwowego, właściwą pracę serca, sekrecję hormonalną, krzepnięcie krwi, przepuszczalność przez błonę komórkową oraz aktywację wielu enzymów. Powszechnie przyjęte jest, że głównym źródłem wapnia dla organizmu człowieka jest mleko i nabiał zarówno ze względu na dużą zawartość, jak i przyswajalność tego pierwiastka z produktów mleczarskich. Zmiany w technologii przetwórstwa żywności są zagrożeniem dla wysokiej biodostępności składników odżywczych z diety.

W pracy podsumowano wyniki wieloletnich badań autorów nad biodostępnością wapnia z produktów mleczarskich oraz uwalnianiem tego pierwiastka z chleba oraz posiłków śniadaniowych składających się z produktów mlecznych z dodatkiem zbożowych. Potwierdzono powszechną opinię o mleku i nabiale jako najcenniejszych źródłach łatwo przyswajalnego wapnia. Jednak w badaniach in vitro wykazano, że dodatek produktów zbożowych do mleka lub jogurtu znacznie zmniejsza ilość wapnia uwalnianego w procesie trawienia, co może wywoływać lub nasilać objawy niedoboru wapnia u człowieka.

Słowa kluczowe: mleko, nabiał, chleb, produkty zbożowe, niedobór wapnia.

INTRODUCTION

Calcium is the most abundant macroelement in a human body (PECKENPAUGH 2011, MICIŃSKI et al 2012, 2013*b*). Out of the average 1200 g of the element detected in an adult organism, 99% is stored in bones and teeth, and the remaining 1% can be found in body fluids and soft tissues. Calcium has a very broad range of physiological functions, of which the most important ones are:

- calcium is the basic structural element (as calcium phosphate-hydroxyapatite) of bones and teeth, necessary for their proper development;
- it is responsible for the regulation of the nervous and muscular system (e.g. it participates in the systolic and diastolic contractions of the myocardium);
- calcium is cne of the main factors in a cascade of blood coagulation process (transformation of prothrombin into thrombin);
- it acts as activator of some biocatalyzers (enzymes), e.g. lipase, ATP-ase.
- it diminishes the passage of serum through capillaries and is used to reduce amounts of allergic exudates;
- calcium mediates secretion of insulin and calcitonin from the cells.

Symptoms of calcium deficiency in humans may be discussed on two levels: deficiency resulting from an insufficient dietary supply or as hypocalcaemia manifested by decreased Ca concentration in blood, most often determined by the occurrence of hormonal disorders (e.g. parathormone – PTH deficiency), insufficient supply, synthesis and/or absorption disorders of vitamin D, resulting in a lower level of 1,25-$(OH)_2D_3$ (1,25dihydroxycholecalcypherol). Calcium deficiency may result in the growth disorders in children and improper bones calcification (rachitis), leading to deformations and reduced mechanical resistance of bones. Elderly people, especially women after menopause, are exposed to bone softening (osteomalacia) and loss of bone mass, which is a risk factor in osteoporosis, which may lead to pathological bone fractures. Most prevalent are lumbar spine fractures, femur neck fractures, wrist and forearm fractures.

Calcium homeostasis means an organism is able of maintain a constant concentration of Ca in blood serum within the range of 2.3-2.7 mmol dm^{-3}, accepted as physiological norm. Calcium homeostasis is achieved by increasing or decreasing the intestinal Ca absorption, its excretion with urine and faeces and utilization of reserves in bone tissue. The latter coincides with an initiation of hormonal regulatory mechanisms. A decrease in the blood calcium concentration is accompanied by excretion of parathormon from parathyroids; the hormone is responsible for conversion of vitamin D to its active, di-hydroxylic form. As a result, the rate of calcium absorption from the gastrointestinal tract increases. PTH and 1,25-$(OH)_2D_3$ simultaneously, influence the Ca release from bones and inhibit the excretion of this element in urine through the process of Ca re-absorption in kidneys. Calcitonin, secreted by the thyroid gland, is a PTH antagonist, which means that it acts opposite to PTH and decreases calcium concentrations in blood serum (Miciński et al 2013*a*).

BIOAVAILABILITY

Minerals, including calcium, occur in food as inorganic and organic compounds, often in water solution, which results in the varied Ca release and absorption. Bioavailability (biological availability, bioassimilation) is understood as a degree to which a nutrient is converted by the human organism into the form ready to be absorbed and utilized in metabolic processes and/or for storage (Fairweather-Tait 1995, Jackson 1996). Bioavailability of nutrients is determined through basic measurements: apparent absorption (A) defined as a difference between amounts ingested and excreted with faeces, equal absorbed amount, and apparent retention (R), understood as a difference between amounts absorbed and excreted with urine, equal amount of nutrient retained within the organism i.e. utilized in metabolism or stored. The bioavailability parameters is expressed in mg(μg) day^{-1} or in percentage units in comparison to ingested amount. The so-called indices of specific bioavailability are used for some bioelements (iron, selenium, zinc); a specific bioavailability index defines the portion of a mineral bound into biologically active compounds (e.g. activity of glutathione peroxidase for selenium or level of ferritin in blood serum as an indicator of iron store in the liver).

Many factors influence bioavailability of minerals in human organisms. Generally, they can be divided into two groups: exogenous, directly connected with food, and endogenous, related to the organism. Among the food-related factors, the most important ones are: properties of a given mineral, type and amount of compounds containing the mineral, oxidation status, solubility, presence of antagonistic (competitive) ions, presence of substances facilitating or hindering absorption of the mineral. Endogenous factors are: genetic conditions, age, sex, physiological status (e.g. pregnancy, breast-feeding), mineral supply status (stock), emotional state, illnesses.

CALCIUM BIOAVAILABILITY FROM MILK AND DAIRY PRODUCTS

Milk and dairy products are an excellent source of calcium (Ca) for a number of reasons. This statement refers to total calcium content in this group of food products, ratio of calcium to protein and of calcium to phosphorous (Table 1). The highest Ca content was found in ripening cheeses (from about 400 to 1400 mg 100 g^{-1} of the product). A lower calcium content was determined in milk fermented beverages (103-170 mg 100 g^{-1}) and fresh cheeses (90-100 mg Ca 100 g^{-1}). However, milk fermented beverages were characterized by a nutritionally good ratio of calcium to phosphorous (1.4:1), but the Ca : P ratio in fresh cheeses was low (0.4), similarly to the calcium to protein ratio, which equalled *ca* 5 mg Ca in 1 g of protein.

It is worth emphasizing that consumption of 1 dm^3 of milk supplies 100% of daily calcium requirement of adult person (1000 - 1200 mg per day). KŁOBUKOWSKI at al. (1997*ab*) found a high calcium content, comparable to that in ripening cheeses, in Ricotta-type non-ripening cheeses, where it ranged from 750 mg in 100 g (cheese No 1) and 600 mg 100 g^{-1} (cheese No 2). The study revealed a highly favourable Ca : P ratio of 1.85 and 1.55, respectively. A higher ratio was found only for Parmesan (1.70) and Ementaler cheese (2.01) – Table 1.

The calcium to protein proportion (mg Ca in 1 g protein) in a diet appeared to be a very helpful index, ready to be used for determination of the influence of dietary protein content on calcium homeostasis and health status of bones. It has been proved that high protein consumption negatively

Table 1

Content of calcium, protein, phosphorus and ratios between these nutrients in milk and dairy products

Dairy product	Content* (g, mg 100 g^{-1} of product)			Ratio**	
	protein (g)	calcium (mg)	phosphorus (mg)	Ca : protein (mg g^{-1} of protein)	Ca : P
Milk 2% of fat	3.4	120	86	35.3	1.40
Natural yoghurt 2% of fat	4.3	170	122	39.5	1.39
Kefir 2% of fat	3.4	103	74	30.3	1.39
Buttermilk 2% of fat	3.4	110	80	32.4	1.38
Whey	0.8	68	40	85.0	1.70
Full-fat Brie cheese	19.8	600	380	30.3	1.58
Full-fat Camembert cheese	21.4	386	310	18.0	1.25
Full-fat Cheddar cheese	27.1	703	487	25.9	1.44
Full-fat Ementaler cheese	28.8	835	416	29.0	2.01
Parmesan cheese	41.5	1380	810	33.2	1.70
Gouda cheese	27.9	807	516	28.9	1.56
Tylżycki cheese	26.1	815	510	31.2	1.60
Acid-type fresh cheese	19.8	96	240	4.85	0.40
Fromage cheese	10.2	55	123	5.4	0.45
Homogenized cream cheese	17.7	88	216	5.0	0.41
Edamski processed cheese	13.5	367	578	27.2	0.63

* protein, calcium and phosphorous content after the Polish tables of food content (KUNACHOWICZ et al. 2008);
** authors' calculations

influences calcium balance in an organism and increased calcium excretion with urine when the Ca : protein index is below 20 (CASHAM 2002, ZITTERMANN 2002). The value of this index is much higher for milk, milk fermented beverages and ripening cheeses (Table 1), which proves that these dairy products are an excellent Ca source. Low values of this index calculated for fresh cheeses were due to the technological process which involved elimination of whey containing even 80% of the milk calcium. Milk souring causes a release of insoluble calcium phosphate from casein micelles, which then dissolves in milk acid, transforms into an ionic form and is transferred into whey after separation of milk curd.

Bioavailability, determined in balance studies, is a very important criterion measuring how a human organism utilizes minerals, including calcium. The highest values (expressed as percentages) of calcium apparent absorption and retention were noted for fresh cheeses produced by both the classical method (acidic coagulation) and with addition of the probiotic *Lactobacillus plantarum*, prebiotics: 2.5% addition of inuline or maltodextrin and synbiotics: *Lactobacillus plantarum* plus 2.5% addition of inuline or maltodextrin. In almost all cases these measures of bioavailability were much above 80% (Table 2). Lower results (by about 20 percent units) of A and R parameters were obtained for diets containing ripening cheeses (Jeziorański and Tylżycki) produced by immersion in traditional (20% NaCl solution) and in modified brine (1 : 1 20% NaCl and KCl solution).

The lowest (about 50-60%) values of A and R (Table 2) were found for Ricotta-type non-ripening cheeses produced by the method of thermal-acidic coagulation of milk protein and for whey and permeate, byproducts of fresh cheese production. It should be stressed that all the values of apparent absorption and retention presented in table 2 are very high, several-fold in excess of the bioavailability of Ca from cereal products (usually <10%).

The highest calcium availability in milk and dairy products among all foodstuffs results from both nutritional and non-nutritional factors. The nutritional factors are connected with the age (higher intestinal absorption by young people), sex, physiological state (pregnancy, breast-feeding), state of organism, saturation with vitamin D, illnesses or medicine taking (e.g. diuretics). It has been proven that young, growing rats absorbed almost three times more calcium from the intestine than mature ones (McErloy et al. 1991). Higher resorption of calcium from bones was observed in women in early menopause due to a lower estrogen level (Nordin et al. 2004). Deficien-

Table 2

Values of calcium apparent absorption (A) and retention (R) coefficients from diet containing dairy products

Dairy products	Calcium bioavailability coefficients* (%)	
	apparent absorption (A)	apparent retention (R)
Ripened cheeses:		
1. Jeziorański (NaCl)	64.4	59.6
2. Jeziorański (NaCl+KCl)	73.0	58.8
3. Tylżycki (NaCl)	78.9	68.0
4. Tylżycki (NaCl+KCl)	81.3	76.3
Ricotta-type unripened cheeses:		
1. Ricotta-type cheese 1	60.5	52.0
2. Ricotta-type cheese 2	57.4	52.2
1. Acid-type fresh cheese	90.3	84.2
2. Acid-type fresh cheese with L. *plantarum*	90.5	86.3
3. Acid-type fresh cheese with L. *plantarum*+inulin	93.5	90.3
4. Acid-type fresh cheese with L. *plantarum*+maltodextrin	84.8	80.1
5. Acid-type fresh cheese with inulin	87.1	80.2
6. Acid-type fresh cheese with maltodextrin	93.3	90.2
Permeate	51.8	49.5
Whey	54.4	52.2

* Bioavailability indexes have been calculated utilizing published (Kłobukowski et al. 1997*a* and *b*, 2004, 2009, Modzelewska-Kapituła et al. 2008) and unpublished data of Kłobukowski et.al.

cy of vitamin D decreases calcium intestinal absorption and utilization in an organism (LIPS 2012). An active person, spending much time outdoors and consuming fish, eggs and fatty dairy products does not suffer from vitamin D deficiency, because it is synthesized in the skin from 7-dehydrocholesterol under the influence of ultra violet rays (ZITTERMANN et al. 1998). The vitamin D synthesis rate decreases in elderly people when the solar exposure is insufficient.

The following nutritional factors affect the bioavailability of calcium from diets containing dairy products: amount and form of calcium, content of lactose, proteins and phosphorous. The beneficial effect of lactose on calcium absorption has been demonstrated in studies on the influence of calcium and milk components on values of Ca apparent absorption indices (BUCHOWSKI, MILLER 1991). Lactose facilitates Ca absorption by stimulating, independently from vitamin D, the diffusive system from the calcium intestinal transfer. In all probability, lactose cooperates with intestinal villi and enhances the Ca penetrability. The resence of calcium bound to casein in the form of colloidal di- and tripeptide (phosphopeptides) may be a barrier to its absorption (TSUCHITA et al. 1995). SORAL-ŚMIETANA et al. (2013) proved that acid whey concentrates produced by various membrane processes did not differ in the calcium bioavailability coefficients determined for rat diets. On the other hand, PANTAKO et al. (1994) proved that whey proteins, especially α-lactoalbumin, may favour calcium absorption by rats.

A diet typical for Poland and many other countries, also highly developed one does not supply enough calcium for a human organism. It has been proved (KERSTETTERET al. 2003) that an insufficient supply of standard value protein and dietary calcium (e.g. a diet without or with too little of milk and dairy products) increases the risk of bone injuries and prolongs the rehabilitation time after fractures. Owing to a high content of easily bioavailable calcium, milk and its products can provide staple human food with Ca, preventing its deficiency which leads to osteoporosis. As a consequence, the diet with sufficient supply of milk products promote proper development and health of humans.

CALCIUM AVAILABILITY FROM BREAD AND CEREAL ADDED TO DAIRY MEALS

Bioavailability can be assessed accurately on a model of living organisms. Experiments with humans are very rare because of restrictions and high costs; most common are experiments on rats. It is easier and less time-consuming to determine the rate of nutrient release during *in vitro* simulated digestion in the human gastrointestinal tract. This way it is possible to assess the amount of a nutrient ready to be utilized by the organism (SKIBNIEWSKA et al. 2002).

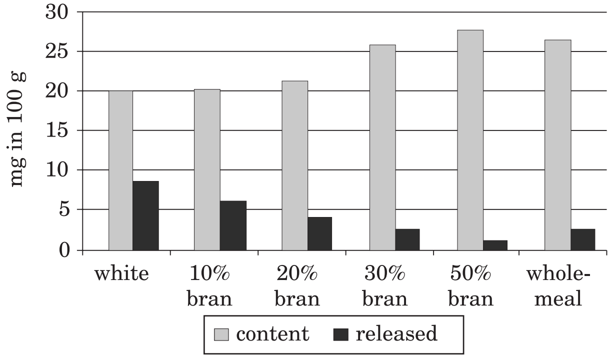

Fig. 1. Content of calcium and its amount released in the process of digestion from bread with various additions of bran

Bread and other cereal products are very popular foodstuffs throughout the world, and are often the main source of energy, nutrients, minerals and vitamins. The content of minerals in bread strongly depends on type of flour: white flour, made mostly from kernel endosperm, contains much less of minerals than darker types of flour, containing some share of germ and bran. Brown bread, especially whole-meal one, contains also phytates and dietary fiber, compounds with anti-nutritional activity which decrease the bioavailability mineral by forming non-digestible, insoluble compounds with minerals (GRASES et al. 2001). Figure 1 presents the content of calcium and its amount released in the *in vitro* digestion process from bread baked from white flour and with addition of various amounts of bran. Brown bread contained not much more of the element, but the amount of released Ca dramatically decreased with an increase in the bran proportion.

While searching for a method to raise amounts of minerals released during digestion, bread baked from dough subjected to prolonged fermentation (no less than 5 h, up to 10-11 h) was examined (SKIBNIEWSKA et al. 2004). The aim was to check whether prolonged fermentation would allow phytase to decompose phytic acid and release minerals. The expected result,

Table 3

Content of calcium in Polish bread (KUNACHOWICZ et al. 2008)

Bread	Ca content (mg 100 g^{-1})
Rye bread (white)	14
Rye bread (brown)	25
Rye bread (Litewski)	61
Rye bread with soya and sunflower seeds	41
Popular bread (wheat and rye)	17
Graham bread	22
Wheat bread	16

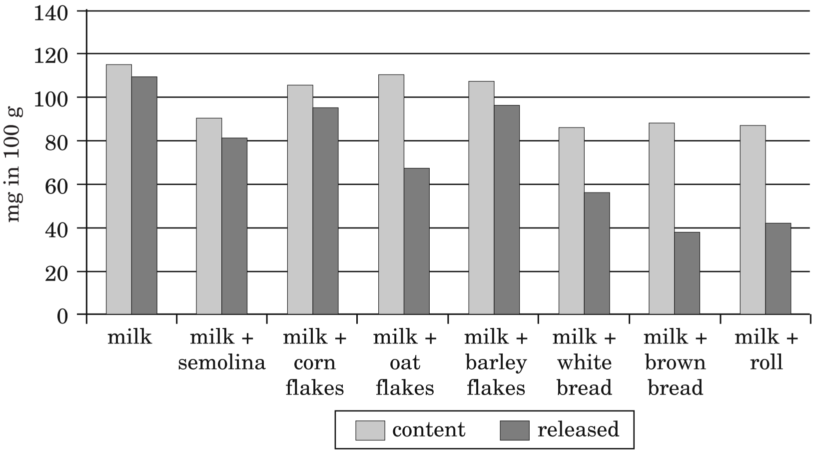

Fig. 2. Content of calcium and its amount released in the process of digestion from breakfast meals

i.e. a high percentage of minerals released from bread produced with this method, was not obtained (63% of Ca was released on average). Another experiment was run to study the influence of various methods of dough preparation on mineral release (SKIBNIEWSKA et al. 2005). Calcium was released in just 5% from whole-meal spelt flour, 16% from whole-meal common wheat flour and 66% (in a range of 34 to 87%) from mixed wheat and rye bread, the most popular type of wheat on the Polish market. SKIBNIEWSKA et al (2002) studied the influence of baking additives (natural leaven, starter cultures and complex dough improver) on the *in vitro* digestibility of minerals from white and whole-meal bread. As in the previous experiments, the share of bran had the strongest influence on the release of minerals: 74-86% of Ca was released from white bread and 16-51% from the whole-meal one (51% was released from bread baked with addition of natural leaven only).

In order to elucidate the influence of intestinal bacteria on mineral release, an experiment with the addition of *Bifidobacterium bifidum* was performed (NALEPA et al. 2012). Amounts of minerals released during enzymatic digestion varied depending on the element, bran content and presence of bacteria. As in all the previous experiments, a higher bran content lowered the release of elements. Addition of bacteria influenced mineral release in a different manner, e.g. the release of calcium (together with iron) was reduced in the presence of *Bifidobacterium bifidum* (bacteria probably utilized some of the elements for their growth).

Bread, even whole-meal one, contains little calcium (Table 3) in comparison to the daily recommended dose (about 1 000 mg depending on age, sex and physiological status), which means that the low availability of calcium from bread is not a problem. But the decreasing Ca availability from food caused by anti-nutrients from cereal products is a cause of worries.

In an experiment carried out in 2005, breakfast meals composed of milk alone or with addition of cereals were digested *in vitro* (Figure 2). Almost all the Ca included in milk was released into the solution. Corn and barley flakes, as well as semolina, decreased only slightly the amount of Ca determined in the solution after digestion, although brown bread and rolls captured about half of the element. Rolls were a surprise because they were made from white flour. They were bought in a shop, hence their exact composition was unknown.

In 2012, a paper on calcium availability from pure yoghurt and yoghurt with an addition of cereal products was published (SKIBNIEWSKA et al. 2010). The yoghurts contained less calcium than reported in the Polish tables of food composition (KUNACHOWICZ et al. 2008). Moreover, a very small portion of the element was released: on average 45%, in a range from 28.5 to 77.9%. At this moment, the results raise a question for discussion: in view of the of general deficiency of calcium in the human diet, should dieticians recommend mixing milk and cereals?

CONCLUSIONS

The above information (high values of apparent absorption and retention indexes) explicitly confirms the opinion that milk and dairy products are the most valuable source of highly available calcium among all the food products is the human diet.

At present, the human bone system is exposed to three problems: a decline in the milk and dairy product consumption causing a dramatic decline in calcium intake, innovations in dairy processing technologies leading to a decreased calcium concentration in food, and dialecticians recommending to increase consumption of whole-meal cereals, which leads to a worse calcium bioavailability. Because osteoporosis has grown to be a global health problem, monitoring calcium bioavailability from food is necessary.

REFERENCES

BUCHOWSKI M., MILLER D. 1991. *Lactose, calcium source and age affect calcium bioavailability in rats*. J. Nutr., 121: 1746-1754.

CASHAM K.D. 2002. *Macroelements, nutritional significance*. In: *Encyclopedia of dairy science*. Amsterdam, Academic Press, 3: 2051-2058.

FAIRWEATHER–TAIT S.J. 1995. *Iron-zinc and calcium-Fe interactions in relation to Zn and Fe absorption*. Proc. Nutr. Soc., 54: 465-473.

GRASES F., SIMONET B.M., PRIETO R.M., MARCH J.G. 2001. *Dietary phytate and mineral bioavailability*. J. Trace Elem. Med. Biol., 15: 221-228.

JACKSON M.J. 1996. *Bioavailability of dietary minerals.* Biochem. Soc. Trans., 24: 775-780.

KERSTETTER J.E., O'BRIEN K.O., INSOGNA K.L. 2003. *Low protein intake impact on calcium and bone homeostasis in humans.* J. Nutr., 133(3): 855-861.

KŁOBUKOWSKI J., KOZIKOWSKI W., CICHON R., WIŚNIEWSKA-PANTAK D., REPS A. 1997*a*. *Effects of brine and salting time on bioavailability of minerals from ripening cheeses*. Pol. J. Food Nutr. Sci., 6/47(2): 137-146.

KŁOBUKOWSKI J., SURAŻYŃSKI A., CICHON R., KOZIKOWSKI W. 1997*b*. *Calcium bioavailability from -type cheeses produced by high heat-acid method of milk coagulation.* Pol. J. Food Nutr. Sci., 6/47(2): 97-104.

KŁOBUKOWSKI J., MODZELEWSKA-KAPITUŁA M., KORNACKI K. 2009. *Calcium bioavailability from diets based on white cheese containing probiotics or synbiotics in short-time study in rats*. Pak. J. Nutr., 8(7): 933-936.

KŁOBUKOWSKI J., SZPENDOWSKI J., WILCZEWSKA J. 2004. *Bioavailability of calcium and phosphorus from curd cheese by-products.* Pol. J. Nat. Sci., Supl., 2: 67-74.

KUNACHOWICZ H., NADOLNA I., IWANOW K., PRZYGODA B. 2008. *Nutritional value of food*. Warsaw, PZWL, pp. 27, 29, 31. (in Polish)

LIPS P. 2012. *Interaction between Vitamin D and calcium*. Scand. J. Clin. Lab. Invest., 72: 60-64.

MCERLOY S.T., LINK J.E., DAVDY R.F., ZINN K.R., EUERSIECK B.F. 1991. *Influence of age and magnesium on calcium metabolism in rats*. J. Nutr., 121: 492-497.

MICIŃSKI J., ZWIERZCHOWSKI G., KOWALSKI I.M., SZAREK J., PIEROŻYŃSKI B., RAISTENSKIS J. 2012. *The effects of bovine milk fat on human health*. Pol. Ann. Med., 19(1): 170-175.

MICIŃSKI J., KOWALSKI I. M., ZWIERZCHOWSKI G., SZAREK J., PIEROŻYŃSKI B., ZABŁOCKA E. 2013*a*. *Characteristics of bovine milk proteins provided for food hyperallergic individuals and its decreasing methods*. Pol. Ann. Med., 20(1) in press

MICIŃSKI J., ZWIERZCHOWSKI G., KOWALSKI I.M., SZAREK J. 2013*b*. *Health-promoting properties of selected milk components*. J. Elem., 18(1): 165-186.

MODZELEWSKA-KAPITUŁA M., KŁOBUKOWSKI J., KORNACKI K., WIŚNIEWSKA-PANTAK D. 2008. *Bioavailability of calcium and phosphorus from diets containing white cheeses supplemented with prebiotics in rats*. Pol. J. Food Nutr. Sci., 58(3): 383-387.

NALEPA B., SIEMIANOWSKA E., SKIBNIEWSKA K.A. 2012. *Influence of Bifidobacterium bifidum on release of minerals from bread with different bran content.* J. Toxicol. Environ. Health, Part A, 75: 1-5.

NORDIN C.B.E., WISHART J.M., CLIFTON P.M., MCARTHUR R., SCOPACASA F., NEED A.G., MORRIS H.A., O'LOUGHLIN P.D., HOROWITZ M. 2004. *A longitudinal study of bone-related biochemical changes at the menopause*. Clin. Endocrinol., 61(1): 123-130.

PANTAKO T.O., PASSOS M., DESROSIERS T., AMIOT J. 1994. *Effets des protein es laitieres sur l'absorption du calcium et du phosphore messuree par les variations temporelles de leurs teneurs dans l'aorte et la veine porte chez le rat*. Int. Dairy J., 4(1): 37-58.

PECKENPAUGH N.J. 2011. *Nutrition essentials and diet therapy.* Wrocław, Poland, Elsevier Urban & Partner, pp. 114. (in Polish)

SKIBNIEWSKA K.A., FIEĆKO M., FORNAL Ł., SMOCZYŃSKI S.S. 2002. *Influence of starter culture and complex dough improver on in vitro digestibility of some minerals from bread.* In. *Current trends in commodity science.* The Poznań University of Economics Publishing House, 2: 635-639.

SKIBNIEWSKA K.A., KOZIROK W., FORNAL Ł., MARKIEWICZ K. 2002. *In vitro availability of minerals from oat products*. J. Sci. Food Agric., 82: 1676-1681.

SKIBNIEWSKA K.A., MAJEWSKA K., KONOPKA I., WIECZOREK J. 2005. *Study on influence of method of dough preparation on the release of minerals*. Bromat. Chem. Toksykol., Supl., 417-422. (in Polish)

SKIBNIEWSKA K.A., WIECZOREK J., HILIŃSKI D. 2004. *Study on release of minerals from bread baked from dough of prolonged fermentation time*. J Elementol., 9(4): 759-765. (in Polish)

SKIBNIEWSKA K.A., ZAKRZEWSKI J., SIEMIANOWSKA E., POLAK-JUSZCZAK L., ALJEWICZ M. 2010. *Cal-*

cium availability from yoghurt by itself or yoghurt-cereal-containing products. J. Toxicol. Environ. Health, Part A, 73:1150-1154.

SORAL-ŚMIETANA M., ZDUŃCZYK Z., WRONKOWSKA M., JUŚKIEWICZ J., ZANDER L. 2013. *Mineral composition and bioavailability of calcium and phosphorus from acid whey concentrated by various membrane processes*. J. Elem., 18(1): 115-125, DOI: 10.5601/jelem.2013.18.1.10

TSUCHITA H, GOTO T, YONEHARA Y. 1995. *Calcium and phosphorus availability from casein phosphopeptides in male growing rats.* Nutr. Res., 15(11): 1657-1667.

ZITTERMANN A. 2002. *Bone health*. In: *Encyclopedia of dairy science*. Amsterdam, Academic Press, 3: 1294-1306.

ZITTERMANN A., SCHELD K., STEHLE P. 1998. *Seasonal variations in vitamin D status and calcium absorption do not influence bone turnover in young women*. Europ. J. Clin. Nutr., 52: 501-506.