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Study of calcium and magnesium levels in dietary supplements containing antioxidants available in the Polish market

Badanie zawartości wapnia i magnezu w suplementach diety zawierających antyoksydanty dostępnych w Polsce

Dietary supplements enjoy a great popularity among the consumers worldwide and in Poland. The application of this type of preparations is an easy way of supplementing the everyday diet in deficit substances indispensable for the proper functioning of the human organism. In 2008, the value of the dietary supplements market in Poland exceeded 1.7 billion PZL (the sum of sales in pharmacies, out of pharmacies and via the Internet), which means that it rose by 24% in relation to the year 2007. The participation of dietary supplements in the general OTC drug sales (medicaments and para-pharmaceutical preparations) in Poland in 2008 amounted to 21%. It is predicted that in the years of 2008–2010 the value of these preparations in the market will be still increasing. A considerable group of these dietary supplements are antioxidants [17].

Human organism produces antioxidants (such as the following enzymes: catalase, glutathione peroxidase, peroxide dismutase, glutamine acid, glutathione, coenzyme Q10, melatonin) to protect itself against the harmful action of free radicals occurring in physiological conditions. The number of endogenic antioxidants recedes with time. Moreover, many environmental factors may increase the number of compounds with reactive oxygen in the organism over the possibility of neutralizing their harmful effects. The number of free radicals in the organism may go up in the inflammatory conditions especially in infectious diseases. Supplementation with the preparations containing antioxidants eases detoxification of cancerogens in the organism; it also prevents the development of many diseases and slows down the aging processes [16].

The content of dietary supplements containing antioxidants frequently abounds in plant components, which are rich in such antioxidants as: phenol compounds – phenol acids, flavonoids (flavones, flavonoles, isoflavones, antocians, isoprene compounds – anaerobic carotenoids (α-carotene, β-carotene, γ-carotene, lykopen), aerobic carotenoids (luteine zeaxantine, cryptoxantine; vitamins – E (tocopherols), C [8].

In the market, one may find many preparations containing an addition of herbs or their dry extracts which were registered as food supplements. Dietary supplements, having plant material recommended for registration each year, are the most abundant group of these preparations. In 2004, as many as 604 such preparations were reported for registration in Poland, and in 2005 – 635, and their number constantly increases [13].

Continuing the research aimed at the determination of the chosen mineral components in dietary supplements, the levels of calcium and magnesium (the macroelemts indispensable for man) in the preparations containing the antioxidants were determined.

MATERIAL AND METHODS

The chosen dietary supplements applied in the prophylaxis and therapy of the organs of sight (*Aronia z luteiną, Bilberin, Maxi Vision, Naturapia wzrok, Oculobon, Pro-wzrok, Vitalux)* and the preparations containing garlic in their composition (*Aktiv Kapseln, Aliovital, Alitol, Bio-czosnek, Czosnek forte, Czosnek z pietruszką, Garlicin, Tabletki z czosnkiem* were analysed (Table I).

Table 1. Name and composition of dietary supplements containing antioxidants

Name of preparation	Composition
Dietary supplements aiding good sight	
Aronia z luteiną (caps.)	Aronia fruit dry extract, calendula dry extract
Bilberin (caps.)	*Myrtilli fructus* extractum siccum, beta-carotenum
Maxi Vision (caps.)	*Myrtilli fructus* extractum siccum, vitamins: E, C; Zn, Se
Naturapia wzrok	Standardized extract from grapes, the fruit of bilberry or blackberry, Zn, β- carotene, lutein, lycopen
Oculobon (caps.)	*Vaccinium myrtillus*, lutein, beta- caroten, vitamin E, C, $B_{1,}$ $B_{2,}$ $B_{5,}$ B_{6}, B_{12}, H, PP, folic acid
Pro-wzrok (caps.)	Billberry dry extract, beta-caroten, vitamin E
Vitalux (caps.)	Beta-carotene, vitamins: C and E, PP, microelements: Zn, Se, Mn
Dietary supplement containing garlic	
Aktiv Kapseln (caps.)	*Allii sativi* maceratio oleosa, *Hyperici herbae* maceratio oleosa, vitamins: E, A; lecithin
Aliovital (caps.)	Garlic macerate (*Allium sativum*)
Alitol (caps.)	Oil garlic extract (*Allii sativi*)
Bio-czosnek (tabl.)	Powdered garlic
Czosnek forte (caps.)	Garlic extract, soy oil
Czosnek z pietruszką (caps.)	*Allii sativi bulbus* siccum, *Petroselini herba*
Garlicin (caps.)	Garlic extract
Tabletki z czosnkiem	*Allii sativi* bulbus pulv.siccum

Four to five series of samples of each preparation were studied, in two parallel runs. Each sample was a separate series of production. The samples (3–4 gram samples) were mineralized dry at the temperature of 450˚C. The process of incineration was fastened by wetting the ashes with a 20% water solution of nitric acid (65% HNO3, Suprapur, Merck), and dissolved in a hydrochloric acid (1+1) v/v (30% HCl, Suprapur, Merck).The levels of calcium and magnesium were determined by the application of the flame absorption method (FAAS) using SOLAAR M5 spectrometer of Thermo Elemental.The optimum parameters for a determination of calcium [10]: wavelength – 422.7 nm; bandpass - 0,5 nm; lamp current (best sensitivity) – 75%; operating current – 5 mA; flame type - air/ acetylene; flame chemistry – stoichiometric; fuel flow rate – 0.9–1.2 l.min.$^{-1}$

The optimum parameters for determination of magnesium [10]: wavelength – 285.2 nm; bandpass–0.5 nm; lamp current (best sensitivity) – 75%; operating current – 5 mA; flame type-air/ acetylene; flame chemistry – stoichiometric; fuel flow rate – 0.9–1.2 l.min.$^{-1}$.

For the creation of calibration curves for calcium and magnesium the pattern solutions of Merck were used. The pattern for calcium is calcium nitrate of the concentration of 100 mg/l (dissolved in the solution of 0.5 mol/l nitric acid). The pattern for magnesium is magnesium nitrate of the concentration of 100 mg/l (dissolved in the solution of 0.5 mol/l nitric acid). Calibration curve for

calcium was in the range of from 0.5 μg/ml to 15.0 μg/ml, and for magnesium from 0.05 μg/ml to 3.0 μg/ml.

The results were transferred to Table 2 and Table 3 also with the specification of the arithmetic mean and standard deviation in μg/g and μg/caps. Additionally, mineralization of the reference material ("Mixed Polish Herbs" INCT-MPH-2) was done and the levels of the studied elements were also determined.

Table 2. Calcium and magnesium content in supplements diet used auxiliary in prophylaxis and therapy the organ of vision (μg/g and μg/capsule)

Name of preparation	Content (min.-max.). arithmetical mean ± standard deviation			
	calcium content (μg/g)	calcium content (μg/capsule tablet)	magnesium content (μg/g)	magnesium content (μg/capsule tablet)
Aronia z luteiną	5.36 – 8.67	0.75 – 1.21	2.76 – 4.05	0.38 – 0.56
(n = 5; $\overline{m}$ = 0.139 g)	6.98 ± 1.25	0.97 ± 0.18	3.34 ± 0.50	0.46 ± 0.07
Bilberin	40.92 – 55.42	29.54 – 40.01	56.05 – 66.69	40.47 – 48.15
(n = 5; $\overline{m}$ = 0.722 g)	46.45 ± 6.23	33.54 ± 4.50	59.15 ± 5.03	43.52 ± 3.40
Maxi Vision	1941 – 2349	881.2 – 1066.5	266.5 – 296.3	120.99 – 134.52
(n = 5; $\overline{m}$ = 0.454 g)	2137 ± 166	970 3 ± 75.2	277.3 ± 11.72	125.95 ± 5.30
Naturapia wzrok	209.7 – 485.1	77.0 – 178.0	264.8 – 365.4	97.2 – 134.1
(n = 5; $\overline{m}$ = 367 g)	364.3 ± 98.8	113.7 ± 57.2	334.4 ± 43.4	122.7 ± 15.9
Oculobon	758.3 – 954.4	249.5 – 314.0	38.94 – 57.49	12.81 – 18.91
(n = 4; $\overline{m}$ = 0.329 g)	856.3 ± 84.0	281.8 ± 27.6	47.53 ± 8.98	15.64 ± 2.95
Pro-wzrok	14.50 – 20.55	6.15 – 8.71	41.00 – 46.70	17.38 – 19.80
(n = 5; $\overline{m}$ = 0.424 g)	16.93 ± 2.39	7.18 ± 1.01	44.13 ± 2.59	18.70 ± 1.09
Vitalux	11.9 – 16.42	7.28 – 10.05	49.74 – 65.52	30.44 – 40.10
(n = 5; $\overline{m}$ = 0.612 g)	14.47 ± 1.72	8.85 ± 1.06	56.54 ± 6.16	34.60 ± 3.77

*n – the number of series, $\overline{m}$ – the mean of mass of caps. or tabl. (g)

The declared levels of these elements were: calcium – 1.08 ± 0.07% and magnesium – 0.292 ± 0.018% and the determined contents equalled: calcium – 1.02 ± 0.07% and magnesium – 0.293 ± 0.006%.

Table 3. The level of calcium and magnesium in dietary supplements contained garlic (µg/g and µg/capsule)

Name of preparation	Content (min.-max.). arithmetical mean ± standard deviation			
	calcium content (µg/g)	calcium content (µg/capsule tablet)	magnesium content (µg/g)	magnesium content (µg/caps. tablet)
Aktiv Kapseln	36.61 – 55.17	14.64 – 22.07	5.54 – 8.80	2.22 – 3.52
(n = 5; $\overline{m}$ = 0.400 g)	46.19 ± 7.54	18.48 ± 3.02	7.21 ± 1.46	2.89 ± 0.58
Aliovital	4.98 – 7.47	1.98 – 2.97	1.21 – 1.89	0.48 – 0.75
(n = 5; $\overline{m}$ = 0.397 g)	5.88 ± 0.94	2.34 ± 0.38	1.48 ± 0.34	0.59 ± 0.14
Alitol	5.53 – 6.49	2.18 – 2.56	2.04 – 2.97	0.80 – 1.17
(n = 5) $\overline{m}$ = 0.394.g)	6.00 ± 0.42	2.36 ± 0.17	2.42 ± 0.36	0.95 ± 0.14
Bio-czosnek	298.6 – 353.8	213.5 – 253.0	346.9 – 500.3	248.0 – 357.7
(n = 4; $\overline{m}$ = 0.715 g)	319.8 ± 26.0	228.7 ± 18.6	419.7 ± 65.3	300.1 ± 46.7
Czosnek forte	4.03 – 7.48	1.74 – 3.22	2.68 – 3.98	1.16 – 1.72
(n = 5; $\overline{m}$ = 0.431 g)	5.67 ± 1.49	2.44 ± 0.64	3.22 ± 0.67	1.39 ± 0.29
Czosnek z pietruszką	4429 – 6500	1869 – 2743	2555 – 3663	1078 – 1546
(n = 5; $\overline{m}$ = 0.422 g)	5299 ± 951	2236 ± 401	2998 ± 439	1265 ± 185
Garlicin	1749 – 2000	539 – 616	1968 – 2150	606 – 662
(n = 4; $\overline{m}$ = 0.308 g)	1827 ± 118	563 ± 36	2056 ± 86	633 ± 26
Tabletki z czosnkiem	260.0 – 378 3	78.0 – 113.5	671.1 – 745.1	201.3 – 223.5
(n = 5; $\overline{m}$ = 0.300 g)	308.0 ± 50.3	90.2 ± 14.1	715.4 ± 28.8	214.6 ± 8.6

*n – the number of series, $\overline{m}$ – the mean of mass of caps. or tabl. (g)

RESULTS

The levels of calcium in the studied dietary supplements were in a broad range – on average from 6.98 ± 1.25 µg/g (*Aronia z luteiną*) up to 2137 ± 166 µg/g (*Maxi Vision*) and up to 5299 ± 951 µg/g in *Czosnek z pietruszką* preparation. The smallest levels of magnesium were also confirmed in *Aronia z luteiną* preparation, on average – 3.34 ± 0.50 µg/g, and the greatest in *Czosnek z pietruszką* preparation, on average – 2997.8 ± 439 µg/g.

Questionnaire studies conducted in the USA, Asia and in Poland showed that the greatest popularity among the consumer is shared by the mineral-vitamin preparations [2, 4, 9, 12, 15]. A good offer are also all supplements containing calcium and magnesium, especially valued by the customers over 50 years of age [3, 7]. It turns out that these elements may constitute the composition of dietary supplements applied in the prophylaxis or in helping the therapy of many illnesses. Substantial amounts of calcium and magnesium salts occur in plant material. The compounds of the above mentioned elements often fulfill the function of tablet or capsule fillers.

Calcium and magnesium are the building material elements of the human organism. They also fulfill many important functions of biochemical character [11, 14].

Hardly soluble calcium salts (hydoxyapatites, phosphates and carbonates) are stored mainly in bones and they constitute a supportive structure for the skeleton, calcium in its ionized form dissolved in intra cellular and extracellular liquids plays the role of an activator or an inhibitor of the enzymes taking part in biochemical processes of the system. This element is a constituent of biological membranes and of the substances integrating the cohesion of cells [11, 16]. Calcium is absorbed from the alimentary canal with a varied intensity– in adults at the rate of 25–35% – and depends on the degree of saturation of the organism with this element, and it depends on the endogen and exogene nutritional factors. Active transport of calcium to bones increases with the fast growth of the organism (in children and youth). The recommended intake of calcium by adult persons (men and women) is 900 mg, and the safe level is 800 mg per day. The recommended intake of calcium by women after menopause is 1200 mg/day in a diet. Safe consumption of this element for women after 60 year of life amounts to 1000 mg/day [16].

In the organism of a healthy man, around 40% of magnesium is distributed in muscles and in soft tissues, 1% in the extracellular fluids and the remaining part – in the bones. Magnesium plays an important role in the mineral homeostasis of bones. In the organism, the majority of the physiological and biochemical processes take place in the presence of or thanks to magnesium ions. The most important are: glycolisis, creation of cyclic AMP and feedback of oxydoreduction with phosphorylation. Magnesium participates in the RNA synthesis and in recreation of cellular DNA. It is the stabilizer of cellular membranes and of different cellular organelles [14, 16].

It was confirmed that in a very well balanced diet (380 mg of magnesium in diet) the intestinal absorption of this element amounts to 60%. If the level of magnesium in a diet rises (550–850 mg), the percentage of the adsorption of this element decreases. The recommended intake of magnesium ranges from, 300 mg per day (for women to 60 years of age) to 370 mg per day (for men till 60 years of age), and the safe level ranges from 280 mg/a day for women to 350 mg/day for men to 60 years of age [16].

In the available literature, one may encounter studies in which the levels of calcium and/ or magnesium were studied in the preparations with declared amounts of these elements [3, 5]. An example may be the study of Krajcowa et al. [5], in which the content of calcium and magnesium in five dietary supplements of magnesium-calcium diet was determined in different laboratories (for verification of results). There are not many publications containing information pertaining to the content of magnesium and calcium in non-multimineral preparations [1].

Leśniewicz et al., in the analyzed curative preparations, determined besides the microelements the levels of calcium and magnesium. The levels of calcium ranged from 3740 µg/g *(Alax)* to 11320 µg of calcium in 1g of *Tonic tablets*. Magnesium levels ranged from 1130 µg/g *(Urogram*) to 5170 µg/g *(Alax)* [6].

In the studied dietary supplements, the increase of calcium level was accompanied by the increase of magnesium level. In some preparations (Pro-Wzrok and Vitalux), the determined level of magnesium was higher than the level of calcium. One cannot say what the reason was for this specific problem, which actually could have been the result of the presence of magnesium stearate – the lubricant. One, however, could not precisely state what was responsible for that difference. Certainly, the levels of magnesium and calcium were due to their quantitative composition, among others, the participation of the plant material or the amount of the lubrication substances.

CONCLUSIONS

1. The levels of calcium and magnesium in the investigated dietary supplement were different and depended on the preparation composition.

2. The determined levels of the studied elements may be a supplementation of daily need of the organism for these macroelements.

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SUMMARY

Dietary supplements enjoy great popularity among consumers as their application is a comfortable way of supplementing an everyday diet in deficit substances indispensable for proper functioning of the human organism. An important group of dietary supplements is constituted by the preparations containing antioxidants protecting the organism from free radicals. The market is abundant with many preparations containing antioxidants, as those applied in the prophylaxis and as supplements in the therapy of eye problems and those containing garlic which present multi-aspect action. Dietary supplements are often plant components which are rich in compounds of antioxidative action such as plant polyphenols (phenolic acids, flavonoids), carotenoids and mineral substances (as parsley or garlic). Continuing the research aimed at the determination of the chosen mineral components in dietary supplements, the levels of calcium and magnesium (the macroelemts indispensable for man) in the preparations containing the antioxidants were determined. The chosen dietary supplements applied in the prophylaxis and therapy of the organs of sight (*Aronia z luteiną, Bilberin, Maxi Vision, Naturapia wzrok, Oculobon, Pro-wzrok, Vitalux)* and the preparations containing garlic in their composition (*Aktiv Kapseln, Aliovital, Alitol, Bio-czosnek, Czosnek forte, Czosnek z pietruszką, Garlicin, Tabletki z czosnkiem)* were analysed. The levels of calcium and magnesium were determined by the application of the flame absorption method (FAAS) using SOLAAR M5 spectrometer of Thermo Elemental. The levels of calcium in the studied dietary supplements ranged in a broad spectrum, on average from 6.98 ± 1.25 μg/g (*Aronia z luteiną*) to 2137 ± 166 μg/g (*Maxi Vision*) and up to 5299 ± 951 μg/g in *Czosnek z pietruszką* preparation. The smallest amounts of magnesium were contained in *Aronia z luteiną* preparation, on average – 3.34 ± 0.50 μg/g while the highest levels of this element were determined in *Czosnek z pietruszką* supplement, on average – 2997.8 ± 439 μg/g. The levels of calcium and magnesium in the studied dietary elements were diversified and they depended on their composition. Determination of these elements may constitute a supplementation of the daily need of the human organism for these macroelements.

STRESZCZENIE

Suplementy diety cieszą się dużą popularnością wśród konsumentów, ponieważ ich stosowanie jest wygodnym sposobem uzupełniania codziennej diety w substancje deficytowe niezbędne do prawidłowego funkcjonowania organizmu człowieka. Ważną grupę suplementów diety stanowią preparaty zawierające antyoksydanty chroniące organizm przed wolnymi rodnikami. Na rynku pojawiło się wiele suplementów z przeciwutleniaczami, m. in. stosowane w profilaktyce i pomocniczo w terapii chorób narządu wzroku, oraz preparaty z czosnkiem o wielokierunkowym działaniu. Suplementy diety zawierają często składniki roślinne, które bogate są w związki o działaniu antyoksydacyjnym, np. roślinne polifenole (kwasy fenolowe, flawonoidy), karotenoidy, oraz w substancje mineralne (np. pietruszka czy czosnek). Kontynuując badania mające na celu ocenę zawartości wybranych składników mineralnych w suplementach diety, oznaczono zawartość wapnia i magnezu (niezbędnych dla człowieka makroelementów) w preparatach zawierających przeciwutleniacze. Przebadano wybrane suplementy diety stosowane w profilaktyce i pomocniczo w terapii narządu wzroku (*Aronia z luteiną, Bilberin, Maxi Vision, Naturapia wzrok, Oculobon, Pro-wzrok, Vitalux) oraz preparaty zawierające w swoim składzie czosnek (Aktiv Kapseln, Aliovital, Alitol, Bio-czosnek, Czosnek forte, Czosnek z pietruszką, Garlicin, Tabletki z czosnkiem).* Zawartość wapnia i magnezu oznaczono stosując płomieniową metodę absorpcji atomowej (FASA) w spektrometrze SOLAAR M5 firmy Thermo Elemental. Zawartości wapnia w przebadanych suple-

mentach diety mieściły się w szerokim zakresie, średnio od 6,98 ± 1,25 µg/g (*Aronia z luteiną*) do 2137 ± 166 µg/g (*Maxi Vision*) i do 5299 ± 951 µg/g w preparacie *Czosnek z pietruszką*. Najmniejsze ilości magnezu zawierał preparat *Aronia z luteiną*, średnio 3,34 ± 0,50 µg/g, natomiast największą ilość tego pierwiastka oznaczono w suplemencie *Czosnek z pietruszką*, średnio 2997,8 ± 439 µg/g. Zawartości wapnia i magnezu w badanych suplementach diety były zróżnicowane – zależały od ich składu. Oznaczone ilości tych pierwiastków mogą stanowić uzupełnienie dziennego zapotrzebowania organizmu na te makroelementy.