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USING TWO METHODS FOR PLANT MATERIAL PREPARATION IN ORDER TO DETERMINE THE CONTENT OF BIOELEMENTS IN RED CABBAGE (*BRASSICA OLERACEA* L. VAR. *CAPITATA* L. F. *RUBRA*)

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Abstract

Mineral components (bioelements) are one of the five major groups of nutrients in human diet, next to proteins, lipids, carbohydrates and vitamins. The required daily amounts per capita are over 100 mg of macroelements and less than 100 mg of microelements. Every industrial processing technology applied to vegetable produce causes precipitation of bioelements and consequently undesirable losses of these components.

The purpose of this study has been to analyze the content of bioelements (Ca, Mg, Zn, and Fe) determined in homogenizates and extracts from three red cabbage cultivars (Koda, Haco POL, Kissendrup SWE). Correlation coefficients served to assess the concordance between the plant material preparation methods used for determination of bioelements. The correlation between the content of Mg versus Ca and Zn versus Fe in edible parts of cabbage (homogenizates) compared to the levels of these metals in red cabbage extracts was tested.

Two methods for the preparation of plant material for chemical analyses were used: homogenization and extraction. Citric acid solution of the concentration of 0.1 mol dm^{-3} was used for extraction. The content of bioelements was determined using atomic absorption spectrophotometry (AAS).

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The content of Ca and Zn in red cabbage depended on the method applied to the plant material preparation, i.e. homogenization or extraction. The content of Mg and Fe depended on the cabbage cultivars. Irrespective of the plant material preparation methods, the red cabbage cultivars Kissendrup SWE and Koda contained the highest average levels of Ca, Mg, Zn, and Fe, while the lowest ones were determined in cv. Haco POL. High correlation obtained for Mg enables us to convert the content of this element in homogenizates (A) into its content of extracts (B) – conversion factor A:B = 0.9. Positive and highly significant correlations were found between the content of the bioelements: Ca and Mg, Zn and Fe in cabbage homogenizates.

Key words: red cabbage, homogenizates, extracts, bioelements, atomic absorption spectrophotometry.

U¯YCIE DWÓCH METOD PRZYGOTOWANIA MATERIA£U ROŒLINNEGO DO OKREŒLENIA ZAWARTOŒCI BIOPIERWIASTKÓW W KAPUŒCIE CZERWONEJ (*BRASSICA OLERACEA* L. VAR. *CAPITATA* L. F. *RUBRA*)

Abstrakt

Sk³adniki mineralne (biopierwiastki) z ¿ywieniowego punktu widzenia stanowi¹ jedn¹ z piêciu podstawowych grup sk³adników od¿ywczych dla cz³owieka, oprócz bia³ek, t³uszczów, wêglowodanów i witamin. Dzienne zapotrzebowanie na sk³adniki mineralne dla 1 osoby wynosi: makroelementy powy¿ej 100 mg i mikroelementy poni¿ej 100 mg. Ka¿da obróbka przemys³owa produktów roœlinnych powoduje wytr¹cenie siê biopierwiastków, i w konsekwencji niepo¿¹dane straty tych sk³adników.

Celem pracy by³o zbadanie zawartoœci biopierwiastków (Ca, Mg, Zn i Fe) oznaczonych w homogenizatach i w ekstraktach z trzech odmian kapusty czerwonej (Koda, Haco POL, Kissendrup SWE). Oceniono zgodnoœæ zastosowanych metod przygotowania materia³u roœlinnego do oznaczania biopierwiastków za pomoc¹ wspó³czynnika korelacji. Poszukiwano korelacji miêdzy zawartoœci¹ Mg a Ca oraz Zn i Fe w czêœci jadalnej kapusty czerwonej (homogenizatach) i w otrzymanych z niej ekstraktach.

Zastosowano dwie metody przygotowania materia³u roœlinnego do analiz chemicznych: homogenizacjê i ekstrakcjê. Do ekstrakcji zastosowano roztwór kwasu cytrynowego o stê¿eniu 0.1 mol dm^{-3}. Zawartoœæ biopierwiastków oznaczono metod¹ atomowej spektrometrii absorpcyjnej.

Zawartoœæ Ca i Zn w kapuœcie czerwonej zale¿a³a od metody przygotowania materia³u roœlinnego do badañ, zawartoœæ Mg i Fe – od odmiany kapusty. Niezale¿nie od metody przygotowania materia³u roœlinnego, odmiany kapusty czerwonej Kissendrup SWE i Koda zawiera³y najwiêcej Ca, Mg, Zn i Fe, a najmniej odmiana Haco POL. Wysoka korelacja w przypadku Mg daje mo¿liwoœæ przeliczenia zawartoœci tego pierwiastka w homogenizatach (A) na zawartoœæ w ekstraktach (B) – przelicznik A:B = 0.9. Stwierdzono dodatni¹ i wysoce istotn¹ zale¿noœæ miêdzy zawartoœci¹ biopierwiastków Ca i Mg, Zn i Fe w homogenizatach z kapusty.

S³owa kluczowe: kapusta czerwona, homogenizaty, ekstrakty, biopierwiastki, atomowa spektrometria absorpcyjna.

INTRODUCTION

For nutritionists, mineral components (bioelements) are one of the five major groups of nutrients for humans, next to proteins, lipids, carbohydrates and vitamins. Mineral components are divided into macroelements (e.g. Ca, Mg, P, Na) and microelements (e.g. Fe, Zn, Mn, Cu, Co). The required daily amounts per capita are over 100 mg of macroelements and less than 100 mg microelements (ZIEMLAŃSKI 2001).

Deficiency of macroelements in an organism, especially that of Ca and Mg, is a problem that appears in many human populations. Supply of essential bioelements is sometimes insufficient and tends to decline (SIKORSKI 1994, WATTS 1995). One possible measure to counteract such problems involves supplementation with mineral preparations (O¯AROWSKI 2001), which in addition to bioelements contain anthocyanins, able to form complexes with metals. Ions of metals enhance the stability of anthocyanins and protect products from decoloration (SHAKED-SACHRAY et al. 2002, SALINAS et al. 2005, SMYK et al. 2008). Such preparations are produced via technological processing of fruit (chokeberry, elderberry, cranberry) or vegetables (red cabbage). It has to be added, however, that any industrial processing of plant products leads to precipitation of elements. Extraction of mineral substances, for example while boiling or scalding vegetables, causes unwanted losses of about 30-65% K, 15-70% Mg and Cu, 20-24% Zn and many other elements (NABRZYSKI 1996). The efficiency of extraction to a large degree depends on the kind of an applied solvent (PLISZKA, HUSZCZA-CIO£KOWSKA 2009b). The literature dealing with the content of bioelements in red cabbage most often discusses homogenizates; in contrast, few references regard the issue of bioelements in extracts (SINGH et al. 2009). The question arises whether such extracts can be considered as a natural and good source of bioelements.

The objective of this study has been to assess the content of anthocyanins and several bioelements (Ca, Mg, Fe, and Zn) determined in homogenizates and extracts from cultivars of red cabbage (Koda, Haco Pol, Kissendrup SWE). Correlation coefficients served to assess the concordance between the plant material preparation methods used for determination of bioelements. The correlation between the content of Mg versus Ca and Zn versus Fe in edible parts of cabbage (homogenizates) compared to the levels of these metals in red cabbage extracts was tested.

MATERIAL AND METHODS

Sample preparation

The material consisted of three cultivars of red cabbage (*Brassica oleracea* L., var. capitata L., f. rubra), which differed in the growing season duration: Koda (an early cultivar), Haco POL (medium early) and Kissendrup SWE (medium late). The cultivars were grown in a one-year experiment (in 2003), in a garden at the Experimental Station of the University of Warmia and Mazury in Olsztyn, Poland.

Having partly removed stalks from cabbage heads, some of the heads were shredded to size 1-5 mm fragments). The plant material was stored frozen (–18^{o}C) until it was analyzed.

Two methods for the preparation of plant material for chemical analyses were used. Edible parts of red cabbage were homogenized in a mortar (method A). For extraction (method B), 30 g of each red cabbage sample was weighed out and macerated in 300 cm^3 of citric acid solution ($C_6H_8O_7 \cdot H_2O$) of the concentration of 0.1 mol dm^{-3}. The samples were left in the dark at 2°C for 24 h, after which they were shaken in a water bath at 37^{o}C. The extracts were filtered through Whatman No 1 filter paper.

The samples were prepared in three replicates.

Analytical measurements

In order to determine the content of bioelements, the plant material obtained from red cabbage: homogenizates (method A) and extracts (method B), was mineralized in a Teflon® microwave digestion bomb.

For mineralization, 0.2 g of homogenizates and 2 cm^3 concentrated nitric acid (HNO_3) were used. When the extracts were mineralized, 1 cm^3 extract and 2 cm^3 concentrated HNO_3 acid were used. Once mineralized, the samples were quantitatively transferred to 25 cm^3 measuring flasks, which were filled up to full capacity with deionized water. The content of bioelements (Ca, Mg, Zn and Fe) was determined using atomic absorption spectrophotometry (AAS).

Analytical grade reagents were used for all the chemical analyses.

Statistical analysis

The results of the determinations of bioelements underwent analysis of variance. Significance of differences in the content of bioelements was tested with Student t-test at $P = 0.01$ (to demonstrate interactions), using Statistica 8.1 programme. In order to test concordance of the results obtained from the two plant preparation methods, correlation coefficients were computed for the content of each bioelement determined in homogenizates and in extracts. Regression analysis was performed on the results. In addition, correlation analysis was completed to test the content of Mg versus Ca and Zn versus Fe in homogenizates and in extracts from red cabbage.

RESULTS AND DISCUSSION

Concentrations of macroelements (Ca, Mg) and microelements (Zn, Fe) in homogenizates (method A) and extracts (method B) from red cabbage are presented in Table 1.

Calcium

High mean concentrations of Ca were determined in two red cabbage cultivars: Kissendrup SWE and Koda (20.70 and 18.76 mg 100 g^{-1}, respectively), which did not differ statistically in the content of this element. In contrast, cv. Haco POL contained significantly less calcium (15.27 mg 100 g^{-1}).

The average Ca content in red cabbage was more strongly dependent on the sample preparation method than on the cultivar of this vegetable. Likewise, the interaction between these factors was significantly high. In the homogenizates (method A), the mean Ca content was ca 5-fold higher than in extracts (method B).

In the material obtained with method A, significant differences in the content of Ca were established for all the three cultivars. The highest Ca content was found in cv. Kissendrup SWE (35.48 mg 100 g^{-1}), and the lowest one – in cv. Haco POL (25.38 mg 100 g^{-1}). According to Majkowska-Gadomska and Wierzbicka (2008), the highest Ca content in red cabbage (in dry matter) appeared in cv. Koda (0.69 g 100 g^{-1}), and the lowest one – in cv. Kissendrup SWE (0.41 g 100 g^{-1}). Other authors (Kunachowicz et al. 2003, 2005) report that red cabbage can contain 46 mg Ca 100 g^{-1} of edible parts.

In our study, the extracts (method B) of red cabbage of all the three cultivars contained similar levels of Ca (from 5.17 to 7.51 mg 100 g^{-1}). Another experiment completed by Pliszka and Huszcza-Ciołkowska (2009a) also demonstrated a low level of calcium in extracts from red cabbage (nearly six-fold less than in edible parts). Further tests are needed to clarify the reasons why Ca appears in such low amounts. Singh et al. (2009) report that the content of Ca in cabbage extracts ranged from 19.3 to 68.5 mg 100 g^{-1} fresh matter.

No significant correlation was found between the Ca content in the plant material prepared by the two methods; the correlation coefficient was low (r = 0.350).

Table 1

Content of bioelements in three cultivars of red cabbage depending on the method of plant material preparation (mg 100 g^{-1} of fresh matter)

Bioelements	Ca			Mg			Zn			Fe		
Cultivar / Method	A*	B**	mean	A	B	mean	A	B	mean	A	B	mean
Koda	30.00	7.51	**18.76**	14.30	12.23	**13.27**	0.39	0.31	**0.35**	0.45	0.66	**0.56**
Haco POL	25.38	5.17	**15.27**	10.81	10.66	**10.73**	0.35	0.24	**0.29**	0.28	0.10	**0.19**
Kissendrup SWE	35.48	5.92	**20.70**	16.31	14.10	**15.21**	0.63	0.17	**0.40**	0.62	0.42	**0.52**
Mean	**30.28**	**6.20**		**13.81**	**12.33**		**0.46**	**0.24**		**0.45**	**0.39**	
LSD for method	1.964			0.288			0.069			n.s.		
LSD for cultivar			2.406			0.353			0.085			0.152
LSD for method × cultivar interaction		3.403			0.499			0.120			0.214	

*Method A – homogenizates from red cabbage
**Method B – extracts from red cabbage
$LSD_{P=0.01}$; n.s. – no significant differences

Magnesium

Among all the tested factors, it was the cabbage cultivar that most strongly differentiated the content of Mg. Independently from the method applied to prepare tested samples of plant material, the mean Mg ranged between 10.73 to 15.21 mg 100 g^{-1}. All the three cultivars were significantly different in the content of this element. The highest Mg level was found in cv. Kissendrup SWE (16.31 and 14.10 mg 100 g^{-1}, method A and B respectively) and the lowest one in cv. Haco POL (10.81 and 10.66 mg 100 g^{-1}, method A and B respectively). Kunachowicz et al. (2005) report that red cabbage contains 12 mg Mg 100 g^{-1} of edible parts, which is similar to the present result (13.81 mg Mg 100 g^{-1}). Majkowska-Gadomska and Wierzbicka (2008) demonstrated that the three cultivars of red cabbage (Koda, Haco Pol, Kissendrup SWE) had identical levels of Mg (0.13 g 100 g^{-1} of dry matter).

The content of Mg in homogenizates obtained from red cabbage (method A) was significantly higher than in extracts (method B), and the values obtained in the tested samples produced with both methods were correlated ($r = 0.974$, significant at $P = 0.01$). Such high correlation suggests that it is feasible to use only one method of sample preparation for determination of Mg in red cabbage. Results obtained from tests on homogenizates can be converted into the ones produced by tests on plant extracts using the following regression equation: $y = 0.6079x + 3.9358$, where: y – content of Mg in extract; x – content of Mg in homogenizates. Both sample preparation methods proved to be comparably efficient in evaluating differences between the three red cabbage cultivars in magnesium content. The conversion factor for converting the content of Mg in homogenizates (method A) into the content of this element in extracts (method B) is A:B = 0.9.

Zinc

Like calcium, the content of Zn in red cabbage was more strongly related to the sample preparation method than to the cultivar. Analogously, the interaction between these factors was high. The highest mean Zn content was determined in cv. Kissendrup SWE (0.40 mg 100 g^{-1}) and cv. Koda (0.35 mg 100 g^{-1}), with no statistically significant differences in the level of this element between these two cultivars. In turn, statistically significant less zinc was found in cv. Haco POL (0.29 mg 100 g^{-1}).

Among the homogenizates, cv. Kissendrup SWE had the highest Zn content (0.63 mg 100 g^{-1}), while the cultivars Koda and Haco POL contained much less zinc (0.39 and 0.35 mg 100 g^{-1}, respectively). Kunachowicz et al. (2003, 2005) report that red cabbage contains 0.43 mg Zn 100 g^{-1} of edible parts.

The average Zn content in homogenizates from red cabbage was twofold higher than in extracts (0.46 mg 100 g^{-1} versus 0.24 mg 100 g^{-1}). As Singh et al. (2009) determined, the content of Zn in red cabbage extracts ranged from 211.7 to 266.7 µg 100 g^{-1} of fresh matter, thus being similar to our determinations. The relationships between the content of Zn in particular cultivars determined in the samples prepared by the two methods were different, which confirmed the significance of the cultivar´sample preparation method interaction.

No correlation was found for the content of Zn between samples prepared by the two methods. The correlation coefficient *r* = –0.499 indicated a tendency towards a negative relationship between the results obtained from homogenizates and extracts.

Iron

The content of Fe in red cabbage, like that of magnesium, was significantly differentiated by the cultivar. It did not depend on the sample preparation method (methods A and B).

High and similar mean content of Fe (0.52-0.56 mg 100 g^{-1}) in red cabbage was determined for two cultivars: Koda and Kissendrup SWE, irrespective of the sample preparation method. Less iron was detected in cv. Haco POL (0.19 mg 100 g^{-1}) (about 2.8-fold less than in the other two varieties). MAJKOWSKA-GADOMSKA and WIERZBICKA (2008) report that the Fe content (converted into dry matter) was the highest in cv. Koda (57.50 mg kg^{-1}), and the lowest in cv. Kissendrup SWE and cv. Haco Pol (52.0 and 50.0 mg kg^{-1}, respectively). Other authors (KUNACHOWICZ et al. 2003, 2005) demonstrated that red cabbage contained 0.5 mg Fe 100 g^{-1} of edible parts. SINGH et al. (2009) in turn report that the content of Fe in red cabbage extracts varied from 327.7 to 1146.0 µg 100 g^{-1} of fresh matter. The present results of the determinations of the Fe content in the extracts are within the limits cited by SINGH et al. (2009).

No correlation was found between the amounts of Fe determined in the tested material obtained by the two methods. The correlation coefficient *r* = 0.559 indicated a tendency towards a positive relationship between the results.

Correlation between the content of Ca and Mg, and between Zn and Fe

Positive and highly significant correlation was determined between the content of the following pairs of elements: Ca and Mg (*r* = 0.930; significant at *P* = 0.01) and Zn and Fe (*r* = 0.860; significant at *P* = 0.01) in homogenizates (method A) – Figure 1. JÊDRZEJCZAK et al. (1999), who determined the content of Ca and Mg in homogenized vegetables and fruit, demonstrated the presence of positive correlation between these bioelements in carrot (*r* = 0.5986) and strawberries (*r* = 0.5764).

In our study, no such correlation was found in red cabbage extracts (method B), either for Ca and Mg (*r* = 0.288), whereas for Zn and Fe, a tendency for positive correlation was observed (*r* = 0.427). There are no

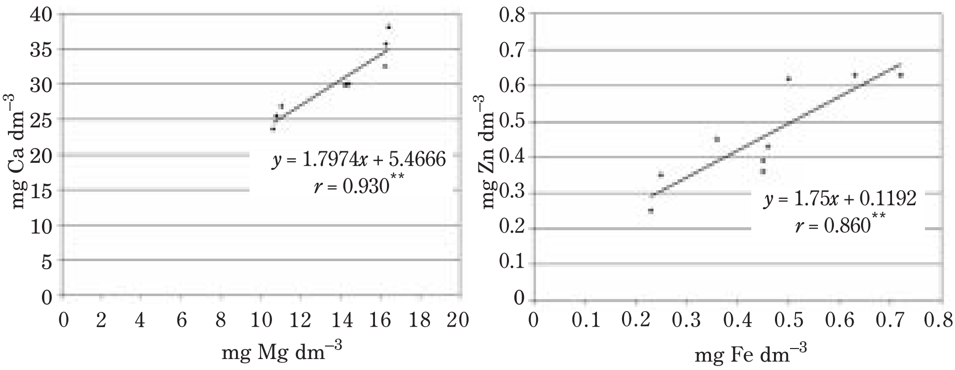

Fig. 1. Correlation between the content of Ca and Mg as well as Zn and Fe in homogenizates from red cabbage (method A)

data in the literature on mutual dependences between Ca, Mg, Zn and Fe in red cabbage extracts.

Some earlier studies on bioelements in fruit extracts revealed correlation between the content of Ca and Mg, which was independent from the extraction method (H_2O and HCl methods). However, no such correlation was proven for the content of Zn and Fe in fruit extracts obtained by the above methods (PLISZKA et al. 2008).

The mean Ca content in the homogenizates of red cabbage (irrespective of the cultivar) was higher than that of Mg, unlike in the extracts, which contained more Mg and Ca. No differences were observed in the mean content of Zn and Fe in homogenizates, while in extracts the content of Fe was higher than that of Zn. Content of bioelements in fruit and vegetable extracts heavily depends on the method applied for extraction (PLISZKA et al. 2008, NASCENTES et al. 2009, PLISZKA, HUSZCZA-CIOŁKOWSKA 2009b, SINGH et al. 2009).

When searching for natural and good sources of bioelements (Ca, Mg, Zn and Fe) from red cabbage, one should take into consideration both cultivar-dependent traits and a method applied to plant material preparation for consumption.

CONCLUSIONS

1. The content of Ca and Zn in the three red cabbage cultivars depended on the sample preparation methods: homogenization and extraction. A very high level of Ca and Zn as well as some differences in the content of this element between the cultivars occurred in homogenizates.

2. Determination of the content of Mg and Fe was mainly dependent on the cabbage cultivar. In general, higher content of Mg and Fe was determined in the homogenizates rather than the extracts from the particular red cabbage cultivars.

3. Irrespective of the plant material preparation method, the red cabbage cultivars Kissendrup SWE and Koda were characterised by the highest mean content of bioelements, while cv. Haco POL was the lowest content of the bioelements among the tested cabbage varieties.

4. It was only for Mg that it proved possible, owing to the high correlation for Mg, to convert the amount of this element present in homogenized material (method A) into its corresponding quantity in an extract (method B). The conversion factor was A:B = 0.9.

5. Positive and highly significant correlations were found between the content of the bioelements: Ca and Mg as well as Zn and Fe in red cabbage homogenizates.

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