



## ENRICHMENT OF SOME LEAFY VEGETABLES WITH MAGNESIUM\*

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### ABSTRACT

Two thirds of the world's population do not consume the recommended amounts of magnesium (Mg). Hence, there is an increasing interest in producing plants enriched with this element. However, while designing any enrichment treatment, the effects of the amended supply of ions on the quantity, quality and safety of food products must be considered. Leafy vegetables are readily consumed and can be a promising source of Mg and antioxidants. An attempt has been made to evaluate the effect of enriching selected cultivars of lettuce (Romaine lettuce cv. Amadeusz and head lettuce cv. Omega) and endive (cv. Burundi) with Mg (40 – optimal, 80, 120 and 160 mg Mg dm<sup>-3</sup>) on the (1) concentration of Mg and other elements (K, Ca, Fe and Zn), and the impact of higher Mg concentrations on the (2) biomass accumulation, (3) efficiency of the photosynthetic apparatus, (4) level of reactive oxygen species (ROS), (5) activity of antioxidant enzymes and (6) the content of phenolic compounds. Although plants enriched with Mg had a higher concentration of Mg, they usually contained less Fe. The content of Mg in plants was sufficiently high for them to be considered as an alternative dietary source of this element, but the results varied from species to species. A higher concentration of Mg had a minor effect on the biomass accumulation and the efficiency of the photosynthetic apparatus, but significantly affected the generation of ROS and changed the activity of the examined antioxidant system's components. These findings demonstrate that enrichment with Mg may simultaneously elevate levels of ROS, which must be taken into consideration prior to implementing any enrichment technology. Among the tested cultivars, endive cv. Burundi proved to be most suitable for Mg enrichment. Plants of this cultivar grown in the presence of Mg in concentrations up to 120 mg Mg dm<sup>-3</sup> accumulated considerable amounts of Mg, without any negative side effects.

**Keywords:** biomass accumulation, efficiency of photosynthetic apparatus, ion concentration, Mg enrichment, phenols, ROS.

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## INTRODUCTION

Worldwide, deficiency of ions in human diet affects half the population (BROADLEY et al. 2008). Nearly two thirds of the global population do not consume the recommended amount of magnesium (Mg), although the main source of this element is found in readily available plant products (HERMANS et al. 2013). The reason could be the fact that plants eaten today, compared to those in the 1960s, are depleted of valuable elements (ROSANOFF 2013).

Magnesium is essential for biochemical functions of cells (PASTERNAK et al. 2010). Its deficiency can be a cause and a consequence of disease (ROSANOFF 2013). Pharmaceuticals used as Mg supplements are characterized by low Mg absorption, which decreases strongly as Mg concentrations increase (FINE et al. 1991). A well-balanced diet is therefore the best solution for regulating Mg homeostasis in the human body and we need to produce a wider range of food enriched with Mg (CZERWIŃSKA, GRZESZCZAK 2013). Fortunately, Mg is easily absorbed from plants, especially from leafy vegetables served fresh, as food processing reduces both the concentration and availability of this element (BROADLEY, WHITE 2010). RIOS et al. (2012) shows that leafy cruciferous vegetables could be enriched with Mg. However, these plants are not eaten in large quantities. Lettuce and endive, which are very popular vegetables owing to their palatability and easy cultivation, seem more promising. They can be eaten fresh all year round, providing people with many nutrients and compounds positively affecting human health, e.g. phenols and inulin (LLORACH et al. 2008, DI BARTOLOMEO et al. 2013). Thus far, lettuce has been successfully enriched with iodine (I) (SMOLEŃ et al. 2014) and selenium (Se) (RIOS et al. 2008), but not with Mg.

Any attempt at enriching plants with nutrients must be preceded by careful consideration of the effects of the amended supply of ions on the quantity, quality and safety of food. Usually, the success of enrichment is evaluated according to the accumulation of a given element in plants. However, an excess of one ion may (1) affect the uptake and metabolism of other ions, e.g. antagonistic and synergistic effects of Mg are reported towards Ca and K (HERMANS et al. 2013), and (2) disrupt various processes in plants, e.g. photosynthesis, in which Mg plays an important role (SHAUL 2002). Negative effects of Mg deficiency have already been reported (HERMANS et al. 2004, SHABALA, HARIADI 2005), but much less is known about a possible adverse impact of excess Mg (CAKMAK, KIRKBY 2008). Therefore, in the present study, an attempt has been made to evaluate the effects of enrichment of selected cultivars of lettuce and endive with Mg on the (1) concentration of Mg and other elements, and the effect of higher Mg concentrations on the (2) biomass accumulation, (3) efficiency of the photosynthetic apparatus, (4) level of reactive oxygen species (ROS), (5) activity of enzymes antioxidative and (6) content of phenolic compounds.

## MATERIAL AND METHODS

### Plant material and growing conditions

The vegetables chosen for this study, that is Romaine lettuce (*Lactuca sativa* L. var. longifolia Lam.) cv. Amadeusz, head lettuce (*Lactuca sativa* L. var. capitata) cv. Omega and endive (*Cichorium endivia* L.) cv. Burundi, are a popular choice in greenhouse production. Seeds (Rij Zwaan, Netherlands) were sown onto rockwool plugs (Grodan, Netherlands) and germinated in a growing chamber (Sanyo MLR-350H, Japan) at 16°C and 8/16 h day/night. Uniform, 2-week-old seedlings were transplanted into a hydroponics system (containers filled with 1200 ml of weekly changed nutrient solution, Table 1)

Table 1

Mineral composition of the nutrient solution

Component	(mg dm <sup>-3</sup> )
N (NO <sub>3</sub> )	200
P	30
K	210
Mg	40
Ca	240
Fe (EDTA)	12
B	0.3
Zn	0.1
Mo	0.16
Cu	0.1
Mn	0.5

with aeration (12 h per day, in cycle: 2 h of aeration followed by 2 h without aeration) and cultivated for 5 weeks at 20/14°C, 10/14 h day/night, light intensity of 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and pH=6.2. During the first week of cultivation, plants were acclimated to the growing medium using ½-strength nutrient solution. An additional dose of Mg as MgSO<sub>4</sub> · 7H<sub>2</sub>O, up to the concentrations of 80, 120 and 160 mg Mg dm<sup>-3</sup> was applied after the acclimation to the full-strength nutrient solution and plants were grown for further 4 weeks, until they reached commercial value. Control plants were grown in a nutrient solution containing recommended 40 mg Mg dm<sup>-3</sup>.

### Efficiency of the photosynthetic apparatus and biomass accumulation

The efficiency of the photosynthetic apparatus was assessed 14 days after starting Mg enrichment, on fully expanded, undamaged leaves from the

middle part of a rosette. Plant gas exchange was evaluated using a LICOR 6400 Photosynthesis System (LI-COR, Inc., USA) equipped with a 6400-40 Leaf Chamber Fluorometer and 6400-01 CO<sub>2</sub> mixer. Chlorophyll *a* fluorescence was determined with a continuous excitation fluorometer in dark-adapted leaves (Handy PEA, Hansatech Instruments, UK). At harvest, fresh weight (FW) and dry matter (DM, after drying for 24 hours at 105°C and for subsequent three days at 75°C) of edible parts were recorded.

### **Generation of ROS and antioxidative system activity**

At harvest, the generation of ROS and the activity of the antioxidant system were assessed spectrophotometrically (Spectrometer UV/VIS U2900, Hitachi, Japan). The levels of superoxide anion-radicals (O<sub>2</sub><sup>•-</sup>) and hydroxyl radicals (OH<sup>•</sup>) were determined in freshly ground leaves at wavelengths of 580 nm and 540 nm, respectively (CHAITANYA, NAITHANI 1994). Samples for determinations of the activity of antioxidant enzymes and phenols were stored at -80°C until analysis. The activity of enzymes was measured at wavelengths of 240 nm and 290 nm, respectively, for ascorbate peroxidase – APX (NAKANO, ASADA 1987, modified by ŁATA et al. 2005) and catalase – CAT (BEERS, SIZER 1952, modified by ŁATA et al. 2005). The content of phenols was assessed at the wavelength of 420 nm with gallic acid (GA) used as standard (MEDINA 2011).

### **Ions concentration**

Before drying (105°C for 2 h and then at 75°C for 48 h), the leaves were rinsed twice in tap H<sub>2</sub>O and then in distilled H<sub>2</sub>O to purify their surface. The dried material was ground in a laboratory mill and wet digested with nitric acid (24 h in room temperature followed by 2 h in 60°C, 2 h in 90°C, 30 min in 110°C and 30 min in 120°C). Atomic absorption spectrometry (Solaar M6, Thermo scientific, USA) was used to determine the amount of K, Mg, Ca, Fe and Zn.

### **Statistics**

Data were subjected to analysis with one factorial Anova using Statgraphics Plus 4.1. Differences between means of combinations were evaluated by HSD of the Tukey test at  $\alpha = 0.05$ . The presented data are mean  $\pm$ SE. The number of biological replications (single plant in a container) was 7. The number of replications (separate sampling) for a given parameter ranged between 3 and 10, and this is indicated in the respective tables and figure.

Two experiments were performed. Since the results obtained in both showed similar trends, data presented here is from the experiment with a wider range of Mg concentrations and more parameters measured.

## RESULTS

### Biomass accumulation

The effect of Mg enrichment on biomass accumulation was not significant, but some trends were recorded (Table 2). The addition of Mg increased biomass accumulation in Romaine lettuce (up to 8% and 18%, FW and DM respectively) and endive (up to 24% and 14%, FW and DM respectively). Conversely, head lettuce accumulated less biomass (Table 2).

Table 2

Fresh weight (FW) and dry matter (DM) of leaves and chlorophyll *a* fluorescence (maximum quantum efficiency of Photosystem II - Fv/Fm and performance index - PI) of Romaine lettuce, head lettuce and endive plants grown in hydroponic system with nutrient solution enriched with Mg. Data are mean  $\pm$ SE,  $n = 7$  (biomass accumulation) or 10 (chlorophyll *a* fluorescence)

Species	Mg (mg dm <sup>-3</sup> )	FW (g plant <sup>-1</sup> )	DM (g plant <sup>-1</sup> )	Fv/Fm	PI
Romaine lettuce	40 control	220.6 $\pm$ 11.4	19.08 $\pm$ 0.82	0.855 $\pm$ 0.001	5.973 $\pm$ 0.22
	80	223.3 $\pm$ 4.7	21.74 $\pm$ 0.36	0.853 $\pm$ 0.001	5.899 $\pm$ 0.28
	120	225.5 $\pm$ 8.8	22.48 $\pm$ 1.06	0.850 $\pm$ 0.001	5.476 $\pm$ 0.31
	160	238.1 $\pm$ 8.0	22.29 $\pm$ 0.69	0.855 $\pm$ 0.001	6.122 $\pm$ 0.31
Head lettuce	40 control	226.2 $\pm$ 7.1	10.44 $\pm$ 0.27	0.859 $\pm$ 0.001	4.398 $\pm$ 0.13
	80	230.3 $\pm$ 11.2	10.97 $\pm$ 0.46	0.859 $\pm$ 0.001	4.735 $\pm$ 0.26
	120	207.4 $\pm$ 4.4	10.15 $\pm$ 0.18	0.848 $\pm$ 0.006	3.672 $\pm$ 0.37
	160	180.3 $\pm$ 11.8	8.920 $\pm$ 0.43	0.855 $\pm$ 0.002	4.402 $\pm$ 0.14
Endive	40 control	128.7 $\pm$ 4.4	8.750 $\pm$ 0.48	0.842 $\pm$ 0.001b*	4.273 $\pm$ 0.21
	80	158.4 $\pm$ 3.4	9.941 $\pm$ 0.20	0.839 $\pm$ 0.002ab	4.373 $\pm$ 0.18
	120	146.7 $\pm$ 8.0	8.309 $\pm$ 0.40	0.835 $\pm$ 0.002ab	4.250 $\pm$ 0.16
	160	159.7 $\pm$ 3.9	9.298 $\pm$ 0.55	0.830 $\pm$ 0.002a	3.931 $\pm$ 0.13

\* Data followed by the same letter do not differ significantly.

### Efficiency of photosynthetic apparatus

The effect of enrichment with Mg on the efficiency of the photosynthetic apparatus was minor (Figure 1a,b). The intensity of photosynthesis increased in endive, hardly changed in Romaine lettuce and decreased, although insignificantly, in head lettuce (Figure 1a). Plants of endive enriched with Mg, as opposed to the lettuce species, had significantly higher stomatal conductance (Figure 1b).

The maximum quantum efficiency of Photosystem II (Fv/Fm), regardless of the level of enrichment with Mg and tested cultivar, was at the optimal level (Table 2). Enrichment with Mg also slightly changed the performance index (PI) – Table 2.

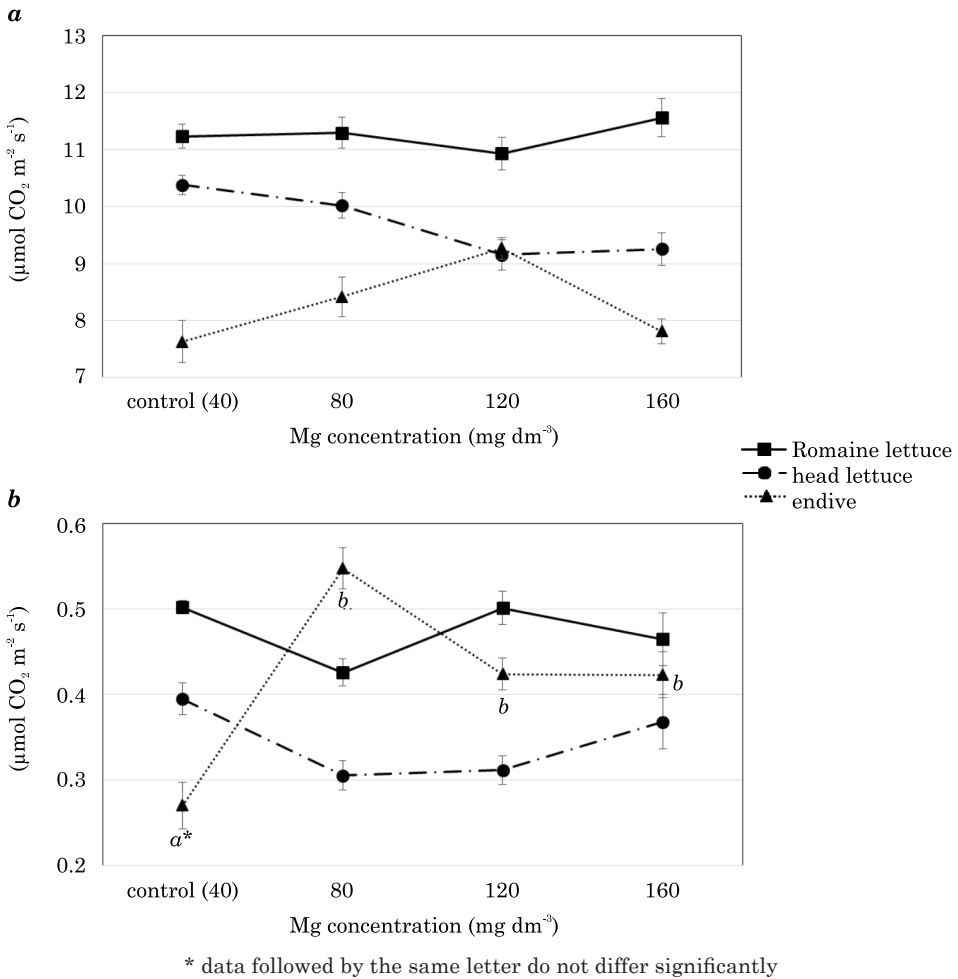


Fig. 1. Intensity of photosynthesis (a) and stomatal conductance (b) in Romaine lettuce, head lettuce and endive plants grown in hydroponic system with nutrient solution enriched with Mg. Data are mean  $\pm$ SE,  $n = 5$

### Generation of ROS and antioxidative system activity

The enrichment with Mg altered the oxidative balance in plants (Table 3). The levels of ROS in endive increased significantly following the addition of 120 and 160 mg Mg dm<sup>-3</sup> (by 89% and 173-213% respectively for O<sub>2</sub><sup>•</sup> and OH<sup>•</sup>). In Romaine lettuce, the level of O<sub>2</sub><sup>•</sup> significantly increased (by 44-78%) but OH<sup>•</sup> decreased (by 28-60%), while in head lettuce the levels of both ROS were usually reduced, although the difference was significant only in the case of OH<sup>•</sup> (by 23-60%).

The activity of antioxidant enzymes insignificantly increased in endive. In Romaine lettuce, the addition of Mg resulted in a slightly higher activity

of CAT, but significantly lower of APX (by 11-29%). In the case of head lettuce, the recorded changes were not significant, but slightly greater activity of APX and lower activity of CAT were noted.

Independently of the cultivar and Mg concentration, the content of phenols increased in plants enriched with Mg. In the case of Romaine lettuce and endive, the recorded changes were significant and amounted to 37-65% and 4-38%, respectively (Table 3).

Table 3

Content of phenolic compounds, anion-radical ( $O_2^\circ$ ) and hydroxyl radical ( $OH^\circ$ ), and activity of ascorbate peroxidase (APX) and catalase (CAT) in leaves of Romaine lettuce, head lettuce and endive plants grown in hydroponic system with nutrient solution enriched with Mg. Data are mean  $\pm$ SE,  $n = 3$

Species	Mg (mg dm <sup>-3</sup> )	Phenols ( $\mu$ g GA g <sup>-1</sup> FW)	$O_2^\circ$	$OH^\circ$	APX	CAT
			relative value		(n kat g <sup>-1</sup> FW)	
Romaine lettuce	40 control	96.38 $\pm$ 3.2a*	0.840 $\pm$ 0.02a	0.276 $\pm$ 0.009c	10.12 $\pm$ 0.20b	0.176 $\pm$ 0.01
	80	132.3 $\pm$ 3.7b	1.495 $\pm$ 0.01c	0.200 $\pm$ 0.015b	11.30 $\pm$ 0.06b	0.212 $\pm$ 0.02
	120	156.0 $\pm$ 1.1c	1.207 $\pm$ 0.04b	0.108 $\pm$ 0.007a	8.971 $\pm$ 0.48ab	0.233 $\pm$ 0.01
	160	159.3 $\pm$ 3.2c	1.303 $\pm$ 0.03b	0.116 $\pm$ 0.007a	7.156 $\pm$ 0.68a	0.238 $\pm$ 0.02
Head lettuce	40 control	73.86 $\pm$ 2.5	0.811 $\pm$ 0.05	0.147 $\pm$ 0.004c	8.447 $\pm$ 0.22	0.198 $\pm$ 0.00c
	80	82.16 $\pm$ 4.5	0.655 $\pm$ 0.04	0.073 $\pm$ 0.002a	8.366 $\pm$ 0.17	0.170 $\pm$ 0.01ab
	120	80.53 $\pm$ 2.5	0.809 $\pm$ 0.03	0.059 $\pm$ 0.006a	8.684 $\pm$ 0.53	0.142 $\pm$ 0.01a
	160	82.82 $\pm$ 1.8	0.628 $\pm$ 0.03	0.113 $\pm$ 0.004b	10.11 $\pm$ 0.10	0.193 $\pm$ 0.01c
Endive	40 control	86.22 $\pm$ 1.3a	0.500 $\pm$ 0.05a	0.052 $\pm$ 0.003a	4.739 $\pm$ 0.47	0.173 $\pm$ 0.01
	80	89.86 $\pm$ 0.4a	0.421 $\pm$ 0.23a	0.066 $\pm$ 0.006a	4.908 $\pm$ 0.04	0.192 $\pm$ 0.00
	120	118.5 $\pm$ 0.8b	0.583 $\pm$ 0.04a	0.142 $\pm$ 0.008b	6.295 $\pm$ 0.35	0.224 $\pm$ 0.02
	160	118.7 $\pm$ 1.7b	0.947 $\pm$ 0.02b	0.163 $\pm$ 0.001b	5.271 $\pm$ 0.21	0.210 $\pm$ 0.01

\* Data followed by the same letter do not differ significantly.

## Ions concentration

The Mg enrichment resulted in a significantly increased concentration of this element in the edible parts of plants (by 48-77%, 14-44% and 21-54% for Romaine lettuce, head lettuce and endive, respectively) (Table 4). The content of Mg was the highest in endive, in which the addition of 160 mg Mg dm<sup>-3</sup> enabled Mg in leaf DM to reach 0.46%. The lowest content was recorded in Romaine lettuce, where it equalled 0.26% of leaf DM.

Mg enrichment usually caused a significant increase in the concentration of K in endive (by 9-26%) and in Romaine lettuce (by 16-24%). The Zn concentration barely changed, except for a significant increase in head lettuce enriched with 120 mg Mg dm<sup>-3</sup>. The addition of 160 mg Mg dm<sup>-3</sup> resulted in a significantly higher concentration of Ca in endive (by 16%), but lower in

Table 4

Content of ions in leaves of Romaine lettuce, head lettuce and endive plants grown in hydroponic system with nutrient solution enriched with Mg. Data are mean  $\pm$ SE,  $n = 3$

Species	Mg (mg dm <sup>-3</sup> )	Mg	K	Ca	Fe	Zn
		(mg g <sup>-1</sup> DM)				
Romaine lettuce	40 control	1.469 $\pm$ 0.05a*	31.26 $\pm$ 0.07a	8.324 $\pm$ 0.04	0.052 $\pm$ 0.002c	0.020 $\pm$ 0.000
	80	2.176 $\pm$ 0.04b	36.14 $\pm$ 1.22ab	8.852 $\pm$ 0.19	0.041 $\pm$ 0.000a	0.017 $\pm$ 0.000
	120	2.582 $\pm$ 0.12b	38.76 $\pm$ 1.09b	8.262 $\pm$ 0.28	0.048 $\pm$ 0.000bc	0.018 $\pm$ 0.001
	160	2.606 $\pm$ 0.93b	31.71 $\pm$ 1.15a	8.123 $\pm$ 0.12	0.044 $\pm$ 0.001b	0.017 $\pm$ 0.001
Head lettuce	40 control	2.554 $\pm$ 0.08a	37.43 $\pm$ 0.51ab	10.13 $\pm$ 0.20b	0.074 $\pm$ 0.000c	0.022 $\pm$ 0.000ab
	80	3.255 $\pm$ 0.23ab	33.14 $\pm$ 1.24a	9.320 $\pm$ 0.29b	0.065 $\pm$ 0.001bc	0.020 $\pm$ 0.001ab
	120	3.673 $\pm$ 0.03b	40.07 $\pm$ 0.21b	9.495 $\pm$ 0.07b	0.054 $\pm$ 0.002ab	0.025 $\pm$ 0.001b
	160	2.908 $\pm$ 0.01ab	35.56 $\pm$ 0.25ab	6.976 $\pm$ 0.14a	0.046 $\pm$ 0.003a	0.020 $\pm$ 0.001a
Endive	40 control	3.005 $\pm$ 0.02a	39.61 $\pm$ 0.68a	11.07 $\pm$ 0.08a	0.103 $\pm$ 0.003b	0.019 $\pm$ 0.000
	80	3.634 $\pm$ 0.07ab	43.38 $\pm$ 1.18a	10.89 $\pm$ 0.12a	0.070 $\pm$ 0.002a	0.020 $\pm$ 0.001
	120	4.295 $\pm$ 0.09bc	49.80 $\pm$ 0.13b	11.93 $\pm$ 0.31ab	0.074 $\pm$ 0.006a	0.020 $\pm$ 0.001
	160	4.654 $\pm$ 0.18c	49.99 $\pm$ 0.97b	12.81 $\pm$ 0.16b	0.105 $\pm$ 0.006b	0.022 $\pm$ 0.001

\* Data followed by the same letter do not differ significantly.

head lettuce (by 31%). Regardless of the Mg addition, the concentration of Fe was reduced (by 8-21%, 12-38% and 29-32% respectively for Romaine lettuce, head lettuce and endive). The only exception was endive enriched with the highest Mg concentration, in which Fe was on the level of control (Table 4).

## DISCUSSION

The challenge faced by modern plant producers is to meet consumers' food preferences, i.e. to make healthy products with a high nutritive value. Therefore, attention is focused on the cultivation of plants enriched with essential elements. Enrichment can be justified when no negative effects on the yield and nutritive value appear parallel to an increased accumulation of the desired element.

### Enrichment with Mg and its effect on concentration of other ions

The results of this work proved that enrichment with Mg is possible. However, differences between the cultivars with regards to Mg accumulation



were recorded. Romaine lettuce presented the greatest increase of Mg when compared to the control, but simultaneously had the lowest Mg concentration, while endive turned out to be the richest in Mg both in the control and after enrichment. Despite the differences in the Mg concentrations, consumption of 50 g FW of all the species contributed to satisfying the Recommended Daily Allowance – RDA (*Magnesium ...* 1997). For females (320 mg Mg day<sup>-1</sup>) Romaine lettuce plant provide 2.0-4.0% of the recommended dose of Mg, head lettuce 1.8-2.8% and endive 3.2-4.2%. In the case of males, these values were slightly lower because of a higher RDA value (420 mg Mg day<sup>-1</sup>).

One of the problems caused by ion enrichment is the depletion of other ions, but this did not occur in our study. The antagonism between Mg and K/Ca has been documented (HERMANS et al. 2013). For example, a decreased content of K and Ca has been demonstrated in sunflower exposed to Mg concentrations similar to those applied in our experiment (LASA et al. 2000). In contrast, the content of K in our study was often higher in Mg enriched plants. Positive effects of Mg on the uptake and translocation of K from the root to the shoot was shown by DING et al. (2006). The synergistic behaviour between Mg and K is possible owing to the role of Mg in Mg-ATP complexes, which are required for active absorption of K across the root cells (DING et al. 2006). In the current experiment, the only element whose concentration significantly decreased after enrichment with Mg was Fe. The effect of excess Mg on concentration of Fe is not well described in literature. HERMANS et al. (2013) proved that the concentration of divalent cations, including Fe in *Arabidopsis thaliana* plants increases in Mg deficient plants, suggesting that in Mg enriched plants a reverse relationship could be observed.

### **Biomass production and efficiency of photosynthetic apparatus**

Magnesium is essential in many metabolic processes and its deficiency has been reported (HERMANS et al. 2004, SHABALA, HARIADI 2005), but less is known about the effects of excess Mg (CAKMAK, KIRKBY 2008). In the present study, Mg had a slight effect on biomass accumulation, suggesting that Mg enrichment can be achieved without compromising of yield. No significant relationships between Mg enrichment and biomass were recorded in cabbage, either (BROADLEY et al. 2008).

Reduction of biomass is often preceded by a decreased efficiency of the photosynthetic apparatus. In Mg deficient plants, the phloem transport of photoassimilates is impaired, the content of chlorophylls is reduced, the activity of enzymes involved in CO<sub>2</sub> fixation is lowered and the stomatal conductance is diminished (HARIADI, SHABALA 2004, HERMANS et al. 2004, CAKMAK, KIRKBY 2008). Increased levels of Mg also impair photosynthesis, although the current knowledge of this phenomenon is limited (CAKMAK, KIRKBY 2008). SHAUL (2002) suggests that high levels of Mg inhibit K transport from the cytosol to the stroma and interfere with Mg homeostasis within the chloroplast, thus decreasing the efficiency of photosynthesis by reducing the stro-

mal pH and disturbing the function of CO<sub>2</sub> assimilating enzymes. In the present study, Mg enrichment affected the efficiency of the photosynthetic apparatus to a small extent, which corresponded well with the changes in biomass accumulation. This can be explained by an elevated, albeit harmless content of Mg in plants after enrichment. For an optimal growth, plants require 0.15-0.35% Mg in dry matter and when this percentage increases to 1.5% it becomes critical to photosynthesis (SHAUL 2002). In this work, the content of Mg in enriched plants was close to the optimum (0.26-0.46%). These results confirmed the few findings demonstrating a lack of negative effects of elevated Mg (up to 200 mg Mg dm<sup>-3</sup>) on the photosynthetic apparatus (LASA et al. 2000, HARIADI, SHABALA 2004).

Chlorophyll *a* fluorescence *A* is another parameter sensitive to non-optimal Mg (HERMANS et al. 2004). This work revealed that the measurements performed on dark and light-adapted (data not shown) plants were unaffected by Mg. LASA et al. (2000) did not record any changes in chlorophyll *a* fluorescence in sunflower grown at 120 mg Mg dm<sup>-3</sup>. HARIADI and SHABALA (2004) demonstrated that 200 mg Mg dm<sup>-3</sup> decreased Fv/Fm, but no earlier than 8 weeks after treatment.

### **Generation of ROS and antioxidative system activity**

Functional food, including plants enriched with essential ions, is considered to be a source of antioxidants. It was shown that enrichment of lettuce with I and Se increased the content of antioxidants (RIOS et al. 2008, BLASCO et al. 2011). However, many diseases may be exacerbated when enriched plants become a source of ROS, as an excess of ions promote their generation (BAZZANO et al. 2002). Under Mg deficiency, the generation of ROS is caused by the accumulation of carbohydrates followed by over-reduction in the photosynthetic electron transport chain (CAKMAK, KIRKBY 2008), while a high concentration of Mg blocks K channels in the inner envelope membrane of the chloroplast stroma, leading to its acidification, inactivation of key enzymes in carbon fixation and consequently to increased ROS production (WU et al. 1991, DING et al. 2006). The novel results of our research indicate that Mg concentrations in leafy vegetables enriched with this element may be high enough to induce greater generation of ROS. The extent to which Mg affects ROS generation depends on many factors, such as a plant cultivar and Mg content. Therefore, enrichment strategies must consider this as a possible risk to customers.

Production of Mg enriched plants can be justified when ROS formation is countered by a sufficiently effective antioxidant system. In the present study, the activity of two enzymes and the content of phenols were often increased after Mg enrichment. Plant phenols are important antioxidants, which reduce the risk of various chronic diseases in humans (RICE-EVANS et al. 1997), but they belong to inhibitors that limit the absorption of many micronutrients. However, no information is available on their influence on Mg

absorption in humans (BOHN 2008). Therefore, a positive relationship between the concentrations of Mg and phenols might be considered as advantageous. Since this study investigated only selected ROS and antioxidants, it is difficult to state unambiguously whether an increase in the antioxidative potential was sufficient to mitigate the negative effects of ROS, but it should be regarded as a threat and be taken into an account.

## CONCLUSIONS

Enrichment with Mg is possible, but results vary between cultivars. The concentration of Mg in plants was high enough for them to be considered as an alternative dietary source of this element. Enrichment can be achieved without depleting other ions, reducing the efficiency of the photosynthetic apparatus and compromising yield. However, the addition of Mg was accompanied by an increased generation of ROS and modified activity of the examined components of the oxidative system, suggesting that leafy vegetables enriched with Mg can be a source of elevated levels of ROS in a diet. Therefore, analysis of compounds that constitute the oxidative balance in plants is necessary before an enrichment strategy is proposed. Among the tested plants, endive cv. Burundi proved to be the most suitable for enrichment with Mg. Plants of this cultivar grown in the presence of Mg in the concentration up to 120 mg Mg dm<sup>-3</sup> accumulate considerable amounts of Mg, without negative side effects.

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