

## **THE EFFECT OF CaCl<sub>2</sub> FOLIAR TREATMENT (BEFORE HARVEST) ON THE ACCUMULATION OF NITRATES AND NITRITES IN FRESH AND STORED BUTTERHEAD LETTUCE**

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**Abstract.** The leaves of lettuce are very rich source of vitamins, macro and microelements and biologically active compounds. However, these plants contain also anti-nutritional compounds as nitrates and nitrites which levels depend on growth and storage conditions. The aim of this study was to determine the effect of foliar application of CaCl<sub>2</sub> treatment before harvest on nitrate and nitrite accumulation, in whole leaves and leaf blades in fresh and stored lettuce. The experimental material was lettuce cv. Omega which was grown in greenhouse. The solution of CaCl<sub>2</sub> was used in the concentration of 0.1M and 0.2M on plants, 20 and 10 days before harvest. After harvest, some plants were directly analysed, while the remaining plants were cold-stored at 4°C for 7 and 14 days in dark polyethylene bags. The higher concentrations of nitrate and nitrite was noticed in fresh whole leaves than in leaf blades. The foliar CaCl<sub>2</sub> treatment of plants before harvest contributed to a statistically decrease in the nitrate levels and nitrite increase in whole leaves in fresh plants. The greatest changes were noticed after using of 0.1M CaCl<sub>2</sub> solution on plants. During the storage time of lettuce in the non-treatment plants were observed the higher concentration of nitrates and nitrites in leaf blades compared to fresh plants. The CaCl<sub>2</sub> treatment on lettuce caused a significant decrease in the nitrite concentration of whole leaves of plants stored for 7 days, while in the leaf blades higher nitrite amounts were accumulated in lettuce which was stored in cold conditions during all experimental time.

**Key words:** *Lactuca sativa* L., CaCl<sub>2</sub> foliar application, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, cold storage

### **INTRODUCTION**

Leaf vegetables are an important component of a balanced diet which promotes the consumption of higher quantities of fruit and vegetables. Nitrate III and V, in slight amounts, are natural components of higher plants and are involved in biochemical and

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physiological processes. The presence of nitrates in vegetables and other food products is a serious threat of human health. The harmful effects of nitrates are related to the dangerous compounds that are synthesized in the organism. Approximately 5% of all nitrate is converted in saliva and the gastrointestinal tract to the nitrite [Pannala et al. 2003, Santamaria 2006]. The most serious danger comes from nitrite which is produced by nitrate reduction and which can lead to methaemoglobinemia or form nitrosamines and nitrosamides by reacting with amines and amides that have carcinogenic action [Bruning-Fann and Kaneene 1993, Mensinga et al. 2003, Santamaria 2006]. On the other hand some components of vegetables, ascorbic acid, tocopherols, carotenoids or flavonoids, inhibit the toxic effect of nitrites [Walker 1990, Steinmetz and Potter 1991]. Also mineral components, potassium and calcium present in soil, affect on assimilation and translocation of nitrates in the plant [Ruiz et al. 1999].

There are some suggestions that the protective effect of certain vegetables on the cardiovascular system is related to their high content of inorganic nitrate which in concert with symbiotic bacteria in the oral cavity is converted into nitrite, nitric oxide and secondary reaction products with vasodilating and tissue-protective properties [Lundberg et al. 2006].

The leafy vegetables are the major vehicle for the entry of nitrate into the human system. Van Velzen et al. [2008] in their study show that nitrate is absorbed from leafy vegetables very effectively, resulting in an absolute nitrate bioavailability of around 100%.

The first step in nitrate assimilation in plant – the reduction of nitrate to nitrite, catalyzed by NAD(P)H: nitrate reductase – is highly regulated. In many plant species, leaves are the primary site of nitrate reduction [Ruiz et al. 1999]. A potentially important factor controlling the phosphorylation status of nitrate reductase is cytosolic  $\text{Ca}^{2+}$  [Huber et al. 1996].

Calcium is considered as an important nutrient for plants and is known to have a rigidifying effect on cell membranes but it is considered necessary for protection against tipburn incidence mainly if perturbations of membranes by supraoptimal levels of active gibberellins are to be prevented [Saure 1998, Michałojć and Szewczuk 2003a, 2003b, Michałojć and Horodko 2006]. It is known to increase the membrane permeability and thus greater availability of other ions to the site of the enzyme action is possible which may cause an indirect regulation of the enzyme activity in the presence of  $\text{CaCl}_2$  [Bharti et al. 1996] and also may act as inhibitor of nitrate reductase [Ruiz et al. 1999].

Therefore the objective of the present work was to investigate the effect of the foliar application before harvest at different concentrations of  $\text{CaCl}_2$ , on the accumulation of nitrate V and III in whole leaves and leaf blades (leaves without midrib) of fresh and cold-stored lettuce.

## MATERIAL AND METHODS

The study involved pot experiment conducted in the years 2006–2007, in spring, in a greenhouse of Department of Cultivation and Fertilization of Horticultural Plants of University of Life Sciences in Lublin. The experimental material comprised lettuce

cultivar Omega. Pots with a volume of 2L were filled with peat with pH of 5.4 and limed to pH of 6.4 with calcium carbonate. There was added 4 g of superphosphate, equivalent to 0.8 g of phosphorus, Cu – 26.6 mg, Mn – 10.2 mg, B – 3.2 mg, Mo – 7.4 mg, Zn – 1.48 mg per pot. Before planting and twice after planting: N – 0.75 g, K – 1.5 g and Mg – 0.45 g, totalling in experiment, were added to the pots. Temperature in the greenhouse was maintained at 18°C at night and 23°C during the day. The three factors experiment was performed in randomized split-plot method in 5 replications where 1 plant in 1 pot was one replication. Experimental factors were as following: calcium chloride in concentrations 0.1M, 0.2M and control; time of storage 0, 7 and 14 days and parts of plants: whole leaves and leaf blades without midrib. Each group involved 15 plants. Plants were sprayed twice, 20 and 10 days before harvest. After harvest, in the half of the second decade of April, one group was directly analysed, while the second and third were cold-stored at 4°C for 7 and 14 days in dark polyethylene bags. Plant were washed in deionised water before analysis. Leaf blades without midrib and whole leaves were extracted from each plant, dried at room temperature and grinded. The prepared material was subjected to chemical analyses.

The content of nitrate V and III was determined with usage of Griess reagent, according to PN-92/A-75112 (neq ISO 6635-1984) and measured at  $\lambda = 576$  nm on Shimadzu spectrophotometer UV-Vis. The levels of nitrate III and V were calculated on the basis of standard curve and expressed as  $\text{mg}\cdot\text{kg}^{-1}$  f.w.  $\text{NaNO}_2$  and  $\text{NaNO}_3$ .

Statistical analysis was made by an analysis of variance (ANOVA), using Statgraphics v 3.1 for Windows. Tuckey's test (at a 5% significance level) was used to evaluate the significant differences among the mean values.

## RESULTS AND DISCUSSION

Nitrate content in vegetables depends on many factors, such as fertiliser usage, cultivation conditions, vegetation period, storage time [Pennington 1998, Tamme et al. 2006], the edible parts of the analysed vegetable species and depended on the cultivar [Majkowska-Gadomska et al. 2009].

The results obtained in this study indicated that nitrate concentrations differed among of the edible part of leaves of cv Omega (tab. 1). The nitrate level expressed in  $\text{NaNO}_3$  varied from 1927.66 to 2481.76  $\text{mg}\cdot\text{kg}^{-1}$  f.w. respectively in leaf blades and whole leaves of fresh control plants. The values are about twice lower than date accepted by Commission Regulation Members States in 2006 for lettuce which was cultivated in greenhouse and harvested from 1 April to 30 September [Gajda-Wyrębek et al. 2009]. The results obtained by Prasad and Chetty [2008] for the content of nitrates in fresh lettuce ranged from 1297 to 5658  $\text{mg}\cdot\text{kg}^{-1}$ , determined as  $\text{NO}_3^-$ -N and were higher than nitrate contents in lettuce assayed by Correia et al. [2010] (1156  $\text{mg}\cdot\text{kg}^{-1}$ ). In our experiments the values of 2481.76  $\text{mg}\cdot\text{kg}^{-1}$  fw expressed as  $\text{NaNO}_3$ , determined for lettuce var. Omega.

The results of this study of  $\text{CaCl}_2$  foliar treatment on lettuce cv Omega before harvest on nitrate and nitrite accumulations in fresh and stored plants are presented in table 1 and 2.

A statistical analysis showed that the  $\text{CaCl}_2$  foliar treatment on plant before harvest had a significant effect on nitrate content in the leaves of lettuce (tab. 1).

Table 1. Concentration of nitrates in fresh and stored lettuce cv Omega after  $\text{CaCl}_2$  foliar treatment (means for 2006–2007)

Tabela 1. Zawartość azotanów w świeżej i przechowywanej sałacie odmiany Omega po traktowaniu dolistnym  $\text{CaCl}_2$  (średnie z lat 2006–2007)

Analysed part of plant Analizowana część rośliny	$\text{CaCl}_2$ concentration (M) $\text{CaCl}_2$ stężenie (M)	$\text{NaNO}_3$ ( $\text{mg}\cdot\text{kg}^{-1}$ fw) – ( $\text{mg}\cdot\text{kg}^{-1}$ św.m.)			
		storage time (days) okres przechowywania (dni)			mean średnio
		0	7	14	
Whole leaves Całe liście	0	2481.76	3385.01	2313.06	2726.61
	0.1	1316.40	1939.47	1489.24	1581.70
	0.2	1938.98	2252.39	2348.14	2179.84
	mean – średnio	1912.38	2525.62	2050.15	2162.72
	$\text{LSD}_{0.05} - \text{NIR}_{0.05}$				
	$\text{CaCl}_2$ storage time okres przechowywania $\text{CaCl}_2 \times$ storage time $\text{CaCl}_2 \times$ okres przechowywania		128.08 127.50 144.25		
Leaf blades Błaszki liściowe	0	1927.66	2253.56	2407.97	2196.39
	0.1	2453.25	2428.99	2423.71	2435.32
	0.2	2586.07	2180.64	2498.55	2421.75
	mean – średnio	2322.33	2287.73	2443.41	2351.15
	$\text{LSD}_{0.05} - \text{NIR}_{0.05}$				
	$\text{CaCl}_2$ storage time okres przechowywania $\text{CaCl}_2 \times$ storage time $\text{CaCl}_2 \times$ okres przechowywania		160.35 161.30 208.12		

It was noticed decreasing the nitrate levels in whole leaves of plants treatment with  $\text{CaCl}_2$  in the concentrations of 0.1M and 0.2M, respectively about 47% and 22%, compared to control. However, in leaf blades these values were higher than in control plants.

Ruiz et al. [1999] in their study with *Nicotiana tabacum* showed that  $\text{NO}_3^-$  availability in different part of the plant was influenced by  $\text{CaCl}_2$  treatment. Nitrate was translocated towards the aerial part and subsequently assimilated in the leaves with the treatments 1.25 mM  $\text{CaCl}_2\cdot\text{H}_2\text{O}$  and 2.5 mM  $\text{CaCl}_2\cdot\text{H}_2\text{O}$ , the latter significantly intensifying these processes.

As shown by experimental data, nitrate concentrations varied significantly during time storage. The highest concentrations of these compounds were noticed after 7 days of cold storage in whole leaves, and in leaf blades after 14 days in non-treatment plants (tab. 1).

The using CaCl<sub>2</sub> foliar treatment on lettuce before harvest influenced on decreasing of nitrate levels in whole leaves during 7 and 14 days of storage plants in cold conditions, but in leaf blades the significant decrease of these compounds was noticed in plants treated with 0.2M CaCl<sub>2</sub> only after 7 days of storage (tab. 1).

According to results obtained by Chung et al. [2004] for fourth vegetables refrigerated storage caused nitrate and nitrite values to be largely unaffected during 7 days. In our experiments the foliar CaCl<sub>2</sub> treatment on leaves of lettuce caused the lower accumulation of this ion during refrigerated storage in whole leaves, compared to non-treated plants.

Table 2. Concentration of nitrite in fresh and stored lettuce cv Omega after CaCl<sub>2</sub> foliar treatment (means for 2006–2007)

Tabela 2. Zawartość azotynów w świeżej i przechowywanej sałacie odmiany Omega po dolistnym traktowaniu CaCl<sub>2</sub> (średnie z lat 2006–2007)

Analysed part of plant Analizowana część rośliny	CaCl <sub>2</sub> concentration (M) CaCl <sub>2</sub> stężenie (M)	NaNO <sub>2</sub> (mg·kg <sup>-1</sup> fw) – (mg·kg <sup>-1</sup> św.m.)			
		storage time (days) okres przechowywania (dni)			mean średnio
		0	7	14	
Whole leaves Całe liście	0	0.265	0.318	0.192	0.258
	0.1	0.508	0.245	0.467	0.406
	0.2	0.358	0.183	0.615	0.385
	mean – średnio	0.377	0.248	0.424	0.349
	LSD <sub>0.05</sub> –NIR <sub>0.05</sub>				
	CaCl <sub>2</sub>		0.020		
	storage time		0.021		
	okres przechowywania				
	CaCl <sub>2</sub> × storage time		0.028		
	CaCl <sub>2</sub> × okres przechowywania				
Leaf blades Błaszki liściowe	0	0.175	0.265	0.372	0.271
	0.1	0.335	0.389	0.357	0.360
	0.2	0.317	0.341	0.430	0.363
	mean – średnio	0.275	0.332	0.386	0.331
	LSD <sub>0.05</sub> –NIR <sub>0.05</sub>				
	CaCl <sub>2</sub>		0.030		
	storage time		0.031		
	okres przechowywania				
	CaCl <sub>2</sub> × storage time		0.041		
	CaCl <sub>2</sub> × okres przechowywania				

The level of nitrite expressed in NaNO<sub>2</sub> in the leaf blades was lower than in whole leaves and was in the level 0.175 mg·kg<sup>-1</sup> f.w. in control fresh plants (tab. 2). In the whole leaves of fresh plants were noticed that CaCl<sub>2</sub> treatment caused increasing of nitrite levels depended of doses about 92% and 35%, respectively for 0.1M and 0.2M CaCl<sub>2</sub>, compared to control fresh plants (tab. 2). During cold storage of plants were noticed increasing nitrite concentrations about 20% after 7 days and after 14 days decreasing about 27.5% compared to control of fresh plants. The using CaCl<sub>2</sub> treatment

in 0.1M and 0.2M affected on decreasing, respectively, nitrite levels in whole leaves about 23% and 43% compared to non-treatment plants stored during 7 days, but increasing 2.5 and 3.2 times after 14 days stored. The  $\text{CaCl}_2$  treatment on lettuce caused increasing the nitrite levels also in leaf blades in fresh plants about 91% and 81%, dependence on doses, compared to control. The storage time also influenced on increasing of the nitrite content in leaf blades about 51% after 7 days and 113% after 14 days, compared to fresh untreated plants.

The obtained results were lower than cited in literature for lettuce. In the plants which were assayed by Correia et al. [2010] the mean of nitrite levels was  $2.6 \text{ mg}\cdot\text{kg}^{-1}$  (as  $\text{NO}_2^-$ ). It is assumed that the nitrite levels in fresh leafy vegetables are less than  $2 \text{ mg}\cdot\text{kg}^{-1}$  [Santamaria 2006].

Nitrate accumulation is not necessarily accompanied by simultaneous nitrite accumulation in the edible part of vegetables which were also assayed by others authors [Amr and Hadidi 2001]. The different capacity to accumulate nitrate can correlate with a different location of nitrate – reductase activity as well as to a different degree of nitrate absorption and transfer in the plant. The vegetable organs can be listed by decreasing nitrate content as follows: petiole, leaf, stem, root, tuber, bulb, fruit, seed [Santamaria 2006].

Table 3. Concentration dry matter in fresh and stored lettuce cv Omega after  $\text{CaCl}_2$  foliar treatment (means for 2006–2007)

Tabela 3. Zawartość suchej masy w świeżej i przechowywanej sałacie odmiany Omega po dolistnym traktowaniu  $\text{CaCl}_2$  (średnie z lat 2006–2007)

Analysed part of plant Analizowana część rośliny	$\text{CaCl}_2$ concentration (M) $\text{CaCl}_2$ stężenie (M)	Dry matter (%) Sucha masa (%)			
		storage time (days) okres przechowywania (dni)			mean średnio
		0	7	14	
	0	3.929	3.846	4.050	3.942
	0.1	3.832	3.915	3.435	3.727
	0.2	3.890	3.711	4.163	3.921
	mean – średnio	3.884	3.824	3.883	3.863
Whole leaves Całe liście	LSD <sub>0.05</sub> –NIR <sub>0.05</sub>				
	$\text{CaCl}_2$		0.382		
	storage time okres przechowywania		0.327		
	$\text{CaCl}_2 \times$ storage time $\text{CaCl}_2 \times$ okres przechowywania		0.316		
		0	3.920	4.083	4.113
	0.1	4.870	4.342	3.988	4.400
	0.2	4.114	4.059	3.984	4.052
	mean – średnio	4.301	4.161	4.028	4.163
Leaf blades Błazki liściowe	LSD <sub>0.05</sub> – NIR <sub>0.05</sub>				
	$\text{CaCl}_2$		0.324		
	storage time okres przechowywania		0.372		
	$\text{CaCl}_2 \times$ storage time $\text{CaCl}_2 \times$ okres przechowywania		0.404		
		0	3.920	4.083	4.113
	0.1	4.870	4.342	3.988	4.400
	0.2	4.114	4.059	3.984	4.052
	mean – średnio	4.301	4.161	4.028	4.163

According to results obtained by Burns et al. [2011], accumulation of nitrate in different cultivars of butterhead lettuce were negatively correlated with their dry matter contents. In our experiments a similar trends in changes of nitrate concentrations after CaCl<sub>2</sub> treatment were noticed in dry matter contents in fresh and stored lettuce in cold conditions, but nonsignificant one (tab. 3).

## CONCLUSIONS

1. Nitrate and nitrite content in leaves of lettuce cv Omega depended on doses of CaCl<sub>2</sub> foliar treatment, storage time and analysed part of plant.

2. The higher concentrations of these compounds were noticed in fresh whole leaves than in leaf blades.

3. The foliar CaCl<sub>2</sub> treatment on plants before harvest contributed to a statistically decrease in the nitrate levels and nitrite increase in whole leaves of fresh plants. The greatest changes were noticed after using CaCl<sub>2</sub> in the concentration of 0.1M.

4. In the leaf blades of fresh control plants was found the lower nitrate and nitrite levels than in lettuce after CaCl<sub>2</sub> treatment in both concentrations.

5. During the storage time of lettuce in the non-treatment plants were observed the higher concentration of nitrate and nitrite in whole leaves after 7 days, but after 14 days the contents of these compounds decreased and was lower than in fresh plants.

6. The using of CaCl<sub>2</sub> treatment on plants caused a significant decrease in the nitrite concentration of whole leaves stored for 7 days, while in the leaf blades higher nitrite amounts than in fresh plants were accumulated in lettuce which was stored in cold conditions during all experimental time.

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## WPLYW DOLISTNEGO TRAKTOWANIA $\text{CaCl}_2$ (PRZED ZBIOREM) NA AKUMULACJĘ AZOTANÓW I AZOTYNÓW W ŚWIEŻEJ I PRZECHOWYWANEJ SAŁACIE MASŁOWEJ

**Streszczenie.** Liście sałaty są bardzo bogatym źródłem witamin, mikro- i makroskładników oraz związków biologicznie aktywnych. Jednakże rośliny te zawierają również związki antyżywniowe, takie jak azotany i azotyny, których poziom zależy od warunków uprawy i przechowywania. Celem badań było określenie wpływu dolistnego stosowania  $\text{CaCl}_2$  przed zbiorem na akumulację azotanów i azotynów w całych liściach i blaszkach liściowych świeżej i przechowywanej sałaty. Materiał badawczy stanowiła sałata odmiany Omega, uprawiana w szklarni. Roztwór  $\text{CaCl}_2$  zastosowano w stężeniach 0,1M i 0,2M na sałatę, 20 i 10 dni przed zbiorem. Po zbiorze, część roślin została bezpośrednio poddana analizie, natomiast pozostałe rośliny były przechowywane w  $4^\circ\text{C}$  przez 7 i 14 dni w ciemnych foliach polietylenowych. Większe stężenie azotanów i azotynów stwierdzono w świeżych całych liściach niż w blaszkach liściowych. Dolistne traktowanie roślin  $\text{CaCl}_2$  przed zbiorem spowodowało spadek poziomu azotanów i wzrost poziomu azotynów w całych liściach roślin świeżych. Największe zmiany zaobserwowano po zastosowaniu stężenia 0,1M  $\text{CaCl}_2$ . Podczas przechowywania sałaty, w blaszkach liściowych roślin nietraktowanych  $\text{CaCl}_2$  zaobserwowano większe stężenie azotanów i azotynów w porównaniu do roślin świeżych. Traktowanie sałaty  $\text{CaCl}_2$  spowodowało istotny spadek zawartości azotynów w całych liściach roślin przechowywanych przez 7 dni, natomiast w blaszkach liściowych wyższe ilości azotynów akumulowały się w sałacie przechowywanej w warunkach chłodniczych podczas całego okresu eksperymentalnego.

**Słowa kluczowe:** *Lactuca sativa* L., dolistne stosowanie  $\text{CaCl}_2$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , przechowywanie w warunkach chłodniczych

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