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CHANGES IN THE CHLOROPHYLL CONTENT IN STORED LETTUCE *Lactuca sativa* L. AFTER PRE-HARVEST FOLIAR APPLICATION OF $CaCl_2$

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Abstract

The aim of the present investigations was to determine the cumulative effect of foliar treatment of lettuce plants with a $CaCl_2$ solution and the cold-storage period on the level of chlorophylls in the leaves of lettuce cv. 'Omega' (*Lactuca sativa* L.). Plants were grown in a pot experiment conducted in the greenhouse of the Department of Cultivation and Fertilisation of Horticultural Plants, University of Life Sciences in Lublin. During the growing period, 10 and 20 days before harvest, the plants received foliar application of $CaCl_2$ at the concentrations of 0.1 M and 0.2 M. Control plants were sprayed with water. Some plants were analysed immediately after the harvest; other specimens were cold-stored at a temperature of 4°C for 7 and 14 days. Whole leaves and blades without the midrib were analysed. The results obtained indicated that the foliar application of 0.2M $CaCl_2$ in lettuce resulted in a decrease in the level of chlorophylls in fresh plants, compared with the control. A beneficial cumulative effect of the $CaCl_2$ application and storage period on the chlorophyll level in lettuce leaves was observed in the leaf blades of plants after foliar treatment with the 0.1M $CaCl_2$ solution and cold-stored for 7 and 14 days, in which increased levels of chlorophyll "a" and "b" and total chlorophyll, compared with the control, were found.

Key words: *Lactuca sativa* L., foliar $CaCl_2$ application, chlorophylls, cold storage

INTRODUCTION

Lettuce is one of the most popular vegetables available throughout the year. It is a rich source of components important for human health as they protect against lifestyle diseases, e.g. cancers and cardiac, cardiovascular, and age-related eye diseases. The major bioactive substances contained in lettuce include vitamins E, C, provitamin A, lutein, folic acid, polyphenolic compounds, and minerals [1–7]. Fresh-cut plants have a short shelf life, during which biochemical changes may occur as a result of post-harvest activities (cutting, packaging) and storage. A typical sign of these processes is browning and loss of colour that are caused by damage to the leaf surface. Loss of colour results from degradation of chlorophylls as an effect of vegetable aging during the storage period [8,9]. Yellowing or spot browning reduce the commercial value and shorten the shelf life of leafy vegetables during storage [9]. The chlorophyll content has been found to decrease already after several storage days. The reduction in the level of these pigments depends on the species, variety, and temperature [10,11]. A low storage temperature of 4°C inhibits reduction of chlorophyll levels. The process is also dependent on the length of the vegetable storage period [9].

One of the methods for delaying the aging processes of plant tissues is increasing the cellular level of Ca^{2+} ions, which play an important role in the process of vegetative aging and maturation of fruits [12], inhibit the rate of chlorophyll and protein degradation [13] and reduce browning of salads prepared from vegetables [14–16]. However, no investigations have been conducted on the effect of Ca^{2+} applied in plants during the vegetation season on the chlorophyll level in lettuce heads during the cold-storage period.

The aim of the present study was to determine the changes in the chlorophyll content in the leaves of lettuce cv. Omega', which received pre-harvest foliar treatment with $CaCl_2$ and was cold-stored for 7 and 14 days.

MATERIALS AND METHODS

The research material was lettuce cv. Omega' grown in pot experiments set up in the greenhouse of

the Department of Cultivation and Fertilisation of Horticultural Plants, University of Life Sciences in Lublin in 2006 and 2007 years. The average temperature in the greenhouse was 18°C at night and 23°C during the day. The plants were grown in pots (2L) filled with peat with pH=5.4 and neutralised to pH=6 with $CaCO_3$. Nutrients were supplied following the specific requirements of the plant species. During the growing period, the lettuce plants were sprayed with $CaCl_2$ solutions at the concentrations of 0.1M (Ca1) 0.2M (Ca2) and with water (control – 0) 20 and 10 days before harvest. After the harvest, lettuce plants in each experimental series (15 plants) were divided into three groups: some plants were designated for immediate analyses and other specimens were cold-stored (4°C) in dark polyethylene bags for 7 and 14 days. Prior to the analyses, the plants were washed with distilled water and whole leaves and leaf blades without the midrib were separated, fragmented into ca. 1 cm^2 pieces, and prepared for the analysis.

Chlorophylls were determined with Arnon's method [17] in acetone extracts prepared from the plant material by acetone homogenization and filtration under reduced pressure. Extracts prepared from whole leaves and leaf blades from fresh and cold-stored lettuce were subjected to the analysis. The chlorophyll content was measured based on extract absorbance at two wavelengths, 645 nm for chlorophyll "a" and 663 nm for chlorophyll "b", using a Shimadzu UV-VIS spectrophotometer.

RESULTS

The results obtained demonstrated that the chlorophyll content depended on the analysed part of the lettuce leaf, concentration of $CaCl_2$ applied through pre-harvest foliar spraying, and the duration of cold storage.

The analysis of the content of chlorophyll "a" in fresh whole leaves and leaf blades revealed an approximately 20% lower concentration thereof in the leaves than in the leaf blades (Fig.1A and B). The treatment of lettuce plants with the $CaCl_2$ solution resulted in an over 30% decrease in the chlorophyll content in the fresh whole leaves upon the application of the 0.1M and 0.2 M concentrations and in the leaf blades of plants sprayed with 0.2M $CaCl_2$.

During the cold storage of control lettuce plants at 4°C, a decrease in the chlorophyll "a" level was found both in the whole leaves after 14 days and leaf blades after 7 days. In the whole leaves of the control plants, the level of chlorophyll "a" decreased by 17.5% in plants stored for 14 days, compared with its content in fresh leaves. In turn, $CaCl_2$-treated plants initially exhibited a decline in the chlorophyll level by ca. 32%; after 7 days, an over 21% increase was noted in plants treated with 0.1M $CaCl_2$ after 14 days storage, in comparison with the control plants analysed in the same period.

The results of assessment of the chlorophyll "a" contents in the leaf blades of $CaCl_2$-untreated and cold-stored control plants indicated an approximately 21% reduction in the level of this type of chlorophyll after 7 days, in comparison with the level in fresh plants, whereas the 14-day storage did not change the content of chlorophyll "a" (Fig. 1.B). It was found, however, that the leaf blades of cold-stored plants sprayed with a 0.1 M $CaCl_2$ solution (Ca1) before the harvest contained higher (by 35% and ca. 40%) levels of chlorophyll "a" than the leaf blades in the control plant samples stored in the same conditions.

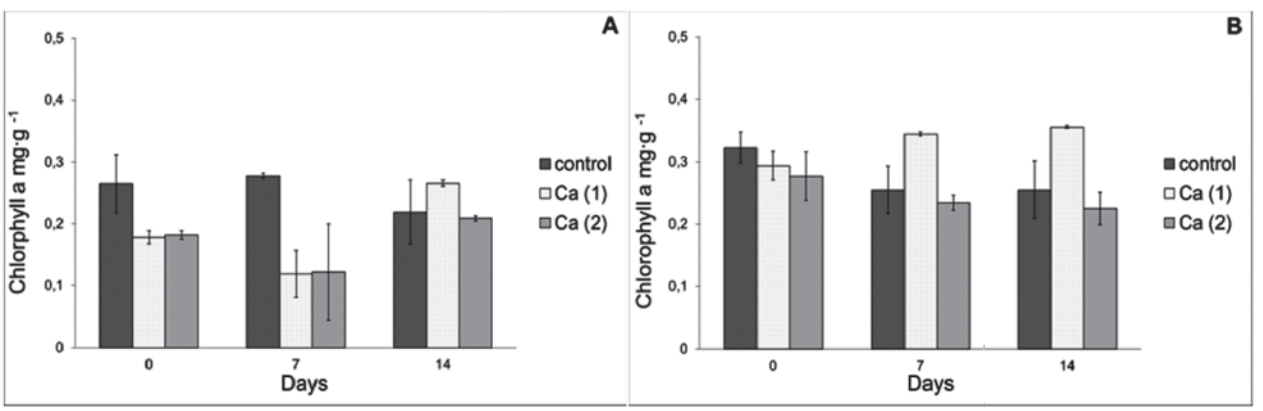

Fig. 1. Content of chlorophyll "a" in whole leaves (A) and leaf blades (B) of fresh and stored lettuce plants cv. 'Omega' after pre-harvest $CaCl_2$ treatment.

Explanations: The figure presents mean values from 2006 and 2007 years with ± SD

The results of the analysis of the chlorophyll "b" content in lettuce showed that the Omega lettuce leaves were characterised by a lower content of chlorophyll "b" than chlorophyll "a" and, as in the case of chlorophyll "a", it depended on the analysed part of the leaf, pre-harvest $CaCl_2$ application, and the length of the cold-storage period (Fig. 2 A and B).

In the whole leaves of the fresh control plants, the level of chlorophyll "b" was by approximately 35% lower than that in the leaf blades. Spraying the plants with 0.1M $CaCl_2$ exerted a beneficial effect on accumulation of chlorophyll "b" in fresh lettuce leaves – a higher content of this chlorophyll was detected in the whole leaves (by 12 %) and leaf blades (by 9.6 %) of fresh lettuce than in the control.

During the cold-storage period, the content of chlorophyll "b" increased in the whole lettuce leaves and decreased in the leaf blades. The strongest effect was observed after 7 days of storage, where the chlorophyll level in the whole leaves increased by 55% and decreased by 23 % in the leaf blades, compared with the fresh control plants (0).

The pre-harvest application of $CaCl_2$ in lettuce had a varied effect on the content of chlorophyll "b" in the whole leaves of cold-stored plants (Fig. 2.A). In plants pre-treated with 0.1M $CaCl_2$, the level of chlorophyll "b" declined by 63% in the whole leaves of plants at 7-day storage, in comparison with the control stored in the same conditions. In turn, after 14 days, the content of chlorophyll "b" increased, compared with the control, by over 22 % and 53.5% in plants pre-treated with $CaCl_2$ at the concentrations of 0.1M and 0.2M, respectively (Fig. 2A).

The favourable effect of foliar $CaCl_2$ application on plants was evident in the leaf blades of cold-stored lettuce, in which after 7 and 14 days of storage the chlorophyll "b" level increased by 36% and 23%, respectively, in plants pre-treated with the 0.1M $CaCl_2$ concentration, compared with the control (Fig. 2.B).

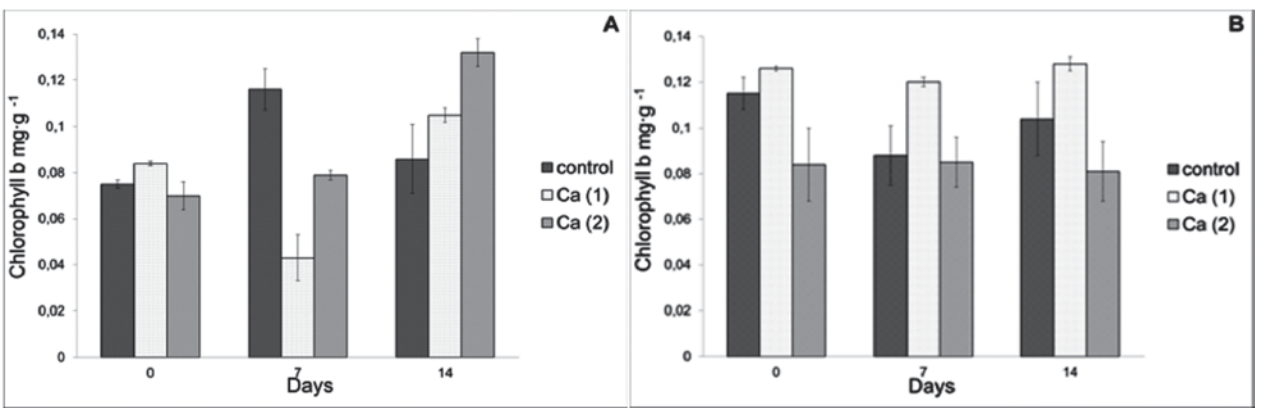

Fig 2. Content of chlorophyll "b" in whole leaves (A) and leaf blades (B) of fresh and stored lettuce plants cv. 'Omega' after pre-harvest $CaCl_2$ treatment

Explanations as in Fig. 1

Changes in the total chlorophyll content induced by $CaCl_2$ application and cold storage in the whole leaves and leaf blades in lettuce are presented in Figure 3 A and B. It can be concluded based on the results obtained that the direction of changes in the content of total chlorophyll was similar to that in the changes in the chlorophyll "a" level (Fig. 1 A and B). Pre-treatment of the plants with $CaCl_2$ lowered the total chlorophyll content in whole fresh leaves by over 33%, in comparison with the control samples (Fig. 3A).

After the 7-days cold-storage period, a slight increase (by ca. 3.6%) in the total chlorophyll content was recorded in the whole leaves; after 14 days, the level decreased by approximately 20%, compared with the fresh control samples. Foliar application of $CaCl_2$ in the plants yielded a varied effect on the total chlorophyll levels in the cold-stored lettuce leaves. In the whole leaves of plants pre-treated with 0.1M and 0.2M $CaCl_2$, the content of total chlorophyll declined by 59% and 49% after 7-day plant storage, and increased after 14 days by 22.5% and 12.2%, respectively, compared with the content in control plants stored in the same conditions.

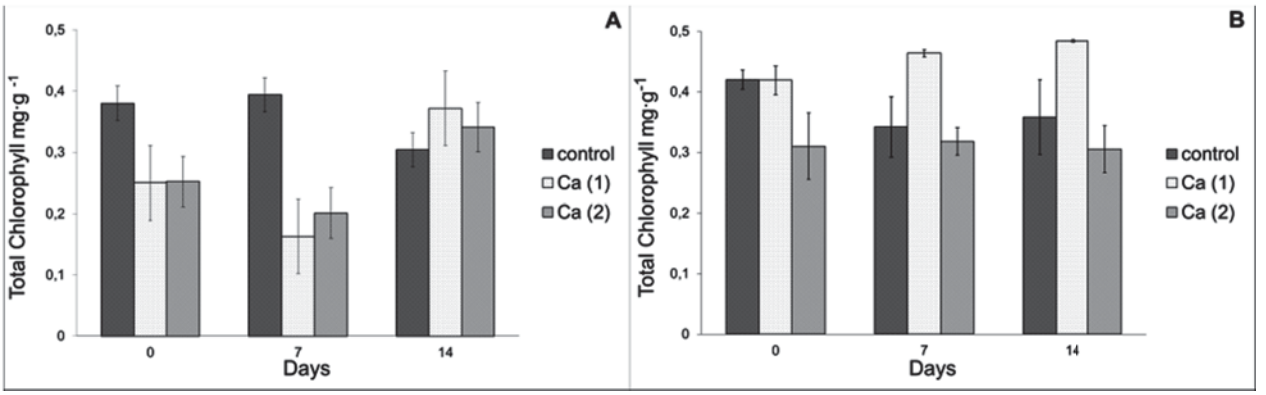

Fig. 3. Content of total chlorophyll in whole leaves (A) and leaf blades (B) of fresh and stored lettuce plants cv. 'Omega' receiving pre-harvest $CaCl_2$ treatment

Explanations as in Fig. 1

A beneficial effect of $CaCl_2$ application on the total chlorophyll content was found in the leaf blades of cold-stored plants. Compared with the control, the content of total chlorophyll was higher by 35.7% and 35.2% in the leaf blades of 0.1M $CaCl_2$-treated plants stored for 7 and 14 days, respectively.

DISCUSSION

Lettuce is a non-durable vegetable, in which degradation of leaf pigments (chlorophylls and carotenoids) or tissue browning occurs during storage [11]. The total chlorophyll content in lettuce leaves begins to decline as early as a few days after harvesting.

The results of the present investigations have demonstrated that the degree of chlorophyll degradation in lettuce depends on the analysed part of the leaf, duration of the cold-storage period, and the concentration of $CaCl_2$ applied through pre-harvest plant spraying. It was found that the whole lettuce leaves were characterized by a lower content of chlorophyll "a", "b", and total chlorophyll than the leaf blades without the midrib. The analyses of the changes in the chlorophyll levels in the leaves of whole lettuce heads stored in dark polyethylene bags at 4°C for 7 and 14 days showed a significant decline in the level of chlorophyll "a" and a decreased content of chlorophyll "b" and total chlorophyll in the leaf blades in the control samples, primarily during the initial storage period, i.e. after 7 days. The pre-harvest application of the 0.1 M $CaCl_2$ solution in lettuce inhibited degradation of chlorophyll "a", "b" and total chlorophyll in the leaf blades, whereas the 0.2 M concentration did not exert such an effect. The degree of chlorophyll degradation during 7-day storage was similar to that in the control plants, whereas the longer storage period (14 days) increased the chlorophyll degradation rate.

Reduction of the chlorophyll content in stored freshly harvested vegetables at different temperatures was reported by other researchers [11]. Investigations conducted by Ferrante and Maggiore [11] demonstrated the effect of sodium hypochloride and storage temperature, on chlorophyll (a+b) stability. The leaves of *Valeriana* lettuce were washed with sodium hypochloride, dried and placed in the plastic bags for storage in 4°C and 10°C. After 5 days chlorophyll content decreased only in leaves stored at 10°C but after 8 days, chlorophyll losses were found in leaves stored at both temperatures. The typical shelf life in most cases is 5–6 days [10].

In the other experiment, Ferrante et al. [9] there were evaluated the effect of cutting leaves of lettuce on chlorophylls (a+b) changes during storage. Before storage, leaves were washed with sodium hypochloride, cut and storage in plastic bag at 4°C. Results of this study showed that chlorophyll content did not change until 5 days, but significantly decreased (- 41%) in the cut leaves after 8 days of storage compared with control.

Available literature does not provide information about the cumulative effect of pre-harvest foliar application of $CaCl_2$ and the cold-storage period on the quality of leafy vegetables. The impact of calcium ions on chlorophyll levels was investigated in other vegetables, e.g. in cucumber cotyledons, where relatively low $CaCl_2$ concentrations were found to reduce the aging effect in the cotyledons, while higher 10^{-3}M concentrations resulted in increased ethylene and carbon dioxide production, H_2O_2 accumulation, and loss of chlorophylls. Calcium is thought to play a regulatory role in hormonal processes occurring in aging tissues [13].

Other factors that may induce changes in chlorophylls, for instance UV-B radiation [15], have also been investigated. The results obtained by Caldwell and Britz [15] have demonstrated that application of UV-B radiation in growing plants increases the content of chlorophyll "a" and "b" as well as total chlorophyll.

CONCLUSIONS

1. Based on the results obtained in this study, it can be concluded that the content of chlorophylls in the Omega lettuce depended on the analysed part of the lettuce leaf, concentration of $CaCl_2$ in the pre-harvest foliar application, and duration of cold storage of lettuce. The leaf blades contained higher levels of chlorophyll than the whole leaves.
2. During the 7- and 14-day cold storage, the content of chlorophylls in the leaf blades decreased, compared with that in the fresh control plants.
3. The pre-harvest application of $CaCl_2$ in lettuce led to a decline in the level of chlorophyll "a" and total chlorophyll in fresh plants.
4. The cumulative effect of the application of 0.1M $CaCl_2$ and the 7- and 14-day cold storage of plants was evidenced by the increased levels of chlorophylls in the lettuce leaf blades, in comparison with those in the control plants, which were stored in the same conditions but not treated with $CaCl_2$.

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Authors' contributions

The following declarations about authors' contributions to the research have made: concept of the study: IP; performance of the experiments: KO; analysis of the experimental data IP, KO, BCH; writing of the manuscript: IP, BCH.

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Zmiany zawartości chlorofili podczas przechowywania sałaty *Lactuca sativa* L. traktowanej dolistnie $CaCl_2$ przed zbiorem

S t r e s z c z e n i e

Celem przedstawionych badań było określenie łącznego wpływu dolistnego traktowania roślin sałaty roztworem $CaCl_2$ i okresu chłodniczego przechowywania, na poziom chlorofili w liściach sałaty odmiany Omega (*Lactuca sativa* L.). Rośliny pochodziły z doświadczenia wazonowego przeprowadzonego w szklarni Katedry Uprawy i Nawożenia Roślin Ogrodniczych Uniwersytetu Przyrodniczego w Lublinie. W trakcie wzrostu rośliny traktowano dolistnie roztworem $CaCl_2$ w stężeniu 0.1 M i 0.2 M, 10 i 20 dni przed zbiorem. Kontrolę stanowiły rośliny opryskane wodą. Jedną serię roślin analizowano bezpośrednio po zbiorze, podczas gdy pozostałe przechowywano w chłodziarce w temperaturze 4°C przez 7 i 14 dni. Analizowano całe liście i blaszki liściowe bez nerwu głównego. Otrzymane wyniki badań wykazały, że zastosowanie dolistne $CaCl_2$ w stężeniu 0.2M na sałatę przed zbiorem spowodowało obniżenie poziomu chlorofili w świeżych roślinach w porównaniu z kontrolą. Korzystny efekt łącznego działania $CaCl_2$ i okresu przechowywania na poziom chlorofili w liściach sałaty zanotowano w blaszkach liściowych roślin traktowanych dolistnie roztworem 0.1M $CaCl_2$ i przechowywanych w warunkach chłodniczych przez 7 i 14 dni, w których stwierdzono zwiększoną zawartość chlorofilu "a", "b" i ogółem w porównaniu z kontrolą.

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