Researches Regarding the Influence of Cold Storage on the Chlorophyll Content in Lettuce

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Abstract
The aim of the present investigations was to determine the effect of the cold storage period on the content of chlorophylls in the leaves of lettuce and arugula (rucola). The research material consisted in two types of lettuce (Lactuca *sativa* L. var. capitata**;** *Lactuca sativa* L. var. crispa**)** and arugula (*Eruca sativa*) purchased from supermarkets in Timisoara. The quantitative determination of chlorophyll pigments in leaves (SPAD) was made by chlorophyll meter (SPAD 502 Konica-Minolta). During the few days cold storage at a temperature of 4ºC, the content of chlorophyll in the leaf significantly decreased, compared with that in the control group. After 3 days of cold storage arugula and lettuce (Lactuca sativa var. capitata) values of chlorophyll content differ statistically very significantly ($p<0.001$) from the values found in the control group which for lettuce (*Lactuca sativa* L. var. crispa) differs statistically significant ($p<0.05$).

Keywords: Chlorophylls, cold storage, *Eruca sativa, Lactuca sativa* L.

1. Introduction

In the last years, the increase of the impact of chronic diseases, including cancer and cardiovascular diseases make us aware of the importance of daily diet. In order to prevent or to reduce the oxidative stress it is recommended to consume fruits and vegetables rich in natural antioxidants. Also, the naturals antioxidants are studied for other possible effects on the health: detoxification, stimulation of the immunity system, cholesterol metabolism changes, on steroid hormones metabolism, blood pressure decrease, antiviral and antibacterial activity, etc.[1]. The chlorophylls are macro cyclic tetrapirol of Mg^{2+}. Between chlorophylls and hem type compounds there are similarities and differences. The presence of magnesium is essential for the chlorophylls functioning. The magnesium can be replaced by $2H^{+}$, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+}. Among them, the species with Zn^{2+} reproduces to a limited extent the chlorophyll behavior. The chlorophyll is the most important natural source of magnesium, a very important element for the metabolism. The chlorophyll is known as being a strong antioxidant, with detoxification and anti-inflammatory properties for the organism. The green vegetables have a significant contribution of chlorophyll for the organism. The natural chlorophyll has surfactant properties similar to detergents.
The lettuce is one of the most popular vegetables with leaves used to prepare salads and can be found over the entire year. It is a rich source of important components for the human health and which protects against diseases.

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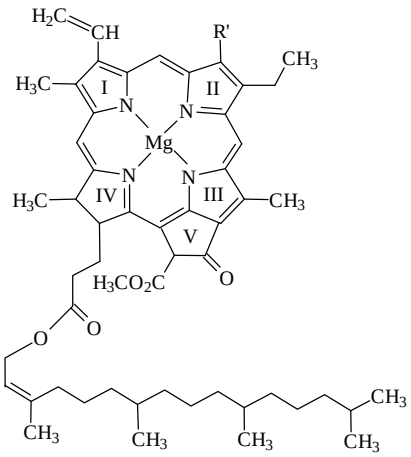

Figure 1. The molecular formula of chlorophylls

The most important bioactive substances contained by the lettuce include vitamins E, C, K, pro-vitamin A, lutein, folic acid and polyphenol compounds. [2-9]. It also contains high quantities of minerals like iron, calcium, magnesium, potassium–important for the organism metabolism. The potassium is an important component of the human body cells and fluids which help to control the heart rhythm and the blood pressure. The manganese is used by the organism as a co-factor for the superoxide dismutase antioxidant enzyme. The copper is necessary to produce red cells from blood. The iron is essential to form the red cells from blood.

The fresh harvested plants have a short shelf life, with biochemical changes as a result of the post – harvest activities (cutting, packing) and storage. The change of color due to the chlorophylls degradation appears as an effect of oldness during the storage period [10, 11]. The yellowing or browning in certain parts reduces the commercial value and shortens the shelf life of vegetables with leaves during the storage period [11]. The chlorophyll content suffers a decrease after few days of storage. The decrease of the content of these pigments depends on a series of factors like: species, variety, temperature, etc. [12, 13] A reduced storage temperature of 4°C prevents the decrease of chlorophyll content. The Arugula (*Eruca Sativa*) is an edible plant from the family Brassicaceae (cabbage family). In our country only the fresh arugula leaves are sold in the majority of supermarkets. The leaves are coming mainly from Italy. It is richer in vitamins than the lettuce. The arugula contains eight times more calcium, five times more vitamin A, C and K and four times more iron than the lettuce. It also contains magnesium, potassium and copper. With a strong taste, bitter, with nutty nuances, arugula can be used fresh in salads but also in cooked meals. Even if it is used as a salad, arugula is part of the cruciferous vegetables family, together with broccoli and cauliflower. The cruciferous vegetables have an important role in the prevention of cancer. As medicinal plant, arugula has also other properties: antioxidant, antiscorbutic, antibacterial, diuretic (the root), improves immunity and adjusts the cholesterol content from blood.

The beneficial effects of a diet rich in vegetables have been partially attributed to an increased consumption of phenols with high antioxidant capacity. Processing vegetables (e.g., salad) for minimally processed products influences the antioxidant capacity of the tissues with different effects in relation to the species used. Many factors can influence the content of secondary metabolites in vegetable products including postharvest processing and storage [14-16].

The purpose of this study was to monitor the changes of the chlorophyll content from the lettuce and arugula leaves bought from REAL supermarkets from Timisoara during the storage period at 4ºC temperature and overall visual quality (OVQ). The idea of this study started with the premise that the majority of the population buys food and implicitly salads from supermarkets and uses them immediately or in few days and they are stored in refrigerators.

2. Materials and methods

The research material was the lettuce and the arugula bought from Timisoara supermarkets. After buying three pieces of each type (lettuce, curly lettuce and arugula), the chlorophyll content was immediately determined and then they were stored in dark colored polyethylene bags at 4ºC temperature in the refrigerator for a period of 7 days, measuring the chlorophyll content when purchased (reference values), after 3 days and after seven days of storage.

The quantitative determination of chlorophyll pigments in leaves was made by chlorophyll meter (SPAD 502 Konica-Minolta). The chlorophyllmeter determine the relative chlorophyll content, by measuring the total leaf

absorbance in two wavelength intervals. Non-invasive measurement; simply clamp the meter over leafy tissue, and receive an indexed chlorophyll content reading (-9.9 to 199.9) in less than 2 seconds.

OVQ is a sensory index closely associated with consumer acceptability. There are different quality components of lettuce OVQ, such as a fresh-looking appearance, bright green color, crispness, and mainly absence of browning. Throughout the sensory analyses, each lettuce was scored independently, making no comparisons among each other. Each sample presented typical organoleptic characteristics mainly differentiated by leaf size, color, and texture. The maxim score initial was 9 (at 0 days storage). The samples with OVQ scores below 5 were unacceptable.

Statistical analysis was performed using OriginPro 8.5 software. Data regarding relative chlorophyll content (SPAD) were expressed as mean _standard error (s_x). The results were processed by using one-way analysis of variance (ANOVA). Differences at $p<0.05$ were considered statistically significant.

3. Results and discussion

Lettuce is a non-durable vegetable, in which degradation of leaf pigments (chlorophylls and carotenoids) or tissue browning occurs during storage [12, 13]. The total chlorophyll content in lettuce leaves begins to decline as early as a few days after harvesting.

From the experimental data presented in table 1 we can notice that the highest chlorophyll content is found in arugula. For the two varieties of lettuce we can notice that for the curly lettuce the chlorophyll content is higher and the decrease during the storage period is lower. At the purchasing moment of the two types of lettuce from the supermarket, the average content of chlorophyll was 25.80 SPAD for the capitata variety and 29.46 SPAD for the crisped variety.

Table 1. Average content ($\pm s_x$) of chlorophyll during the storage period at 4°C

Chlorophyll content/lettuce type	0 days	3 days	7 days
Lettuce-*Lactuca sativa* L.-var. capitata	25.80±0.93	18.26±1.19	13.26±1.48
Curly lettuce-lettuce *Lactuca sativa* L.–var crispa	29.46±2.71	22.77±1.54	21.40±1.10
Arugula–*Eruca Sativa*	30.93±0.74	26.19±0.92	23.10±1.04

After 3 days of storage in the refrigerator at 4ºC temperature, the chlorophyll content of the capitata variety decreased to 18.26 SPAD and after 7 days to 13.26 SPAD. For the crispa variety, the chlorophyll content reached 22.77 SPAD after 3 days and 21.40 SPAD after 7 days. The decreases of the chlorophyll content for the capitated variety were significant ($p<0.001$; $F=26.63549$). The decreases of chlorophyll content for the crispa variety were significant ($p=0.011666$; $F=5.109268$).

For arugula, the chlorophyll content decreased from 30.93 SPAD at purchasing to 26.19 SPAD after 3 days and 23.10 SPAD after 7 days of storage. The decreases of chlorophyll content during the storage in the refrigerator at 4ºC temperature were significant ($p<0.001$; $F=18.91376$).

Table 2. The average content ($\pm s_x$) of chlorophyll during the storage period at 4°C fonction of the analyzed part of the lettuce

Chlorophyll content/lettuce type	Lettuce part	0 days	3 days	7 days
Lettuce-*Lactuca sativa* L.-var. capitata	Outside leaves	27.95±1.16	21.43±0.22	16.80±1.63
	Inside leaves	23.64±0.48	15.09±1.13	9.72±0.99
Curly lettuce-lettuce *Lactuca sativa* L.–var. crispa	Outside leaves	34.47±3.32	23.47±1.73	22.79±0.80
	Inside leaves	24.45±3.36	22.06±2.69	20.01±1.97

In figure 1 and table 2, we can notice that for both types of lettuce the chlorophyll content is higher for the outside leaves. The results demonstrated that chlorophyll content depends on the analyzed part of the lettuce and on the storage period.

Storage time introduces some vegetable degradation in lettuce heads appearance, mainly loss of texture, discoloration extension, and development of browning. Figure 2 shows the evolution of OVQ during refrigerated storage. The decrease in chlorophyll content observed in the external leaves strongly correlated with the decrease in OVQ [17].

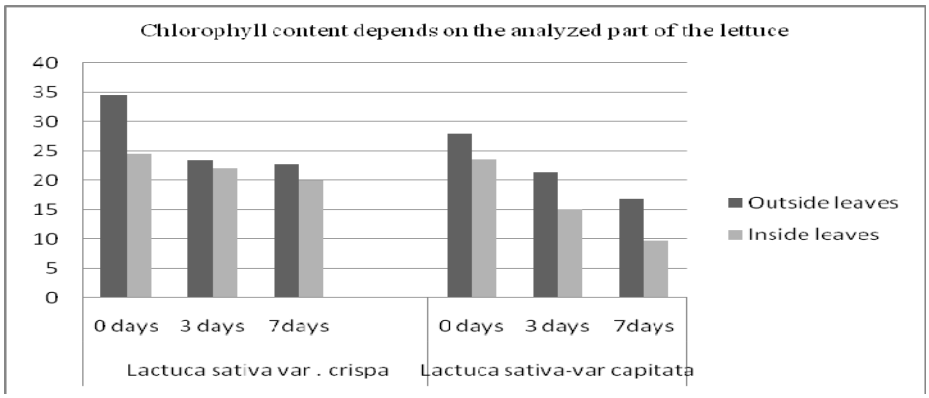

Figure 1. Dependence of the chlorophyll content according to the analyzed part of the lettuce

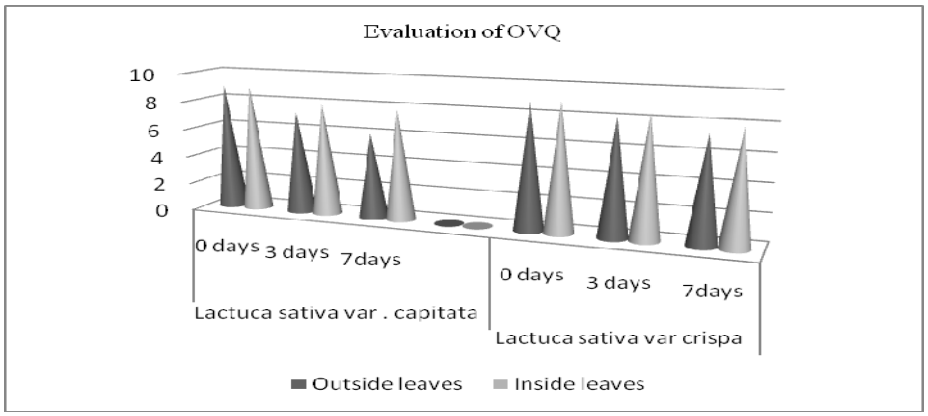

Figure 2. Evaluation of OVQ at lettuce

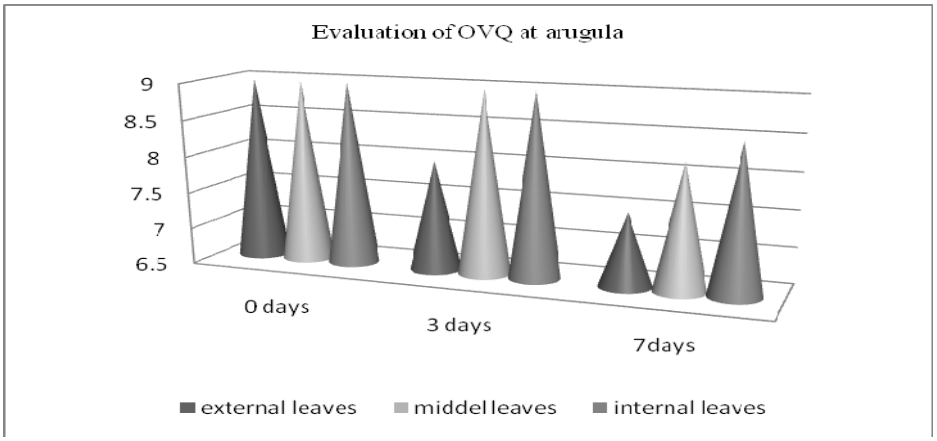

Figure 3. Evaluation of OVQ at arugula

At arugula leaves while the external leaves showed a decrease in OVQ from the 1st sampling day and during all the storage period, the middle and internal leaves showed no reduction in OVQ until 7 days of storage (Figure 3).
It is important to mention that the vegetables must be consumed fresh as much as possible in order to get the chlorophyll contribution.

4. Conclusions

Based on the results obtained in this study, it can be concluded that the chlorophyll content in the lettuce depended on the analysed part of the lettuce leaf and duration of cold storage of lettuce. The arugula contained higher contents of chlorophyll than the lettuce leaves. The chlorophyll content has been found to decrease already after several storage days.

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