COMPARATIVE ANALYSIS OF CHLOROPHYLL AND CAROTENOID PIGMENTS CONTENT OF ARUGULA (*ERUCA SATIVA*) AND LETTUCE (*LACTUCA SATIVA*) LEAVES

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Abstract
The aim of this study was to analyze the chlorophyll and carotenoids content of fresh arugula (*Eruca sativa*, Ruca variety) and lettuce (*Lactuca sativa*, Marilena variety) leaves from the Romanian market. The pigments concentration in the analyzed samples was determined by a spectrophotometric method. In both analyzed samples, chlorophyll "a" predominates to chlorophyll "b". The highest content of chlorophyll pigments was found in leaves of arugula: 31.47 mg/g, while in the lettuce leaves was found a concentration of 27.14.Arugulaleavesare alsothe richestincarotenoids (245.50 μg/g).

Introduction

In recent years, increasing awareness concerning the promotion and protection of health exerted by the bioactive compounds from plant products, has resulted in increased attention to green leafy vegetables as vital components of the daily diet [1].
Green leafy vegetables are an excellent source of fiber, folate, carotenoids and chlorophylls. This plants has also a high content of vitamins C and K and minerals, are rich in antioxidants and removes free radicals from the body, before becoming dangerous [1,2].
Chlorophyll is a specific pigment for green vegetables, which plays a critical role in photosynthesis. It is a fused polycyclic aromatic hydrocarbon; has a structure similar to heme, but contains magnesium instead of iron. There are several different forms of chlorophyll which are in different proportions depending on plant [3]. Until the present we known 12 chlorophylls types, of which the most well researched are chlorophylls a and b [4]. Chlorophyll a is greenish –yellow in solution and chlorophyll b is blue- green [3]. In the green normal leaves, there is a greater amount of chlorophyll a than b, the report of which is 2: 0.7 [4]. All forms of chlorophyll are oil-soluble. Among the therapeutic effects of chlorophyll can include: stimulating the immune system, help prevent cancer, help combat anemia, purifying the blood and the organism, help to rejuvenate and energize the body, normalize blood pressure, combating bad odors, bad breath as well as body odor [3].
Carotenoids are pigments without nitrogen with a polyisoprene structure, universal spread both in the plant and animals tissues. There are two major types of carotenoids: hydrocarbon class and oxygenated class or xanthophylls. These compounds cause a yellow, orange or red color in the tissues where they are, due to the large number of conjugated double bonds which they contain [5]. Carotenoid pigments are synthesized only by plants. Until the present have been identified over 700 carotenoid compounds. Is known the essential role of carotenoids for plants: in the process of photosynthesis, in breathing, in fertilization, in transferring of absorbed energy from chlorophyll, in protection against destructive photooxidation. Also carotenoid compounds involved in the smooth running of important biochemical processes in animals and human life: in the vision (provitamins A), growing and breeding; more recently have been recognized protective effects of carotenoids against serious disturbances such as:

cancer, heart disease, and were stimulated intensive research on the role of carotenoids as antioxidants and as regulators of the immune response system [6].
Arugula (*Eruca sativa*) is an edible plant of the family *Brassicaceae* (cabbage family). From arugula are consumed: the leaves (rich in chlorophyll, vitamin C, glucosides and potassium), flowers and seeds (for flavored mustards) [7,8]. Arugula possess anti-secretory, cyto-protective, aphrodisiac and anti-ulcer properties [9].
Lettuce (*Lactuca sativa*) is worldwide cultivated, being one of the most consumed green leafy vegetable in the raw form, for its pleasant taste and high nutritional value. It is a rich source of important bioactive compounds such as carotenoids, chlorophylls, vitamins, polyphenols, minerals [10].
In this work we aimed to analyze the content of chlorophyll a, chlorophyll b and total carotenoids in leaves of arugula and lettuce from the Romanian market.

Experimental

For this study were purchased from the Romanian market following materials: leaves of arugula (*Eruca sativa*)- Ruca variety and lettuce (*Lactuca sativa*)- Marilena variety.

Chlorophyll pigments analysis

Determination of chlorophyll pigments in the studied plants was done by a spectrophotometric method [11].
The vegetal samples were weighed on analytical balance and then were subjected to trituration with quartz sand in the presence of acetone 80% (v/v). The obtained homogenate was then centrifuged at 3500 rpm, for 5 minutes, and the supernatant was collected in amber glass containers. The precipitate was taken up with solvent until the extract is no longer present any coloration. The combined supernatants were analyzed at 646 nm and 663 nm in a UV-VIS Spectrophotometer Perkin Elmer Lambda 25.
According to the relationship of Lichtenthaler and Wellburn [12], chlorophyll content was quantified as follows:

$$Chl\ a = 12.21 \cdot (A_{663}) - 2.81 \cdot (A_{646}),$$

$$Chl\ b = 20.13 \cdot (A_{646}) - 5.03 \cdot (A_{663}),$$

$$Chl_{total} = 17.32 \cdot (A_{646}) + 7.18 \cdot (A_{663}),$$

where: Chl a - chlorophyll in mg/l,
Chl b - b chlorophyll in mg/l,
Chl_{total} - total chlorophyll content in mg/l,
A663 - absorbance of the sample at 663 nm,
A646 - absorbance of the sample at 646 nm.

Carotenoid pigments analysis

Determination of the carotenoid pigments was carried out spectrophotometrically, using the same acetone extracts as for chlorophylls analysis.
Calculation of the carotenoid content in the studied raw materials was based on the relationship[]:

$$Carotenoids = [(1000 \cdot A_{470}) - (3{,}27 \cdot Chl\ a) - (1{,}04 \cdot Chl\ b)] / 229$$

where: Chl a - chlorophyll in mg/l,
Chl b - b chlorophyll in mg/l,
A_{470} – absorbance of the sample at 470 nm.

Results and discussion

Results on chlorophyll content in the analyzed samples are shown in Figure 1.

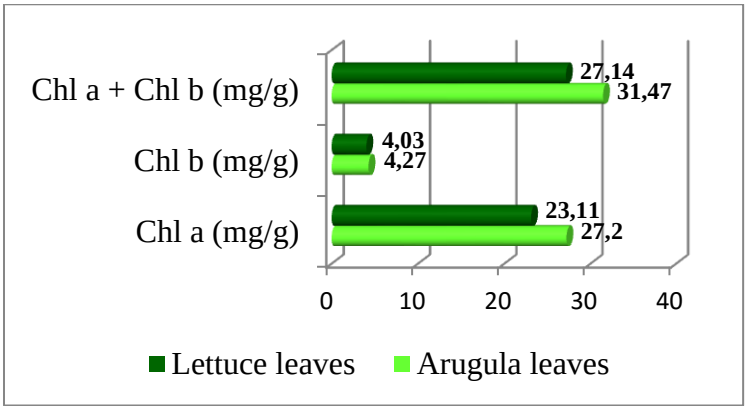

Figure 1. Content of chlorophyll pigments in the analyzed samples

From the experimental results is seen that, for both analysed plants, chlorophyll apredominates to chlorophyll b. Arugula leaves are the richest in chlorophyll pigments (chlorophyll a: 27.20 mg/g; chlorophyll b: 4.27 mg/g), but in the case of lettuce values are only slightly smaller (chlorophyll a: 23.11 mg/g; chlorophyll b: 4.27 mg/g).
The results concerning the carotenoid pigments content in the arugula and lettuce leaves are showed in figure 2.

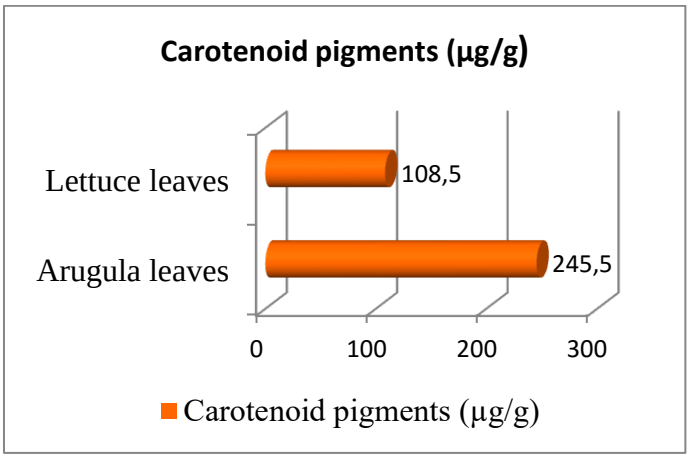

Figure 2.- Carotenoid pigments content in the analysed samples

From the above values is observed that the leaves of arugula (245.5 µg carotenoids/g) are of 2.26 times more rich in carotenoids than lettuce leaves (108.5 µg/g).

Conclusion

1. Spectrophotometric analysis of chlorophyll pigments revealed that both in arugula leaves and in the lettuce leaves, chlorophyll a predominates versus chlorophyll b.
2. The concentrations of chlorophyll pigments in the leaves of arugula and lettuce are close in value, arugula having slightly higher amounts than lettuce.
3. Carotenoids are found in amounts of over than two times higher in the arugula leaves than in lettuce leaves.

References

[1] F.I., Smith,P., Eyzaguirre, African Journal of Food, Agriculture, Nutrition and Development, 7(3), (2007) pp. 1-8.
[2] K.A. Steinmetz, J.D. Potter, Epidemiology. Cancer Causes Control, 1991, 2, pp. 324-57.

[3] A.L. İnanç, Chlorophyll: Structural properties, health benefits and its occurrence in virgin olive oils, Akademik Gıda, 9(2), (2011) pp. 26-32.
[4] N. Sălăgeanu, Fotosinteza, Ed. Academiei Române, Bucureşti, 1972.
[5] V. Tămaş, G. Neamţu, Pigmenţi carotenoidici şi metaboliţi, vol. 1, Ed. Ceres, Bucureşti, 1986.
[6] D.G. Dumbravă, Contribuții la studiul, izolarea și purificarea pigmenților caotenoidici din material vegetale, Ed. Politehnica, Timișoara, 2008, pp.8-9.
[7] F. Nuez, J.E.Hernández Bermejo. Neglected horticultural crops, 1994, pp. 303-332. In: Hernándo Bermejo JE, León J (Eds.). Neglected Crops: 1492 from a different perspective. Plant Production and Protection Series 26. FAO, Rome, Italy.
[8] D. Pignone, C. Gómez-Campo. Eruca, 2011 pp. 149-160. In: Kole C (Ed). Wild crop relatives: genomic and breeding resources, oilseeds. Springer-Verlag, Berlin, Heidelberg, Germany.
[9] S. Alqasoumi, T. Al Howiriny, M. Al Yahya, R. Syed. Rocket "*Eruca sativa*": A salad herb with potential gastric anti ulcer activity. World J Gastroentero, 15, (2009) pp.1958-1965.
[10] R. Cruz, P. Baptista, S. Cunha, J.A. Pereira, S. Casal, Carotenoids of Lettuce (*Lactuca sativa* L.) Grown on Soil Enriched with Spent Coffee Grounds,Molecules,17, (2012) pp. 1535-1547.
[11] I. Ianculov, R. Palicica, M. Butnariu, D. Dumbravă, I. Gergen, Obţinerea în stare cristalină a clorofilei din cetină de brad (*Abies alba*) şi de pin (*Pinus sylvestris*), Revista de Chimie,56(4), (2005) pp 441-443.
[12] H.K. Lichtenthaler, A.R. Wellburn, Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions, 11, (1983) pp 591 – 592.