CHLOROPHYLL AND CAROTENOID CONTENT IN LETTUCE (*Lactuca Sativa L.*) AND NETTLE LEAVES (*Urtica Dioica L.*)

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Abstract

Leaf vegetables are widely used in human diet, they are low in calories and fat, but high in dietary fibres, content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others.The main pigments found in vegetables are chlorophyll a and b. Chlorophyll a converts light energy into chemical energy through photosynthesis process. The content of chlorophyll pigments varies by species.

The aim of the research was to determine the chlorophyll and carotene pigments from nettles leaves and lettuce, using two organic solvents, in order to obtain the best extraction solution, and the contribution of these solvents to the extraction in lettuce and nettles was examined comparatively.

For nettles the best extraction solvent was acetone for chlorophyll a and methanol for carotene. Althought the methanol was a good extractant for lettuce, it should not be forgetten that it was toxic.

Because the methanol is toxic, extraction with acetone is preferred, providing reliable results that must be corrected with a suitable coefficient according to the vegetable species.

Key words: Chlorophyll, carotenoid, extraction, lettuce, nettle leaves, extraction.

INTRODUCTION

Different vegetables are considered as sources of human health promoting components.

Leaf vegetables are widely used in human diet, they are low in calories and fat, but high in dietary fibres, content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others.

The main pigments found in vegetables are chlorophyll a and b. Chlorophyll a converts light energy into chemical energy through photosynthesis process.

The content of chlorophyll pigments varies by species (E.J. Arar, 1997).

Chlorophylls belong to the group of the most important bioorganic molecules; they are the principal pigments in photosynthesis, capable of light energy absorbing and its conversion to “chemical energy” by the formation of chemical compounds rich in energy (needed for the biosynthesis of carbohydrates and other compounds in photosynthetic organisms such as plants, algae an photosynthetic bacteria.

Carotenoid pigments can be located in chromoplasts, contributing to the color of vegetables and fruits, or in chloroplasts, together with chlorophhylls, are involved in the two phytosystems (A.M.Brata, 2012).

Carotenoids group and their derivatives, include about 70 compounds that are present in most vegetables and fruits (GH. Campeanu et all, 2011).

Genotype along with growing and management conditions can affect the content and the composition of phytochemicals in plants (E.J. Arar, 1997).

Direct and indirect methods can be used to investigate primary organic production.

Indirect methods are often used in practice of an approximate of the value of organic production because it is fairly difficult to employ direct methods in plant communities (A.M.Brata, 2013).

As indirect methods, it is possible to monitor and measure all phenomena and processes correlated with productivity (R.G. Barlow et all, 1997).

Chlorophyll content is one of the indices of photosynthetic activity (GH. Câmpeanu, et all, 2011).

It is of particular significance to precision agriculture as on indicator of photosynthetic activity (E.J. Arar, 1997).

The greatest chlorophyll content in plants accurs at the outset of the flowering phase, and chlorophyll is believed to take part in the proces of organoganesis (M. A. Costache et all, 2011).

There is usually 4-5 mg of chlorophyll per unit of leaf surface.

Common nettle (*Urtica dioica L*), a herbaceous perennial flowering plant, is a member of the Urticaceae family.

Traditional herbal medicine in the Balkan countries uses stinging nettle leaves in the form of an herbal infusion as a remedy for the treatment of diarrhea, vaginal discharge, internal/external bleeding.

Being rich in chlorophyll, nettle leaves are used for the treatment of anemia as well as general well-being, and more recently as natural food colorant.

The nettle extract is a common ingredient in cosmetics, e.g. in shampoos and hair growth lotions.

MATERIAL AND METHOD

The aim of the research was to determine the chlorophyll and carotene pigments from nettles leaves and lettuce, using two organic solvents, in order to obtain the best extraction solution, and the contribution of these solvents to the extraction in lettuce and nettles was examined comparatively.

Absorptive properties of the pigments were followed up in quantitative analysis using a spectrophotometric method.

It is know that chlorophyll pigments have broad absorption band from blue to red (M. A. Costache et all, 2011).

Also the coextracted carotenoids have maximum absorption in blue band so that the chosen determination method is based on measuring the absorbance at three wavelengths for each type of matrix and then calculate chlorophyll and carotene using the three "trichromatic equations" (R.G. Barlow et all, 1998).

The weighed samples, having been put separately in 100% methanol and 100% acetone, were homogenized with the NF 200 type homogenizer at 3000 rpm for 15 minutes.

The supernatant was separated and the absorbances were read at 400-700 nm on Schimadzu UV-MINI 1240 spectrophotometer.

The experiments were repeated three times.

Top fresh nettle (*Urtica dioica L.*) leves were collected for the purposes of this study from the forest community in March of 2015 and the fresh lettuce (*Lactuca Sativa L.)* leves were collected from Fiziș, Bihor community in March of 2015.

The preparation of plant material was performed according to the following analysis: Chlorophyll a and b and carotenoids were extracted from the fresh nettle leaves and fresh lettuce in acetone (0.5 g of the plant material in 5 mL acetone).

After the centrifugation at 3000 rpm for 15 min, the supernatant was used and the absorbance of extract was measured at 662 nm, 645 nm and 470 nm, for fresh nettle leaves and at 662 nm, 645 nm and 470 nm, for fresh lettuce.

Same for the methanol, but different absorbance of extract was measured at 666 nm, 653 nm and 470 nm.

RESULTS AND DISSCUSIONS

Analyses performed on lettuce leaves showed a large variety of chlorophyll a and b and also of carotene content.

Table 1

Chlorophyll a and b and total carotenoids content in leaves of *Lactuca Sativa,* variety Riga, mg/100g fresh product

Time extraction	METHANOL			ACETONE		
	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b
Fresh lettuce	12,28	8,15	20,44	17,21	5,42	22,63
Total carotene	6,12			6,04		
24 hours	10,52	7,21	17,73	15,08	5,98	21,07
Total carotene	2,45			4,56		
48 hours	9,00	4,23	13,24	14,0	1,76	14,78
Total carotene	2,54			4,72		
72 hours	6,38	0,79	7,18	13,02	1,76	14,78
Total carotene	2,65			4,26		

It was determined that the solvents used were important in the pigment extraction, and the best solvent was methanol (table 1).

In the case of methanol extraction, the contents were higher, varying from 7,18 mg/100 g (after 72 hours) to 20,44 mg/100 g fresh lettuce.

The acetone extraction of chlorophyll a, the highest values of chlorophyll extracted in acetone ranged between 17,21 mg/100 g in fresh lettuce leaves.

Comparing the values of the two extraction variants of chlorophyll a, best results were obtained with acetone.

Extraction with acetone resulted in high values of chlorophyll b in lettuce, the lettuce values ranged between 1,76 mg/100 g (after 72 hours) and 5,42 mg/100 g fresh lettuce.

Results of the extraction of chlorophyll b showed that methanol solvent determined the best results followed by extraction with acetone. Acetone extraction determined higher levels of carotene pigment in lettuce, varying between 4,26 mg/100 g (after 72 hours) and 6,04 mg/100 g in fresh lettuce.

Analyses performed on nettles leaves showed a large variety of chlorophyll a and b and also of carotene content.

Table 2

Chlorophyll a and b and total carotenoids content in leaves of *Urtica Dioica L.*, mg/100g fresh product

Time extraction	METHANOL			ACETONE		
	Chl a	Chl b	Chl a+b	Chl a	Chl b	Chl a+b
Fresh nettles	28,06	13,86	41,91	28,11	17,45	45,56
Total carotene	5,49			7,38		
24 hours	16,62	12,00	28,62	25,72	11,30	37,02
Total carotene	6,28			4,18		
48 hours	14,73	10,26	24,99	19,80	9,93	29,73
Total carotene	6,30			3,28		
72 hours	16,28	5,36	21,64	16,51	8,55	25,07
Total carotene	6,02			0,94		

In the case of methanol extraction (table 2), the contents were higher, varying from 21,64 mg/100 g (after 72 hours) to 28,06 mg/100g fresh product.

The acetone extraction of chlorophyll a, the highest values of chlorophyll extracted in acetone ranged between 28,11 mg/100 g in fresh nettles leaves.

Comparing the values of the two extraction variants of chlorophyll a, best results were obtained with acetone.

Extraction with methanol resulted in high values of chlorophyll b in nettles, the nettles values ranged between 5,36 mg/100 g (after 72 hours) and 13,86 mg/100 g fresh nettles.

Results of the extraction of chlorophyll b showed that methanol solvent determined the best results followed by extraction with acetone. Acetone extraction determined higher levels of carotene pigment in nettles, varying between 0,94 mg/100 g (after 72 hours) and 7,38 mg/100 g in fresh nettles.

Comparing the results of the there extractions of carotene, acetone extraction determined the best results in analyzed nettles.

CONCLUSIONS

In conclusion, in the present study, the Chl a, Chl b and carotenoides have suffered degradation after 72 hours.

In fact, acetone and methanol were used in the studies with high plant leaves, and it was determined that the extraction rate was various in every solvent.

That is why we believe that the selection of the method and the solvent to be used in the studies in connection with pigments according to the species will be more usefull.

For nettles the best extraction solvent was acetone for chlorophyll a

and methanol for carotene.

Althought the methanol was a good extractant for lettuce, it should not be forgetten that it was toxic.

Because the methanol is toxic, extraction with acetone is preferred, providing reliable results that must be corrected with a suitable coefficient according to the vegetable species.

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