QUANTIFICATION OF CAROTENOIDS AND CHLOROPHYLL LEAF PIGMENTS FROM AUTOCHTHONES DIETARY

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Abstract: *Chlorophylls are the preponderant photosynthetic pigments of the verdant tissues of vascular plants, liverworts, and various algae. Carotenoids are essential for the survival of photosynthetic organisms. They function as light-harvesting molecules and provide photoprotection. Information gathered from the screening of secondary plant metabolites is vital for the accurate determination of the dietary intake of these micro-nutrients, and in the development of comprehensive food tables. Determination of basal levels is also necessary for the rational engineering of health-promoting phytochemicals in food crops. In addition this approach can also be applied to the routine screening of products to determine metabolic differences between varieties and cultivars, as well as between genetically modified and the corresponding non-genetically modified tissue. Beta -carotene accounts for more than 90% of total carotenoids in vegetables. In human beings, beta-carotene not only serves as valuable source of vitamin A, but also serves as a potent antioxidant, scavenging free radicals and quenching singlet oxygen. By this latter property, beta-carotene is understood to reduce the risk of development of certain types of cancer*
This study therefore is aimed at determining the beta-carotene and chlorophyll contents of same selected autochthones plants (Allium ursinum, Alliaria petiolata, Urtica dioica) from Macin Mountains harvested on spontaneous flora.
Photosynthetic pigments of investigated plants were extracted from leaves using appropriate solvents. The pigment quantification of individual plants was investigated by spectrophotometric analysis.
The levels of carotenoids, and chlorophyll varied in each plant and the results were similar to the previously results reported in the literature.

Keywords: *Allium ursinum, Alliaria petiolata, Urtica dioica*

Introduction

Carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits and vegetables. Carotenoids are the most ubiquitous and widespread pigments which are characteristic for organisms of all taxa[1].
Ability of carotenoids in modifying structure, properties, and stability of cell membranes, and thus affecting molecular processes associated with these membranes, may be an important aspect of their possible beneficial effects on human health [2].
Some carotenoids, including β-carotene (Fig.1), quench highly reactive singlet oxygen under certain conditions and can block free radical-mediated reactions. In epidemiological researches, the intake of carotenoid-rich fruits and vegetables has been correlated with protection from some

forms of cancer. Analogically, serum β-carotene levels have been associated with a decreased chance of developing lung cancer. It must be stressed, however, that these epidemiological associations do not show cause and effect. In this regard, long-term intervention trials with beta-carotene supplements are in progress.

Whatever the results of these trials, carotenoids clearly show biological actions in animals distinct from their function as precursors of vitamin A[3].

Chlorophyll is the pigment that gives plants and algae their green color. Plants use chlorophyll to trap light needed for photosynthesis [4]. Chlorophyll a and b differ from one to another through the radical put in position three.

Chlorophyll a contains one methylic radical (CH_3), and chlorophyll b one radical CHO (Fig.2).

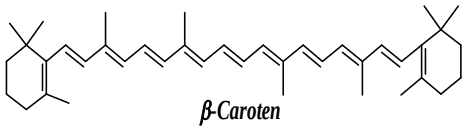


Fig.1-β-carotene chemical structure

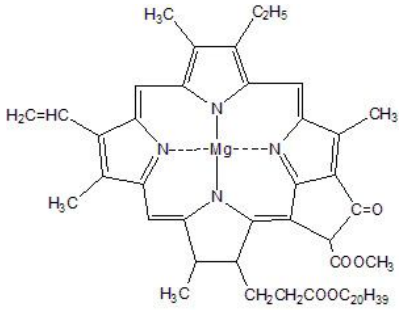
β-carotene is under the violet cristal form following ever the chlorophylla and is soluble in organic solvents.

Through oxidative enzimatic hydrolysis, β-carotene transforms in two molecules of vitamine A.

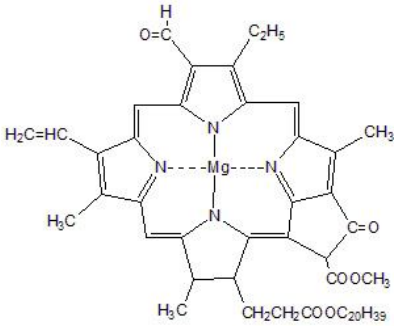
The average content of carotenoids from vegetables and fruits is different on species between 6,0 and 24,0 mg/100g dry product of the carrot and 0,1 mg/100g dry product of the plums tree. Carotenoids pigments are spread in all of the plant˙s section with or without chlorophyll (leaves, fruits, stems, bulbs, seeds, etc.).

However, the contend in carotenoids pigments depends of the species but also the influence of the enviromental conditions is very important[7].

The present paper consists in the determination of the contend of β-carotene and chlorophyll from three species of plants- *Allium ursinum(*Fam. *Alliaceae), Alliaria petiolata(*Fam. *Brassicaceae), Urtica dioica(*Fam. *Urticaceae)* –harvested from spontaneous flora of the Macin Mountains.



Chlorophyll a



Chlorophyll b

Fig. 2-Chlorophyll a and b[5,6]

Some representative examples indentified on the area are stored in the Herbarium of Botanical Garden Galati and of the Pharmacy and Medicine Faculty of "Dunarea de Jos" University Galati.

Materials and methods

Methods of the determination and extraction, for chlorophyll and β-carotene, used, are in according with the official methods AOAC [8,9,10].

For the extraction and for the spectrophotometric determination of clorophyll from the biological material harvested (folium) was used 1 g dry vegetal product from every plant. The cold mortar was done with quart sand and the

extraction was done with 85% acetone. The extinction was determined at 660 and 642nm.

The calculation of the totale clorophyll concentration and of the clorophyll a and b(mg/L) is done with the help of the next relationships:

(1) Total chlorophyll = (7.12 $A_{660.0}$ + 16.8 A_{642}) f_d

(2) Chlorophyll *a* = (9.93 $A_{660.0}$ - 0.777 A_{642}) f_d

(3) Chlorophyll *b* = (17.6 A_{642} - 2.81 $A_{660.0}$) f_d

For the calculation of the chlorophyll concentration in mg/100g plant, we remind of the acetone extract volume obtained from vegetal material and of the partition from filtrate solution submissived to the extraction with ether.

For the separation and spectrophotometrical determination of the β-carotene was used extract from 1 g vegetal product removed with ether brew of petrol-benzene (v:v=1:1).

Qualitative determination of the β-carotene was put in evidence with the help of the UV-VIS spectrometry.

Spectrometrical analysis was realized with the UV-VIS Double Beam PC spectrometric and scanned with the auto device Cell UVD-3200, in comparison with specter β-carotene absolute Merck provenience.

The separation of the carotenoidic pigments by the chlorophyll and xantophyll pigments, consists into a pass through an adsorbtion column, with Al_2O_3 (4 – 7 cm height).

Obtained carotenoidic extract was measured at 436 nm. The concentration in β-carotene (Cx) of the analyzed samples was derived from a standard curve of β carotene, ranging from 2 to µg/mL (Pearson˙s correlation coefficient: r^2:0,9913).

The contain in mg carotine/100g vegetal poduct is determined with the help of the relationship [11]:

$$mg\ carotene\ \% = \frac{Cx \cdot fd \cdot Vex}{m} \cdot 10^{-1}$$

where:

C_x – concentration in carotene of the analyzed samples,removed from the standard curve(µg/mL);

f_d – the factor of dilution applied on the analyzed samples, in order to frame their absorbance;

V_{ex} – the volume of obtained extract(mL);

m – the mass of the analyzed sample(g).

Results and discussions

The results obtained from the study are presented in Tables 1 and 2.

The chlorophylic extracts were diluated with anhidrous ether in raport 1:2, for obtaining of some optime values of the absorbance, at the wavelength used(660 nm and 642nm).

After the obtaining of the results phisyco-chimical analysys, we observed the following: the graphic representation with the auto device Cell UVD-3200, is between 400 and 500 nm (Fig. 3, Fig. 4), values wich appart of the UV-VIS spectrum of the β carotene standard absolute (Merck).

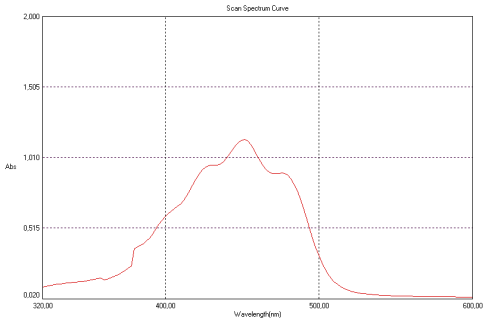


Fig. 3-Spectrometry UV-VIS β carotene absolute (Merck) ethalon

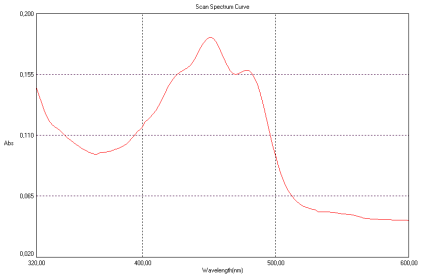


Fig. 4-Spectrometry UV-VIS β-carotene of *Allium ursinum*

The values obtained of analyzed samples can be observed at standard curve characteristical β carotene with the values R^2=0,9913 (Fig.5).

New researches concerning the contains of chlorophyll and carotenoids from *Allium* genus put in evidence valoric differences that can be compared with the obtained results(by us). So, the highest content of investigated pigments was obserwed in leaves of wild garlic(*Allium ursinum*) : 999mg/100g for carotenoids and 287 mg/100g for chlorophyll a, and 135mg/100g for chlorophyll b [12].

The contain of chlorophyll a, determined by us from wild garlic is higher so of 374,95mg/100g from the dried plant on natural way but the contend of chlorophyll b was lower (104,77mg/100g). It must be observed that like in all freeze samples from three species, the values of the chlorophyll a and b were lowed in comparison with the dry samples by natural way. Also, the greatest contain, of the total chlorophyll, can be found on the *Urtica dioica* species (2604.4mg/100g dry plants and 2291.94mg/100g lyophilised plants). The lowest values are in *Alliaria petiolata* cases.

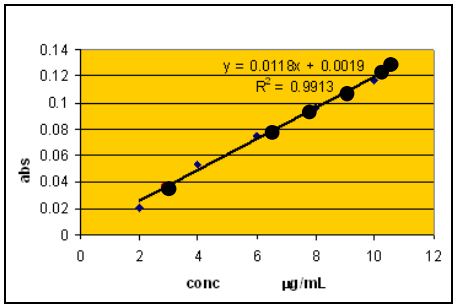


Fig.5-β-carotene determination of samples
Legend:
♦ carotene standard curve
● sample analyzed

Table 1

Determination of chlorophyll content (a and b) on samples(f_d=1:2)
Legend:dp-dry plant; lp-lyophilised plant

Samples	*Urtica dioica*		*Allium ursinum*		*Alliaria petiolata*	
	dp	**lp**	**dp**	**lp**	**dp**	**lp**
A_{660}	0.641	0.581	0.217	0.201	0.175	0.164
A_{642}	0.282	0.241	0.112	0.110	0.093	0.081
Total chlorophyll (mg/L)	18.603	16.371	3.4266	2.2254	2.8084	2.4921
chlorophyll *a* (mg/L)	12.292 ±0,002	11,1642 ±0,004	2.6782 ±0,006	2.0201 ±0,002	1.6655 ±0,003	1.4871 ±0,00
chlorophyll *b* (mg/L)	6.634 ±0,001	5.218 ±0,002	0.7484 ±0,005	0.2053 ±0,004	1.1429 ±0,001	1.005 ±0,001
Total chlorophyll (mg/100g plant)	2604.4	2291.94	479.72	480.02	395.64	351.63
chlorophyll *a* (mg/100g plant)	1720.9 ±0,02	1562.9 ±0,02	374.95 ±0,06	283.47 ±0,01	233.71 ±0,05	206.41 ±0,02
chlorophyll *b* (mg/100g plant)	928.76 ±0,05	730.52 ±0,04	104.77 ±0,02	196.55 ±0,00	161.93 ±0,06	145.49 ±0,03

Tabel 2

Determination of β carotene content by samples(f_d=1:20)
Legend:dp-dry plant; lp-lyophilised plant

Samples	*Urtica dioica*		*Allium ursinum*		*Alliaria petiolata*	
	dp	**lp**	**dp**	**lp**	**dp**	**lp**
A (λ= 436 nm)	0.036	0.079	0.125	0.136	0.090	0.115
Conc. (μg/mL) β-carotene	2.89	6.53	10.43	11.36	7.46	9.58
V_{ex} (mL)	158	80	24	24	25	25
mg β-caroten %	913.24 ±0,01	1044.8 ±0.02	500.64 ±0.06	545,28 ±0,04	373 ±0.01	479 ±0.02

The β carotene contain determinate in present paper is highered in case of *Urtica dioica* species (1044,8 mg/100g lyophilised sample) and lowered at *Alliaria petiolata* species (373g/100g dry plant). However, we must do an specification about lyophilised samples wich have superior values in comparison with the ones on natural way.
Allium ursinum(wild garlic), *Alliaria petiolata* (garlic mustard) and *Urtica dioica* (common nettle) are spread on large areas in Macin Mountains zones, in special near the rivers. The growing of the plants is conditioned by the trophicity of the soil but also the atmospheric humidity[13]. This plants are used by the humans in the early spring to prepare salads or another types of foods. The harvested leaves in optime periods can have good effects to the human organism, this was proved and through thir contining of βcarotene with antioxidant effect.[14,15].

Conclusions

The highest contend in total chlorophyll (a and b) from the three analyzed plants can be found in common nettle.
The lyophilised samples have high values in comparison with the dry samples on natural way only in case of determination of β-carotene.

Our results indicates that leaves of *Urtica dioica, Allium ursinum* and *Alliaria petiolata* could be used as potential sources of natural untoxic antioxidants in food and pharmaceutical industries.

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