Academy of Romanian Scientists *Volume 1, No. 2, 2012, pp 74 - 92*
Annals Series on Biological Sciences Online Edition ISSN 2285 - 4177
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Study on the content of active principles of some native plants with effect in making the stability and thermal resitance to fried sunflower oil

Received for publication, october, 1, 2012.
Accepted, november, 15, 2012

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Abstract.
In this paper, there have been studied some species of plants through phytochemical analysis (flavonoids, β-carotene, chlorophyll), for testing the antioxidant effect with usage in the food industry. The analyzed plants were harvested from the spontaneous flora of the Macin mountains (Luncavita forest) at a height 150 - 200m and from the Botanical Garden of Galati.(42 m height, landmark to The Black Sea).
There were analysed the flavonoids and polyphenolic compounds from some natural herbs of families: Alliaceae (Allium ursinum), Brassicaceae (Alliaria petiolata) and Urticaceae (Urtica dioica) as crude hydroalcoolic extracts. The content of phenolic compounds was determined colorimetrically with the Folin–Ciocalteu (FC) reagent and was expressed in gallic acid equivalents (GAE). The flavonoid contents was determined using a method based on the formation of complex flavonoid-aluminium and was expressed in quercetin equivalents (QE). Also there was analyzed the β -carotene and chlorophyll contents by spectrophotometric method. Finally we tested the thermal resistance of sunflower oil after the incorporation of three species of natural herbs (the aerial part of plants). By this treatment, we seek to preserve almost unchanged the oil quality during thermal treatment, by increasing the level of antioxidants from oil. For this study, four different frying temperatures i.e. 110, 150, 180 and 200ºC were applied for 30 minutes to sunflower oil before and after addition of plants. We have also realized a kinetic study of samples stability in time at 110 ºC.

Key words: heat treatment; PV; FFA ,polyphenols, flavonoids, carotenoids, chlorophyll,

Introduction

In the last years much attention has been devoted to natural antioxidant and their association with health benefits (Arnous et al., 2001). Plants are potential sources of natural antioxidants. They are sources of various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Huda-Faujan et al. 2009). Phenolic compounds are responsible for major organoleptic characteristics of plant, particularly color and taste properties. They are also known as to contribute to the health benefits associated with consumption of diets high in fruits and vegetables or plant-derived beverages. Innumerable studies have been

devoted to polyphenols, their occurrence in plants and their effects on quality of life. However, plant polyphenol composition is still poorly understood. Furthermore, polyphenols are highly reactive compounds and good substrates for various enzymes, including polyphenoloxidases, peroxidases, glycosidases, and esterases. They undergo numerous enzymatic and chemical reactions during postharvest food storage and processing. Although the occurrence of such reactions and their roles in the development or degradation of food quality are well documented, the structures of the resulting products are still poorly understood and their concentrations in food are usually unknown.

Plant polyphenols comprise a great diversity of compounds, among which flavonoids and several classes of non-flavonoids are usually distinguished (Harborne, 1989). The latter (Figure 1) are mostly rather simple molecules, such as phenolic acids (which are subdivided into benzoic acids and hydroxycinnamic acids, based on C1-C6 and C3-C6 skeletons, respectively) and stilbenes, but also include complex molecules derived from them (eg, stilbene oligomers, gallotannins, ellagitannins, and lignins). The flavonoids (Figure 2) have a common nucleus consisting of 2 phenolic rings and an oxygenated heterocycle. They are divided into several groups differing in the oxidation state of the heterocyclic pyran ring (eg, anthocyanins, flavonols, and flavanols). More than 4000 flavonoids have been identified in plants, and the list is constantly growing (Harborne and Williams, 1992). This is because of the occurrence of numerous substitution patterns in which primary substituents (eg, hydroxyl, methoxyl, or glycosyl groups) can themselves be substituted (eg, additionally glycosylated or acylated), sometimes yielding highly complex structures. Moreover, flavanols are also encountered as oligomers and polymers, referred to as condensed tannins or proanthocyanidins.

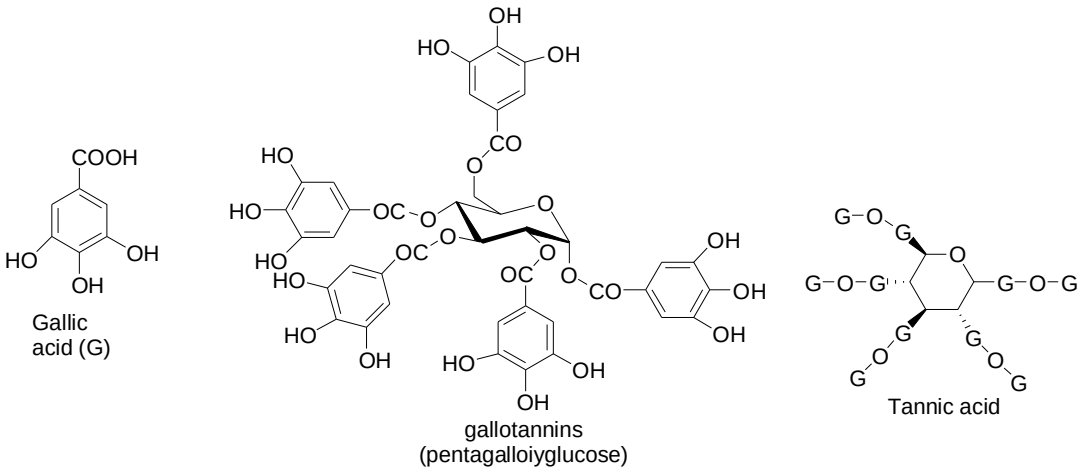

Fig. 1 - Chemical structures of the main classes of nonflavonoid polyphenols

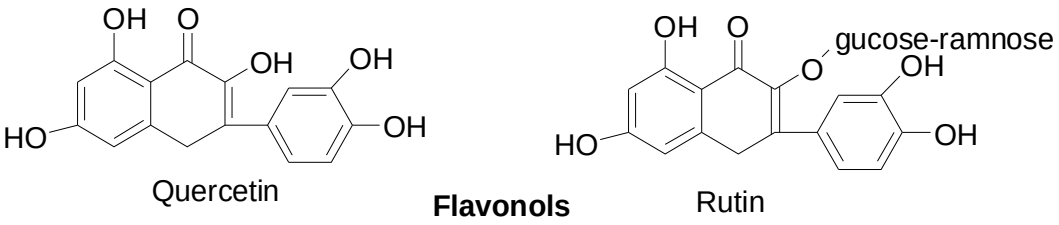

Fig. 2 - Chemical structures of the main classes of flavonoids

Plant polyphenol composition is highly variable both qualitatively and quantitatively; some of the compounds are ubiquitous, whereas others are restricted to specific families or species (eg, isoflavones in legumes). Polyphenol diversity in fruits (Macheix et al., 1990) and in plant foods (Shahidi and Naczk, 1995) has been described in excellent reviews. Within a single species, large variations may also occur, particularly because of genetic factors, environmental conditions, and growth or maturation stages. Recently, there are a lot of methods that have been developed to evaluate the polyphenol content of complex mixtures such as plant extracts (Anatolovich et al., 2002) and to identify all possible mechanisms characterizing an antioxidant activity (Frankel and Meyer, 2000; Gorinstein et al., 2009).

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 (Corol et al., 2003). 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 (Britton, 1995). Some carotenoids, including β-carotene (Fig. 3), 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 β -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 (Bendich and Olson, 1989).

Chlorophyll is the pigment that gives plants and algae their green color. Plants use chlorophyll to trap light needed for photosynthesis (Matthews and Holde, 1996). Chlorophyll *a* and *b* differ from one to another through the radical in position three. Chlorophyll *a* contains one methylic radical ($-CH_3$), and chlorophyll *b* one radical -CHO (Fig. 4).

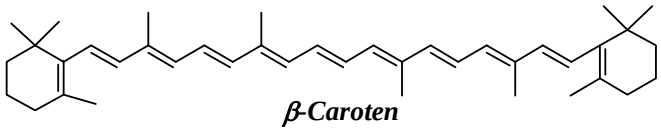

Fig. 3 - β-carotene chemical structure

β-carotene is under the violet cristal form following ever the chlorophyll and is soluble in organic solvents. Through oxidative enzimatic hydrolysis, β-carotene transforms in two molecules of vitamine A (Dashwood, 1997). 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 content in carotenoids pigments depends of the species, but also the influence of the enviromental conditions is very important (Neamtu, 1983).

Oils used in the home and oils used commercially require significantly different properties. Perhaps most importantly, commercial oils need to withstand intense heat and frying for longer periods of time. During the frying process, a number of changes take place in fats and oils, depending on the type of oil used and the food fried (Demir and Bas–Han, 1998; Demir and Otludil, 1997). So complex thermolytic and oxidative reactions occur, leading to the formation of new compounds such as diacylglycerols, monoacylglycerols, free fatty acids (FFAs), monomers, polymers, and so on, which are harmful to the human body (Clark and Serbia, 1991; White 1991). However, the most interesting transformations are FFA content, viscosity and color change of the vegetable cooking oil as well as formation and decomposition of hydroperoxides and polymerization via complex free radical processes at elevated temperatures above 160ºC (Blumenthal, 1991).

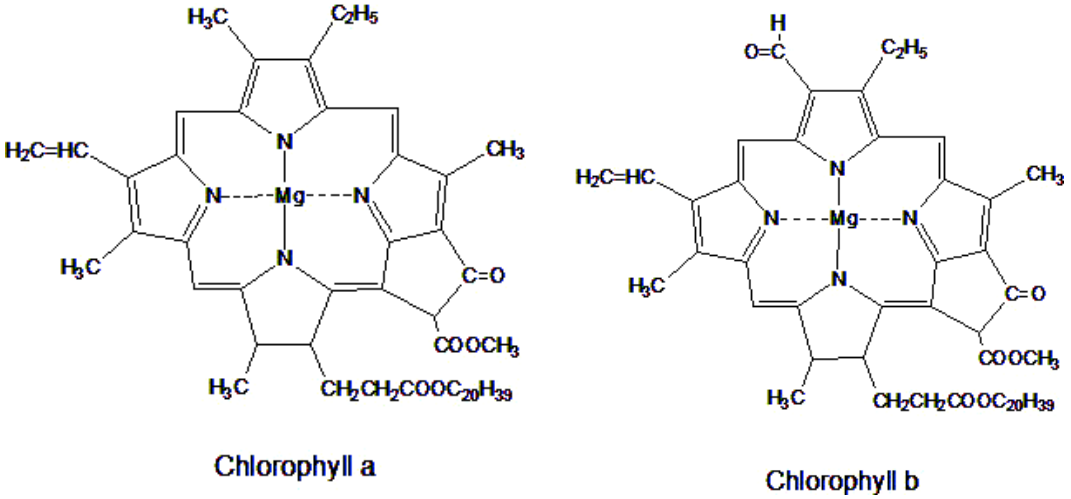

Fig. 4 - Chlorophyll *a* and *b* (Dashwood, 1997)

Extending the frying life of oil is of commercial and economic importance. Therefore, improving the thermal stability of cooking oils could provide considerable savings to the food processors.In order to increase stability, many healthy oils must be hydrogenated for commercial use, a process that adds unhealthy transfats.Therefore, the present work aims to evaluate the thermal resistance of sunflower seed oil before and after treatment with three species of aromatic plants, from *Salvia, Urtica* and *Allium*, used in alimentation as condiments or in other forms. By this treatment, we believed that the quality of frying oils can be kept almost constant throughout heat treatment, by increasing of oil's antioxidants content. The choice of the sunflower oil has been determined by its very frequent use in home and food industry. Sunflower (*Helianthus annuus*) is one of the four major annual oilseed crops produced in the world (Schmidt, 1991). Being an excellent source of the essential fatty acids required by the human body, sunflower oil is among the healthiest vegetable oils available. The linoleic acid is one of its essential unsaturated fatty acids (Bourre et.al., 1989). Also, sunflower oil is an important source of vitamin E (45 mg α-tocoferol/100g). Aromatic plants have been used since ancient times in food flavorings, pharmaceuticals, cosmetics and perfumery. Essential oils or some of their constituents such as polifenols, flavonoids and carotenoids present biological activities, including antimicrobial and antioxidant properties (Loo and Richard, 1992; Prakash, 1990).

Since antiquity, *Salvia* species have been well known plants and widely used as folk medicines with antibacterial, antituberculosis, antiviral, cytotoxic, cardiovascular, liver protective and other properties (Ulubelen et al., 2001; Ulubelen et al., 1994; Topcu et al., 2003; Ulubelen, 2003; Ulubelen et al., 2002; Zhou et al., 2005). Sage is also used to preserve foods, especially meat and cheese, due to its antioxidant properties, as well as being employed as a spice for flavoring. Phytochemical investigations have shown that *Salvia* species are mainly rich in diterpenoids and triterpenoids (ursolic acid, oleic acid) as well as in flavonoids and other phenolic compounds (tannins, cholorogenic, p-cumaric, cafeic and nicotinic acids) (Topcu and Ulubelen, 2007; Topcu et al., 2004; Topcu et al., 1995; Lu and Foo, 2002; Yesilyurt et al., 2008).

Allium sp. is the largest and most important representative genus of the *Alliaceae* family and comprises 450 species, widely distributed in the northern hemisphere. Besides the well known garlic (*Allium sativum* L.) and onion (*Allium cepa* L.), several other species are widely cultivated for culinary use, such as leek (*Allium porrum* L.), scallion (*Allium fistulosum* L.), shallot (*Allium ascalonicum* Hort.), wild garlic (*Allium ursinum* L.), chive (*Allium schoenoprasum* L.) etc. *Allium* species are a rich source of phytonutrients, useful for the treatment or prevention of a number of diseases, including cancer,

coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia), (Lanzotti, 2006). Due to its rich composition in volatile oils, minerals and oligoelements, flavonoids, polifenols, vitamins (C and B complex), sulfur componds, wild garlic (*Allium ursinum*) has garlic's properties but stronger curative power.

The aim of this study is to evaluate the total phenolic and flavonoid, β-carotene and chlorophyll content of some Romanian plants which are in relationship with antioxidative activity of the plants. According to our knowledge, there are very few data regarding the potential antioxidant properties related to phenolic and flavonoid fractions, β-carotene and chlorophyll of *Allium ursinum* (Fam. *Alliaceae*)*, Alliaria petiolata* (Fam. *Brassicaceae*)*, Urtica dioica* (Fam*. Urticaceae*), which are widely used as salads and foods in most of the countries of the Balkan Peninsula. Considering their interesting properties, we tested in particular the effect of *Allium ursinum* in comparison with *Salvia officinalis* (Fam. *Lamiaceae)* and *Salvia sclarea* (Fam*. Lamiaceae)* plants, which are widely used as condiments and salads. In order to test the contribution of natural herbs to the thermal resistance of sunflower seed oil, the evolution of peroxide value (PV) and free fatty acids content (FFA) during heat treatments, was investigated before and after oil aromatization with the three species of plants. We studied the evolution in time of those parameters at a determined frying temperature, and the evolution at different frying temperatures for a constant period of time, too. With respect to this, in the paper we present our investigations on the total phenolics and flavonoids and β-carotene and chlorophyll content of the mentioned above plant extracts.

Materials and methods

Biological material analyzed in the present paper was collected from the North Dobrogea (Luncavita Forest) from spontaneous flora of the Macin Mountains and is made from following vegetal products: folium of *Allium ursinum* (wild garlic), *Alliaria petiolata* (garlic mustard) and *Urtica dioica* (nettle). 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. The harvesting was realized when the leaves grew until maturity before the bloom of the plants and were macroscopic determined in Botanical Garden laboratory of Galati.

We have developed our extraction and determination methods for chlorophyll and β-carotene accordingly with the AOAC (1999) methods.

Chemicals Chemicals were purchased from Sigma Co. Folin–Ciocalteu (FC) reagent was purchased from Merck (Germany). All other chemicals and reagents were of analytical grade.

Extracts preparation. The ground air-dried immature plants (5 g), were extracted using a method of extraction with ultrasounds with 70% ethanol for 24 h, at room temperature. After the extraction, the extracts were collected and filtered. To remove chlorophylic pigments, ethanolic extract is subject to repeated extraction with petroleum ether until disappearance of its green color. Ethanolic phase obtained after extraction is used for the determination of flavonoids and polyphenols, the volume being adjusted to 100 mL with cold 70% ethanol.

Analysis of total phenolic content The total polyphenol content (TPC) of the extracts was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1(ISO 2005; Singleton et al.1999). Briefly, 1.0 mL of the diluted sample extract was transferred in triplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added. The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm (in a UV– Vis spectrophotometer) was measured against water. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 g material. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 μg/mL (Figure 5, Pearson's correlation coefficient: R^2 : 0.9988).

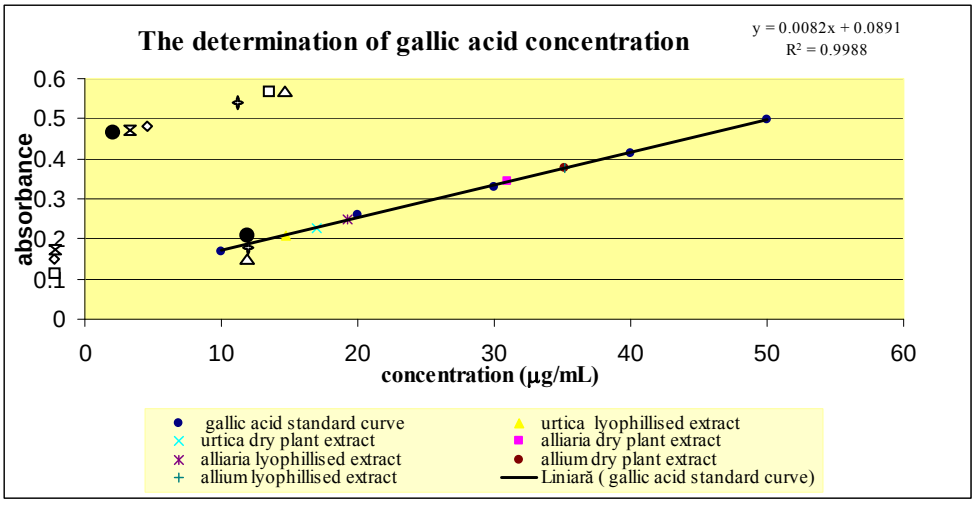

Fig. 5 - Standard curve of gallic acid and concentration in polyphenols of analyzed extracts

Estimation of total flavonoid content

Measurement of total flavonoid content in the investigated extracts was determined spectrophotometrically[12] using a method based on the formation of complex flavonoid-aluminium with the maximum absorbtivity at 430 nm. The aqueous dilutions of samples, in the amount of 1 ml, were separately mixed with 1 ml of 2% $AlCl_3$. After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoids content was expressed as quercetin equivalents (QE) in mg/100 g material, by using a standard graph. (figure 6, Pearson's correlation coefficient: r^2 : 0.9732)

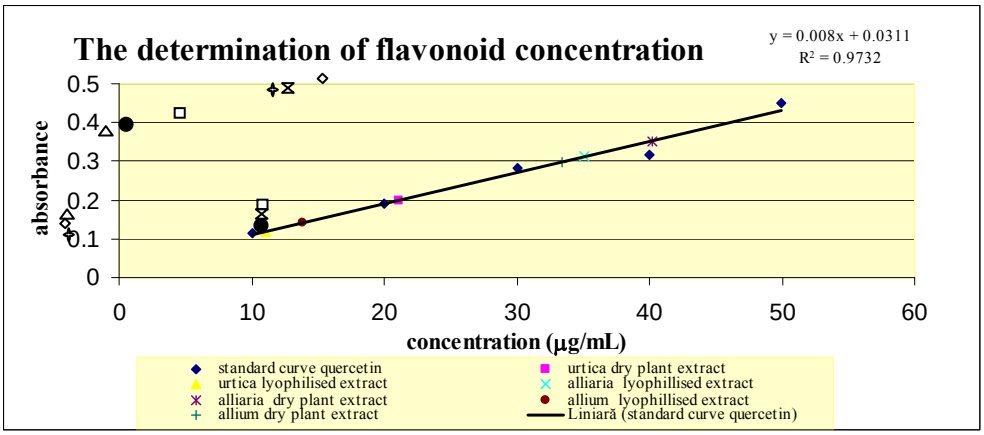

Fig. 6 - Standard curve of quercetin and concentration in flavonoid of analyzed extracts

For the extraction and spectrophotometric analyse of chlorophyll, 1 g dry vegetal product (folium) from every plant was used. 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 carotene/100g vegetal poduct is determined using the relationship (Dinică et. al., 2009):

$$mg\ carotene\ \% = \frac{C_x \cdot f_d \cdot V_{ex}}{m} \cdot 10^{-1}$$ 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).

Test of free fatty acids content (FFA) and peroxide value (PV)

We used sunflower oil directly from a technological flow, without added antioxidants. Plants were bought from the Botanical Garden of Galati (*Salvia officinalis* and *Salvia sclarea*) or harvested from the North Dobrogea (Luncavita Forest) from spontaneous flora of the Macin Mountains. All chemicals used were of analytical grade.

Oil treatment with natural herbs

The aerial part of plants dried in air, mortar and sieved to 630 μm, was incorporated into sunflower oil at 2% (w/v). After ultrasonic stirring for 30 minutes, samples were kept in refrigerator for 7 days. After this treatment, the undesirable color observed in the mixture was removed by an additional decolorizing step, using Cameroun clay (50 μm).

Thermal stability evaluation

In order to evaluate the effect of sunflower oil treatment with an aromatic herb on its thermal resistance, both untreated oil and treated oils were heated for 30 min at 110, 150, 180, and 200° C, respectively. Also, a kinetic evaluation of samples was made at 110°C.

Peroxide value determination

Peroxide value (PV) was determined by using the *AOAC method* (AOAC, 1999). About 1 g of oil was weighed into a 250 mL iodometric flask. Previously prepared acetic acid–chloroform (1:2) solution (6 mL) and saturated potassium iodide (1 mL) were added. After 3 min. stirring and 5 min. rest, the mixture was titrated with 0.01 N $Na_2S_2O_3$ until yellow color is almost gone. Approximately, 5 drops of 1% starch solution was added, and titration was continued with shaking vigorously to release all iodine from $CHCl_3$ layer, until the blue color just

disappeared. Parallel, a blank sample is similarly treated. PV was calculated by using the following equation:

$$PV=S\cdot N\cdot 1000/m_p \text{ (meq } Na_2S_2O_3\text{/Kg oil)}$$

where, S is the ml $Na_2S_2O_3$ (blank corrected), N is the normality of $Na_2S_2O_3$ solution and m_p is the mass of oil sample (g).

FFA determination

FFA content was determined in triplicate, by the titration method of AOAC (AOAC, 1999). About 1 g of oil was weighed into a 250 ml flask. 10 mL benzene:alcohol (1:1) mixture and 3-4 drops of 1% phenolphthalein, as indicator, were added. The mixture was titrated with 0.1 N NaOH with vigorous shaking until permanent faint pink color appeared and persisted at least 1 min. The FFA value was calculated according to the following equation:

$$I.A = 3.9998\ V/m_p \text{ [mg NaOH/g sample]}$$

where m_p is the mass of the oil test portion (g), and V the volume of NaOH consumed (mL).

Results and Discussion

Although the most antioxidant activities from plant sources are derived from phenolic-type compounds, these effects do not always correlate with the presence of large quantities of phenolics. Therefore, both sets of data phenolic and flavonoid compounds need to be examined together. With respect to this, the investigated plant extracts were analysed for total phenolic and flavonoid contents.

Polyphenols content. The Folin-Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols. In this work, the total polyphenol content of 3 samples of food plants, belonging to the Romanian autochthones flora, was analyzed.

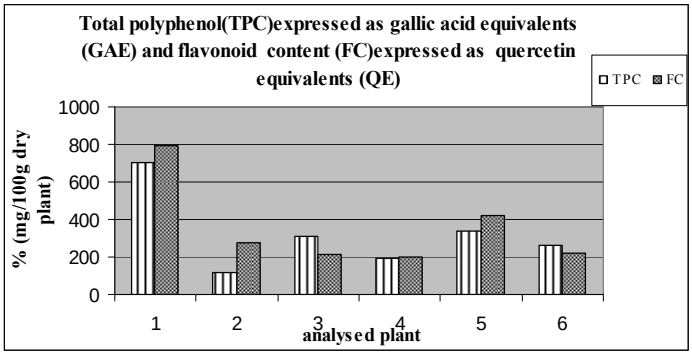

Fig. 7 - The total polyphenol content and flavonoid content of analyzed plants
Legend: *Allium ursinum* (1-dp, 2-lp), *Alliaria petiolata* (3-dp, 4-lp), *Urtica dioica* (5-dp, 6-lp); dp-dry plant; lp-lyophilised plant.

The amount of total phenolics varied widely in plant materials and ranged from 265 mg to 594 mg GAE/100g dried plant. Results are presented in Figure 7.

As shown the total polyphenol content in plants was found greater in air dried plant than lyophilized plant. Our experiments show that the wild garlic is the richest in polyphenol compounds.

Total flavonoid contents. Furthermore, the results obtained from evaluation of total flavonoid content also indicate great variations (Fig. 7). In the wild garlic plant, the content of quercetin equivalents was notably higher than it was in nettle and mustard garlic. Also, the total flavonoid content in plants was found greater in air dried plant than lyophilized plant. The decreases of total phenolic and flavonoid contents in lyophilized plants are most probably caused by the solubility of compounds in the water removed by lyophilization.

The results concerning β-carotene and chlorophyll content obtained 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 phisyco-chimical analyses results, we observed the following: the graphic representation with the auto device Cell UVD-3200, is between 400 and 500 nm (Fig. 8, Fig. 9), values wich appart of the UV-VIS spectrum of the β-carotene standard absolute (Merck).

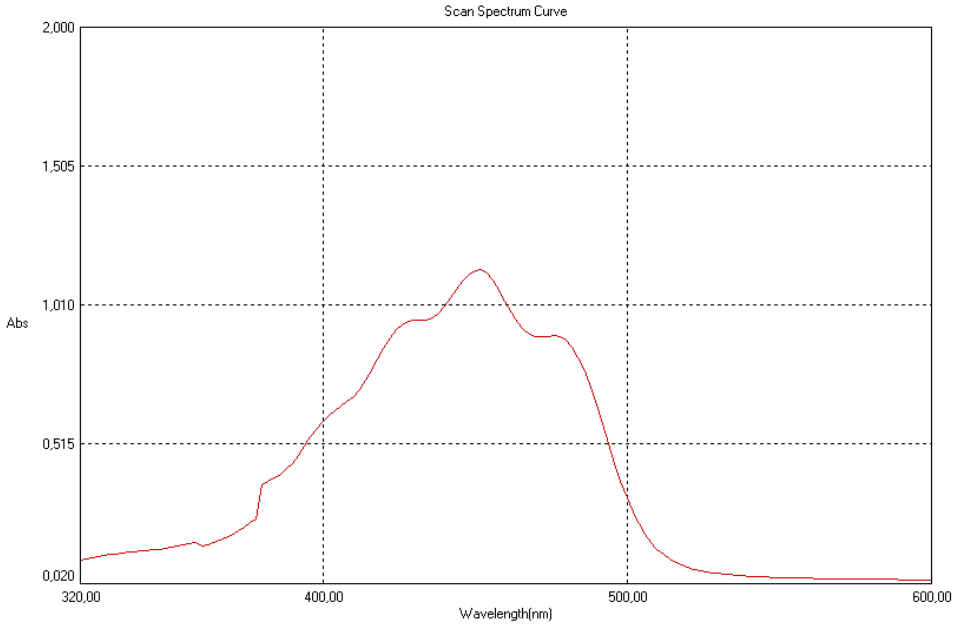

Fig. 8 - Spectrometry UV-VIS β carotene absolute (Merck) ethalon

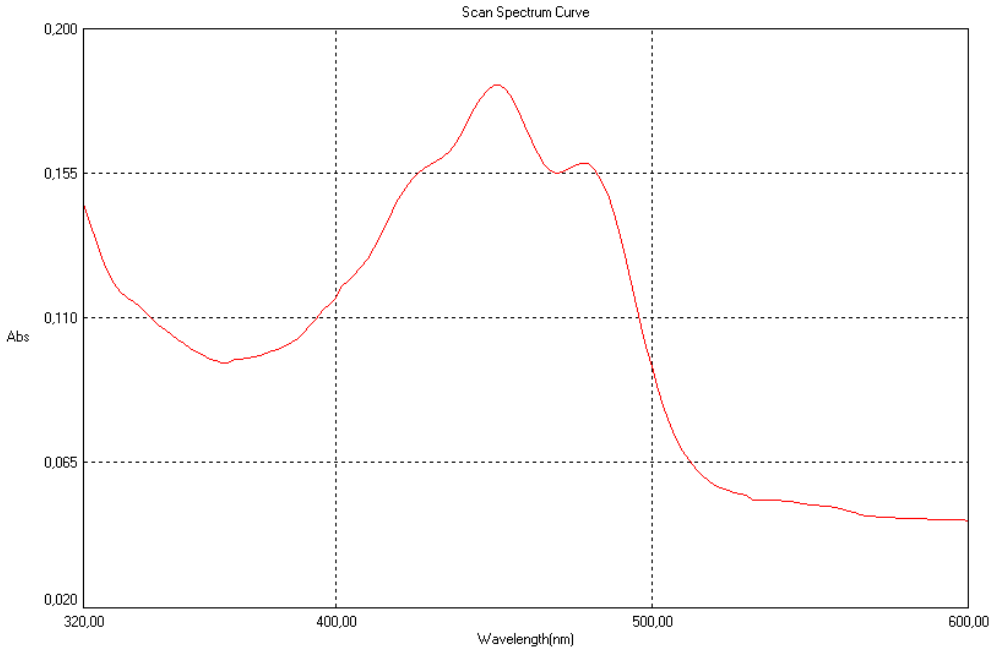

Fig. 9 - Spectrometry UV-VIS β-carotene of *Allium ursinum*

The values obtained of analyzed samples can be observed at standard curve characteristic β-carotene with the values R^2=0,9913 (Fig.10).

New researches concerning the content of chlorophyll and carotenoids from *Allium* genus put in evidence valoric differences that can be compared with our obtained results . So, the highest content of investigated pigments was observed in leaves of wild garlic (*Allium ursinum*): 999mg/100g for carotenoids and 287 mg/100g for chlorophyll *a*, and 135mg/100g for chlorophyll *b* (Stajner and Varga 2003).

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

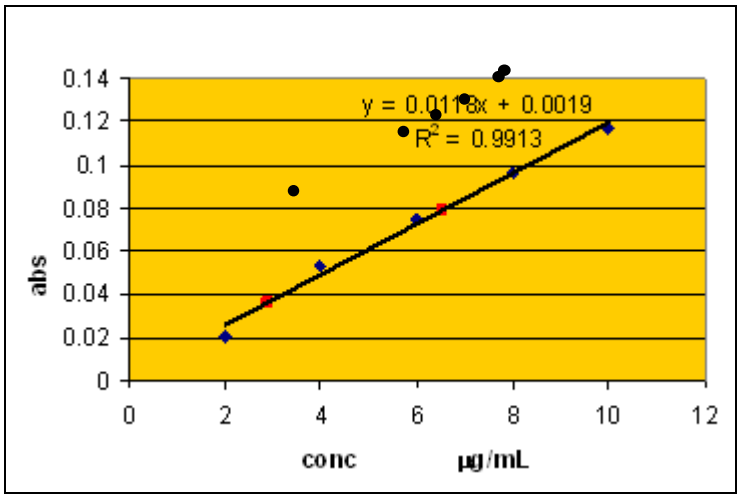

Fig. 10 - β-carotene determination of samples

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

The β carotene content analyzed in present paper is higher in case of *Urtica dioica* species (1044,8 mg/100g lyophilised sample) and lower at *Alliaria petiolata* species (373g/100g dry plant). However, we must do an specification about lyophilised samples wich have higher values in comparison with the natural ones. *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 (Ciocârlan, 2000).

Table 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

This plant is 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, regarding the containment of β-carotene with antioxidant effect (Gayathri et. al., 2004; Herdan et. al., 1995).

Peroxide formation is a major concern from the point of view of rancidity and toxicology of fried oils. Food lipid oxidation products such as peroxides, malonaldehyde, and several cholesterol oxidation products are reported to promote atherosclerosis and coronary heart disease (Subramanian et. al., 2000).

The evolution in time of PV (meq. of peroxide per kg of sample) of the sunflower oil samples during heat treatment at the 110°C is shown in Figure 11.

Our results showed a significant difference between PV of treated (sunflower oil with *Salvia sc*., *Salvia off*. and with *Allium ursinum*) and PV of untreated (original sunflower oil) samples during heating. Although the same temperatures were applied to all samples, the amount of peroxides found in untreated sample was higher than that of peroxides contained in treated samples. However, no big differences were observed between PV of treated oil samples. Peroxides values increased in 90 minutes from 7 to 20 meq/kg for original sunflower oil, from 6.25 to 12 meq/kg for sunflower with *Salvia sc*., from 6 to 13 meq/kg for sunflower with *Salvia off*. and from 6.5 to 12.5 meq/kg for sample with *Allium ursinum*, after a heating at 110°C. As the temperature was raised from 25 to 200°C respectively, PV

for original sunflower oil increased from 7 to 16 meq/kg and for treated oils the increase was smaller, about from 6 to 12 meq/kg. Another important indicator of oil deterioration during heating is the FFA content. The released fatty acids, resulted during frying at elevated temperatures (160–180°C) in the presence of air and moisture, are more susceptible to thermal oxidation under frying temperatures. The oxidized products of fatty acids give the off-flavors and odors (hydrolytic rancidity) to the frying medium and fried foods. Therefore, controlling the level of FFA within a reasonable range would prevent the breakdown of fats.

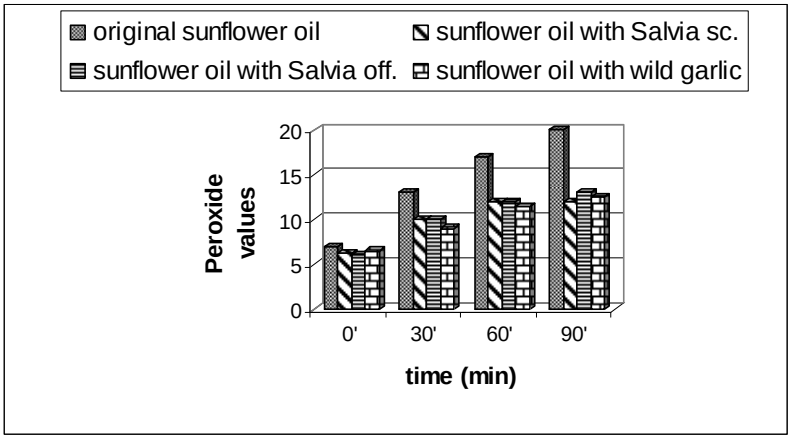

Fig. 11 - Effects of *Salvia* and wild garlic adittion to sunflower oil on time evolution of peroxides (PV) formed after exposure at 110°C temperature

The change in PV of the untreated and treated sunflower oil during heat treatment, at different frying temperatures is shown in Figure 12.

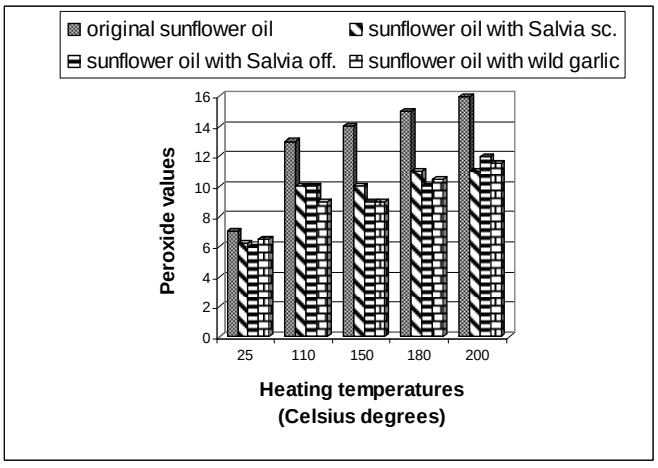

Fig. 12 - Effect of *Salvia* and wild garlic addition to sunflower seed oil on peroxides (PV) formed after exposure to different frying temperatures.

In the present study, the FFA content increased from 1.2 to 2.6 (mgNaOH/g sample) for original sunflower oil, from 1.2 to 1.5 (mgNaOH/g sample) for sunflower oil with *Salvia sc*. and *Salvia off*. and from 1.2 to 1.45 (mgNaOH/g sample) for sunflower oil with wild garlic, by heating from 25 to 200 °C, respectively, as shown in Fig. 13. Analysis of results presented in this paper revealed the existence of a difference between treated and untreated samples of oil during heating. However, it did not show any difference between sunflower oil with *Salvia* species and sunflower oil with wild garlic samples.

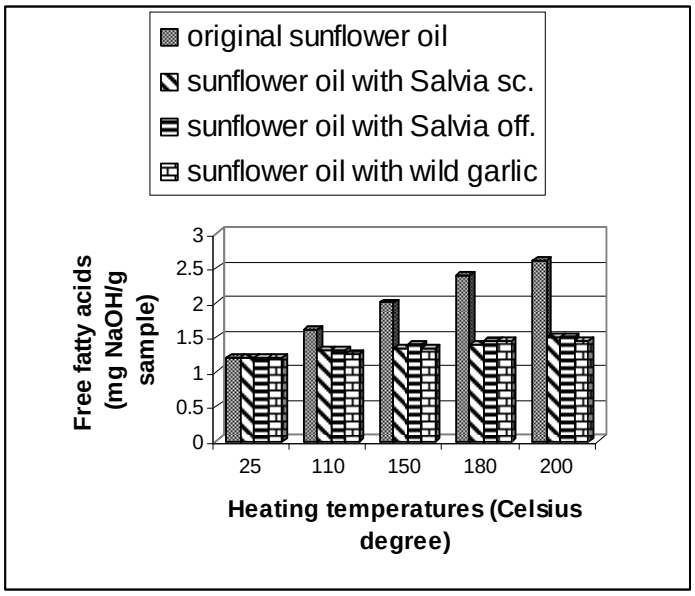

Fig. 13 - Effect of *Salvia* and wild garlic addition to sunflower seed oil on free fatty acids (FFA) formed after exposure to frying temperatures.

Conclusions

Our studies show that Romanian plants may be potent sources of natural antioxidants because the total phenolic content had positive correlation with antioxidant capacity (Jastrzebski et al., 2007; Jianxiong et al., 2008). According to our results, the plants from the autochtones flora are of very good quality. The total polyphenol and flavonoid content are important chemical parameters for plants regarding their biological properties. Both parameters should be applied to the antioxidant capacity studies.

The highest content in total chlorophyll (*a* and *b*) from the three analyzed plants was found in common nettle. The lyophilised samples had high values in comparison with the dry samples on natural way only in case 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.

The obtained results concerning effect of aromatizing plants from *Salvia* sp. and *Allium ursinum* (wild garlic) species added to sunflower oil, during heat treatments showed the sunflower oil quality growth rate.

Though the PV and FFA increased with temperature for all samples, however, the evolution of studied parameters in treated oil was found to be slower than the one observed in untreated oil. So, sunflower oil with Salvia and wild garlic samples exhibited relatively reduced peroxides values and FFA contents, following heat treatment, comparatively with original sunflower oil.

Conclusively, we have proved that these plants (*Salvia officinalis*, *Salvia sclarea* and *Allium ursinum*) used in alimentation for their aromatizing and curative properties, show good properties as antioxidants and/or free radicals scavenger. Thus, their incorporation in sunflower oil improved its thermal resistance and stability, which support their use to control lipid oxidation during food processing. This may be due to the abundance of natural antioxidants (such as polyphenols and flavonoids) present both in salvia and wild garlic which were transferred into sunflower seed oil following its treatment with natural herbs. These natural components can react with free radicals of the frying oil, acting synergistically as free radical scavengers and/or contributed to the protection of tocopherols, susceptible to heat-induced loss, particularly to the protection of α-tocopherols, the main antioxidant contained in original sunflower seed.

Our results are in according with other studies regarding effect of natural herbs on oil stabilization (Bensmira et al., 2007). Further studies will be conducted to better understand the factors influencing antioxidant activity of those aromatizing plants.

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