Bioactive Compounds and Antioxidant Activity in Some Fresh and Canned Aromatic Herbs

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Abstract
The chlorophylls, carotenoids, total polyphenols contents and antioxidant activity in fresh and stored Parsley, celery and dill leaves were comparatively determined by spectrophotometric and high performance liquid chromatography (HPLC) methods. Results indicated that freezing does not induce significant changes in chlorophyll concentration. Drying induces significant decreases, varying according to the analyzed plant; parsley (60%), followed by dill (56.4%) and celery (45.3%). The carotenoids identified in all plants were lutein, β-carotene and cis-β-carotene. Fresh parsley leaves showed the highest total carotenoid concentration (21.64 mg/100g FW), followed by celery (15.41 mg/100g FW) and dill (14.95 mg/100g FW). Total polyphenols had an average concentration of 2.15 mg/g FW in parsley; 2.7 mg/g FW in dill and almost the double, 4.13 mg/g FW in celery. Drying of leaves induced a decrease with approximately 50% of the total polyphenol concentration, while freezing, did not significantly affect it. The highest antioxidant activity was observed in the dill, followed by parsley and celery. In both types of methods (freezing and drying in the oven), decreases in antioxidant activity were observed.

Keywords: aromatic herbs, antioxidant activity, carotenoids, chlorophylls, total polyphenols

Introduction
Green leafy vegetables are important constituents of the human diet, contributing to the daily intake of minerals, vitamins and other biologically active compounds.

Parsley (Carum petroselinum, Apium petroselinum, L., Petroselinum hortense (crispum)) is a biennial or perennial plant, indigenous to the Mediterranean regions. It is cultivated in Europe, USA and Japan. The most common uses of parsley are as flavoring, in combination with other herbs, both as fresh or dried leaves. Parsley is a component of the “bouquet garni” in French cuisine (Shelef, 2003). Parsley has a very high content of vitamins (β-carotene, thiamin, riboflavin and vitamins C and E), but also it is a rich source of calcium, iron and folate, fatty acids, essential oils and other micronutrients. The essential oil of the leaves is considered superior to that obtained from the seeds and is used in condiments and seasonings (Parthasarathy et al., 2008).

Celery (Apium graveolens L.) is a biennial native to the Near East, southern Europe, and North Africa. It is cultivated for its seeds in India, Europe, United States of America (USA) (Shelef, 2003). The major bioactive compounds in celery include a class of phenolic compounds called furanocoumarins as psoralen, xanthotoxin and bergapten (Tyagi et al., 2008). Celery has been used in traditional medicine and aromatherapy due to its many health benefits such as lowering the blood pressure, treatment of bronchitis, liver and spleen disease or arthritic pain, this natural holistic approach to health becoming more and
more popular now a days. Celery stimulates healthy and normal functioning of kidney by helping in the elimination of the body toxins (Parthasarathy et al., 2008; Alsuhaibani, 2013).

Dill (Anethum graveolens L.) is a native of the Mediterranean and Russia. It is cultivated in many European countries, USA and Canada, India, Pakistan, and Japan. Leaves and seeds of dill contain various vitamins, carotenoids, phenolic compounds, volatile oils and minerals. Essential oils in seeds contain over 40 compounds, the most important components being limonene (responsible for flavor) and carvone (responsible for spicy taste) (Jirovetz et al., 2003; Shelef, 2003; Karklelienė et al., 2014).

Free radicals cause the oxidation of biomolecules (e.g., protein, amino acids, lipid and DNA) which leads to cell injury. Antioxidants or molecules with radical scavenging capacity are thought to exert a potential protective effect against free radical damage. Many studies showed that the natural antioxidants from various aromatic and medicinal plants can be involved in the reduction of chronic diseases induced by DNA damage, mutagenesis and carcinogenesis (Zhang et al., 2006; Jung et al., 2011). Increased intake of vegetables is generally associated with a reduced risk of cancer and cardiovascular disease. This association is based on the presence of different phytochemicals in vegetables like carotenoids and phenolic compounds, with either potential or proven beneficial effects on human health. Processing and preparation, particularly thermal treatment, which are applied prior to consumption, may affect these phytochemicals (Bunea et al., 2008).

Due to the seasonality of many fruits and vegetables, it is necessary to use affordable preservation methods in order to extend the time of use of such foods. Drying of vegetables can be carried out by using a variety of different processes, ranging from solar drying, air-drying, to microwave and freeze drying. All these processes influence the properties of the resulting product, causing changes in both the general chemical composition and the composition in phytochemicals (Dadan et al., 2018).

The objectives of this study were to determine the effects of leaf preservation, by drying or freezing, on the chemical composition and the antioxidant activity in three aromatic plants commonly used in Romanian cuisine, namely parsley, celery and dill. At the same time, the chlorophyll content of these plants was determined as a qualitative index of the specific color. To achieve these objectives the parameters studied in fresh, dried and frozen leaves were: chlorophyll concentration; total carotenoid concentration and their profile; the total phenolic content and total antioxidant activity.

**Materials and methods**

All the samples, parsley (Petroselinum crispum), celery (Apium graveolens) and (dill (Anethum graveolens) were harvested in the Cluj county area (Romania). The fresh leaves were cleaned and washed under running tap water, divided in parts and weighed exactly. The fresh parts were analyzed on the same day. The second group of samples from each plant was blanched for 30 seconds (as a pretreatment for storage) and frozen at -20°C. In the case of vegetables, blanching is performed in order to reduce the microbial load, to stabilize colours and to inactivate particular enzymes which degrade phytochemicals (Bunea et al., 2008). The third group was dried in the

![Figure 1. Storage and processing conditions of each leaves sample](image-url)
oven at 30°C during one week. All samples were weighed just prior to processing, so all results were expressed on fresh material (FW).

Storage and processing conditions of each type of leaves are presented in Figure 1.

**Determination of carotenoid profile and total carotenoid concentration**

Total carotenoids were extracted from 5 g fresh or preserved leaves, which were homogenized using a Miccra Art D9 type homogenizer (MICCRA GmbH, Heitersheim, Germany) at a speed of between 10000 and 39000 rpm. The homogenate was extracted with a mixture consisting of methanol:ethyl acetate:petroleum ether (1:1:1, v/v/v). After filtering the extract, the residue was re-extracted two times with the same solvent mixture, following the procedure described by Bunea et al., (2008). The extracts were combined and partitioned in a separation funnel, successively with water, diethyl ether and saturated NaCl solution. The ether phase was evaporated to dryness under vacuum, using a rotary evaporator at 35°C. The oleoresin (evaporated residue) was dissolved in 15 ml of petroleum ether and divided into two parts. The total carotenoid content was estimated by reading the sample absorbance at λ max = 442 nm, using 2500 as the specific absorption coefficient (Britton, 1995). All results were expressed as mg/100g FW.

Half of the oleoresin was dissolved in diethyl ether and saponified overnight, in the dark, at room temperature using an equal volume of 30% methanolic KOH. The saponified extract was washed with saturated saline solution and distilled water. The organic layer containing carotenoids was dried over anhydrous sodium sulphate and evaporated to dryness (Bunea et al., 2008).

**HPLC analyses for individual carotenoids**

were carried out on a Shimadzu LC20 AT system (Shimadzu Europe LTD., Duisburg, Germany), with PDA detector, using a reversed phase C30 YMC column (250x4.6 mm; 5 μm). A binary gradient mobile phase was used, consisting in methanol / tert-butyl methyl ether / water (81:15:4) (solvent A) and tert-butyl methyl ether/ methanol/ water (90:7:3) (solvent B). The gradient started with 1% B at 0 min and increased to 100% B at 160 min, according to the method described by Giuffrida et al., (2012). The flow rate was adjusted to 0.8 ml/min. All chromatograms were monitored at 450 nm. The HPLC peaks were identified by using parallel HPLC runs with available carotenoid standards as well as by recording the UV–Vis spectra specific to each carotenoid peak.

**Determination of chlorophylls**

Samples were homogenized and extracted with 90 % acetone in water, using a magnetic stirrer until the residue was colorless. The absorbance was read at 645 and 663 nm (Dulf et al., 2010). In order to quantify the chlorophylls a (Chl a) and b (Chl b) the following formulas were used:

\[
\text{Chl a (mg/g)} = (11.75 \times A_{663} - 2.35 \times A_{645}) \times V/g
\]

\[
\text{Chl b (mg/g)} = (18.61 \times A_{645} - 3.96 \times A_{663}) \times V/g
\]

where: \( A_{645} \) – absorbance at 645 nm; \( A_{663} \) – absorbance at 663 nm; \( V \) – volume of the extract (ml); \( g \) - sample s weight (mg).

**Determination of total phenolic content**

Total phenolic compounds were determined using the Folin–Ciocalteau reagent according to Bunea et al., (2008) procedure. The concentration of the total phenolic content was determined as mg of gallic acid equivalent (GAE) by using an equation obtained from gallic acid calibration curve (in the range 0.5–1.3 mg/100 ml). The results were expressed as milligrams of GAE per g FW.

**Determination of total antioxidant activity**

In order to determine total antioxidant activity, a silver nanoparticle method proposed by Ozyurek et al., (2012) was used and modified by Pintea et al., (2015). The extraction of the hydrophilic compounds was performed in methanol: water (80:20, v/v). The spectrophotometric method applied is based on the reduction reaction of \( \text{Ag}^{+} \) ions (from silver seed solutions stabilized with sodium citrate) to \( \text{Ag}^{0} \). The absorption of the stabilized solution of silver nanoparticles was read at \( \lambda = 423 \) nm. In order to calculate total antioxidant activity, a standard curve obtained from standard gallic acid solutions was used.

**Statistical analysis**

All assays were performed in triplicate. The statistical data were expressed as mean ± standard deviation (SD). In order to determine the significant differences between the fresh and canned herbs, analysis of variance (ANOVA) followed by student's t-test were performed. The criterion for statistical significance was set at \( p < 0.05 \) or \( p < 0.01 \).
Results and discussions

In higher plants occur two types of chlorophyll - a and b. Chlorophyll and its derivatives exert a positive effect on human body by complexing selected chemical compounds claimed to be carcinogenic agents (Kuźma et al., 2014). In the case of fresh leaves (Table 1), the highest concentration of Chl a was observed for dill leaves (5.20 mg/g), followed by parsley (4.63 mg/g) and celery (2.21 mg/g), while the highest concentration of Chl b was recorded for parsley (2.27 mg/g), followed by dill (1.31 mg/g) and celery (1.25 mg/g).

In terms of conservation, both methods influence the concentration of chlorophyll pigments. Color is often degraded during the preservation of leaves, especially during the drying process. In the case of drying preservation, there are significant decreases in chlorophyll levels, depending on the plant analyzed. Thus, the most significant decrease has occurred in parsley (p < 0.01), followed by dill and celery (p < 0.05). The data obtained in the case of drying preservation are similar to those reported by Kamel (2013), which determined the variation of the biochemical components of the parsley and dill leaves dried in microwave oven, over various periods of time. A decrease in the concentration of chlorophyll in plants maintained at low temperature (4°C) was also observed by Cătunescu et al., (2012).

Carotenoids are important compounds because of their biological activities: vitamin A activity, prevention of age-related macular degeneration, skin protection against UV radiation. Some studies of the anti-oxidant activity of carotenoids demonstrate the antioxidant capacity of carotenoid standards or food extracts (Andrei et al., 2007; Bunea et al., 2012).

The total carotenoid concentration in fresh parsley leaves showed the highest value, the average of the analyzed samples being 21.64 mg/100g FW. In the case of fresh leaves of celery and dill, similar concentrations were observed, averaging 15.41 mg/100 g FW for celery and 14.95 mg/100 g FW for dill (Figure 2). Regarding the fresh parsley leaves the results are in agreement with those reported by El-Qudah (2008), who analyzed the total carotenoid concentration in various aromatic plants commonly used in the human diet. However, the present results are higher than those observed by Daly et al., (2010), with an average of 11.1 mg/100 g and Arnold et al., (2013) with 12.63 mg/100 g FW (lutein, zeaxanthin and beta-carotene), but they are lower than those found by Kuźma et al., (2014), where the analyzed parsley leaves contained average values of 31.28 mg/100 g.

In dill leaves (Figure 2), the concentration determined in our study is similar to that observed by Lisiewska et al., (2006). According to this study, the total carotenoid concentration also depends on the size and degree of maturation of the plant, the recorded values ranging from 9.2 to 16.9 mg/100g.

Table 1. Chlorophylls content of samples (mean ± S.D.)

<table>
<thead>
<tr>
<th>Leaf type</th>
<th>Sample type</th>
<th>Chlorophyll a (mg/100g)</th>
<th>Chlorophyll b (mg/100g)</th>
<th>Total chlorophylls (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parsley</td>
<td>Fresh (P)</td>
<td>4.63±0.23</td>
<td>2.27±0.18</td>
<td>6.90±0.40</td>
</tr>
<tr>
<td></td>
<td>Dry (Pd)</td>
<td>1.90±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.76±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen (Pf)</td>
<td>3.98±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.90±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Celery</td>
<td>Fresh (C)</td>
<td>4.07±0.07</td>
<td>1.25±0.16</td>
<td>5.33±0.26</td>
</tr>
<tr>
<td></td>
<td>Dry (Cd)</td>
<td>2.20±0.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.70±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.91±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen (Cf)</td>
<td>3.81±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.11±0.07</td>
<td>4.92±0.24</td>
</tr>
<tr>
<td>Dill</td>
<td>Fresh (D)</td>
<td>5.20±0.08</td>
<td>1.31±0.05</td>
<td>6.51±0.15</td>
</tr>
<tr>
<td></td>
<td>Dry (Dd)</td>
<td>2.17±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65±011&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.83±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Frozen (Df)</td>
<td>4.16±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.14±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Different letters within columns represent: <sup>a</sup>p ≤ 0.05 (significant); <sup>b</sup>p ≤ 0.01 (highly significant)
of fresh leaves. Higher values were reported by Arnold et al., (2013), with 32.83 mg/100 g FW (lutein, zeaxanthin and beta-carotene).

An important factor contributing to the change in carotenoid concentration in leaves is the preservation mode. As shown on Figure 2, the concentration of carotenoids decreases in dry leaves but is higher in the case of leaf preserved by freezing. Preservation by drying, even if it was conducted in a closed environment protected from light, decreased the concentration of carotenoids, more pronounced in the case of celery (with 66.7%), followed by dill (49.5 %) and parsley (40%). In case of frozen samples, the carotenoid concentration increased compared to the fresh ones for all the plants analyzed. As shown in our study, blanching and freezing of the leaves may facilitate the release of carotenoids from the specific matrix. Carotenoids are more stable during preservation processes than other classes of compounds, such as chlorophylls.

The total carotenoid extracts were separated by HPLC, individual carotenoids being identified by comparison with standards and based on their specific UV-VIS absorption spectra. The chromatograms obtained in the case of fresh leaf samples are shown in Figure 3. The main carotenoids identified were lutein, all-trans-beta-carotene and cis-beta-carotene. Neoxanthin and violaxanthin were also present in all extracts but were not quantified. Other studies have shown that these plants also contain, in small amount, other carotenoids, namely zeaxanthin and beta-cryptoxanthin (Rodriguez-Amaya, 1997; Furtado et al., 2004; Daly et al., 2010). The profile of carotenoids is a typical one for photosynthetic tissue, with lutein as major pigment (40-50 %) (Britton and Kachik, 2009).
As depicted in Figure 4, the highest concentrations were registered for lutein, in all samples, ranging from 48.3 to 52.8% of total carotenoids. After the different treatments applied to the samples, lutein was the most stable carotenoid. The lowest values were observed for cis-β-carotene, with concentrations of 5.86 - 9.08%. By comparison, fresh parsley contains the highest proportion of lutein (52.8%) and smallest cis-β-carotene (5.86%) content. In the case of dill, the concentrations were 51.2% for lutein and 6.87% for cis-β-carotene. Celery was characterized by the lowest concentrations of lutein (48.3%) and highest concentrations of cis-β-carotene (9.08%). Regarding β-carotene, it had similar concentrations in all samples, ranging from 41.2 to 42.6%. Carotenoids from food have been associated with numerous health benefits such as a reduced risk of cardiovascular diseases, age-related macular degeneration (AMD) and cataract, or certain types of cancer. Lutein particularly has been proved to be efficient in preventing AMD and to be beneficial for the cognitive function (Eggersdorfer and Wyss, 2018).

Polyphenols constitute one of the most numerous and ubiquitous groups of plant metabolites...
and are an integral part of the human diet. Vegetables are known to possess a variety of antioxidant effects and properties. Flavonols and flavones in plant materials are closely associated with their antioxidant function mainly due to their redox properties exerted by various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity (Ciz et al., 2010).

The results obtained in the determination of total polyphenols concentration are presented in Figure 5. Total polyphenols had average concentrations of 2.15 mg/g FW in parsley; 2.7 mg/g FW in dill and much higher; 4.13 mg/g FW in celery. Our results are similar to those presented by some authors but in contrast with others. The relatively high variability of quantitative data from various studies can be due to the plant sample (cultivation conditions) but also to the extraction method. Thus, according to the article published by Kuźma et al., (2014), the total phenolic content of parsley leaves varies between 362.93 mg/100 g DW for 80% methanol extraction and 723.58 mg/100 g for acetone extraction. Similarly, for celery leaves, the concentration of polyphenols is dependent on the extraction mixture. In the article published by Jung et al., (2011) four different types of extracts were studied. The extract in methanol had the highest concentration of phenolic compounds (51.09 mg/g DW), followed by the extract in water (46.40 mg/g DW), ethyl acetate (22.70 mg/g DW) and butanol (19.43 mg/g DW).

As can be seen from Figure 5, drying the leaves in the oven induces a decrease of about 50% in the concentration of the polyphenols. In the case of freezing, polyphenols losses are very low, the values being close to those in fresh leaves.

Starting from the high content in water-soluble antioxidant compounds, in the present study, it was of interest to carry out a determination of the total antioxidant activity using a new method based on stabilized silver nanoparticles. The results obtained (µmoles gallic acid /g) are detailed in Figure 6. Antioxidant activity is influenced by leaf preservation, for both types of methods, freezing and drying. In the case of fresh samples, the highest antioxidant activity was observed in dill (with an average of 8.03 µmoles gallic acid /g) followed by parsley (5.45 µmoles gallic acid /g) and the lowest in case of celery (4.38 µmoles gallic acid /g).

Preservation of plants, both by drying and freezing, causes significant decreases in antioxidant activity. There are many papers attempting to rank the antioxidant properties of different plant materials using different methods. The antioxidant capacity assays measure the combined effect of many antioxidants present in the sample, which are able to scavenge free radicals generated in the assays (Ciz et al., 2010). In complex plant matrices, such as aromatic plants, characterized by a wide variety of polyphenols, ascorbic acid and other phytochemicals with antioxidant action, some discrepancies between polyphenols content and total antioxidant activity could be expected.
Conclusion

The leaves of the Romanian aromatic herbs studied have a high nutritional value, being rich in carotenoids and phenols and characterized by a significant antioxidant activity. The conservation of leaves by freezing is a better method compared to the drying technique, this fact being proved by the low decreases of the chlorophylls content and antioxidant compounds, thus better preserving their nutritional values.

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References