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EFFECT OF COLD STORAGE ON ANTIOXIDANTS FROM MINIMALLY PROCESSED HERBS

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Abstract

Vitamin C and total polyphenols are quality markers used to assess the effect of treatments and storage on foodstuffs. Although, the effect of other conventional shelf-extension method is well established, refrigeration was taken for granted and rather neglected. The present study aims to describe its influence on three minimally processed herbs stored at 4°C for 12 days: parsley (Petroselinum crispum), dill (Anethum graveolens) and lovage (Levisticum officinale). The content of ascorbic acid and total polyphenols was determined on methanolic extracts. Ascorbic acid was separated, identified and dosed using HPLC coupled with an UV–VIS detector. Total polyphenols were determined spectrophotometrically, following Folin-Ciocalteu method. On the first day of storage, the content of vitamin C was above 170 mg/100 FW for the three herbs: dill had the highest content, followed by parsley and lovage, statistically similar. During the 12 days of storage, the content of vitamin C decreased by 18% for parsley, by 8% for lovage and by 3% for dill. At the beginning of the study, lovage had the highest content of total phenols followed by parsley and dill. On day 5 of storage, the content increased, reaching the maximum values for the three herbs and then it decreased below the levels of the first day. It was noted that during the 12 days of study, the evolution of total polyphenols at refrigeration temperature was given by a function of second degree. Thus, the present study confirms that vitamin C can be successfully used as a quality marker for herbs due to its low stability during storage. The evolution of total phenols is polynomial, reaching its peak during the shelf-life of herbs.

***Key words**: ascorbic acid, dill, lovage, parsley, phenols.*

INTRODUCTION

Consumers are becoming more literate about the benefits of fresh products containing bioactive compounds (Parfitt et al., 2010). Aromantic herbs are believed to provide antioxidant compounds; vitamins, phenolic acids, flavonoids, sterols and coumarins are the compounds with functional properties in herbs (Charles, 2012; Santos et al., 2014). But, the continuous race against time of modern society has led to an increasing demand for ready-to-eat products. This generated a steady increase of minimal processing industry of fruit and vegetables (Parfitt et al., 2010). The commodities are fresh-like containing all the valuable phytochemicals and consumers perceive them as convenient, of high quality, less wasteful and with a reasonable price (Alvarez et al., 2013).

Although minimal processing keeps the products alive, every operation in the production chain promotes senescence processes (ethylene production, respiration and browning) and reduces resistance to microorganisms (Alvarez et al., 2013). This lowers quality, shortens shelf-life and enhances microbial contamination.

Hydrosoluble vitamins, such as vitamin C, are the compounds with the highest variation during the storage of minimally processed products, because the changes are more intense in the water fraction (Santos et al., 2014). Thus, vitamin C and total polyphenols are quality markers traditionally used to assess the effect of treatments and storage on vegetables. Although, the effect of other conventional shelf-extension method is well established (Śledź et al., 2013; Mezeyová et al., 2016), refrigeration was taken for granted and rather neglected. Herbs from *Lamiaceae* family (basil, lemongrass, marjoram, mint, oregano, rosemary, thyme) are thoroughly studied (Blasa et al., 2010; Śledź et al., 2013; Curutchet et al., 2014; Santos et al., 2014), while *Apiaceae* family (coriander, dill, lovage, parsley) has been less considered (Mezeyová et al., 2016; Tsamaidi et al., 2016). Moreover, parsley, dill and lovage are the main herbs consumed in Romania (Glăman et al., 2015).

The aim of this study was to assess the evolution of the content of antioxidants (vitamin C and total polyphenols) in three minimally processed herbs stored at 4°C for 12 days: parsley (*Petroselinum crispum* (Mill.) Fuss var. neapolitanum), dill (*Anethum graveolens* L.) and lovage (*Levisticum officinale* Koch.).

MATERIALS AND METHODS

Preparation of samples. Fresh parsley (*Petroselinum crispum* (Mill.) Fuss var. neapolitanum), dill (*Anethum graveolens* L.) and lovage (*Levisticum officinale* Koch.) were purchased from a local market and minimally processed as previously described (Cătunescu et al., 2012b; Cătunescu et al., 2016) by cutting of stems to approximately 6 cm, washing with tap water, draining and packing in polyethylene bags (Krups Vacupack Plus F380). The samples were stored at 4°C for 12 days and analyzed in the 1st, 5th, 8th and 12th day (Santos et al., 2014).

Extraction procedure. The content of antioxidants was determined on methanolic extracts. The extraction procedure proposed by Cătunescu et al. (2012b); Cătunescu et al. (2017) was followed: 1 gram of fresh herbs was manually extracted with 10 ml of acidified methanol (99.09:0.01 v/v, MeOH:HCl) in a mortar, at shade. The extracts were later filtered, dried in a vacuum rotary evaporator at 37°C and redissolved in 5 ml of pure methanol. The samples were stored at -20°C in opaque containers.

Determination of ascorbic acid content. A previously proposed method was employed (Cătunescu et al., 2012b; Roman et al., 2013; Cătunescu et al., 2017): the extracts were filtered (Teknokroma Syringe Nylon Filters 0.45 μm; 13 mm diameter) and the ascorbic acid was separated, identified and dosed in a HPLC Agilent 1200 system coupled with UV–VIS detector (DAD). The mobile phase was water/acetonitrile/formic acid (94/5/1; v/v/v) and it isocratically eluated an Eclipse XDB-C18 column (5 μm; 150 x 4.6) at a flow rate of 0.5 ml/min. The chromatograms were registered at a wavelength of 240 nm. A calibration curve of L-ascorbic acid (Sigma 99%) was used to dose the ascorbic acid in the samples. All results were presented as mg/100 g of fresh herbs (FW).

Determination of total polyphenols content. The Folin-Ciocalteu method proposed by Roman et al. (2013) and Cătunescu et al. (2017) was used. A calibration curve was plotted using 5 concentrations of a 1 mg/ml solution of gallic acid in 40% ethanol. One ml of each dilution, 5 ml of Folin-Ciocalteu reagent and 60 ml of distilled water were mixed. A volume of 15 ml of 7.5% Na_2CO_3 solution were added after 1 minute and the absorbance was measured after 2 hours at a wavelength of 750 nm (Biotek multidetector UV-Vis spectrometer). The same procedure was used for the samples prepared in the wells of spectrophotometric plates as follows: 2.375 ml of distilled water; 0.025 ml of methanolic extracts; 0.150 ml of Folin-Ciocalteu reagent and 0.450 ml Na_2CO_3. The blanks contained 0.025 ml of 40% ethanol instead of extracts. The content of total polyphenols in the samples was expressed in mg GAE/100 g FW using the calibration curve.

Statistical analyses. The results were obtained in triplicate and expressed as mean ± SD. XLSTAT software (Addinsoft, New York, USA, Version 2016.03.31333) was used to compute one-way ANOVA ($p < 0.05$), Fisher pairwise comparisons (LSD, $p = 0.05$) and correlational analyses (Pearson coefficient).

RESULTS AND DISCUSSIONS

Effect of cold storage on vitamin C content. Ajayi et al. (1980) concluded that the content ascorbic acid was close to 100% of the total content of vitamin C in leafy vegetables, thus, it was approximated as such for the current study (Cătunescu et al., 2012b).

Table 1. Vitamin C content of minimally processed parsley, dill and lovage during a 12-days storage (mg /100g fresh herbs)

Storage day	parsley	dill	lovage
1	179.75±5.92 $^{A, b}$	204.53 ± 5.06 $^{A, a}$	173.49 ± 3.37 $^{A, b}$
5	161.67 ± 1.15 $^{B, c}$	198.00 ± 2.65 $^{AB, a}$	171.83 ± 1.53 $^{A, b}$
8	154.67 ± 4.16 $^{BC, c}$	193.67 ± 2.52 $^{BC, a}$	171.33 ± 5.48 $^{A, b}$
12	147.91 ± 3.71 $^{C, c}$	188.53 ± 3.18 $^{C, a}$	168.76 ± 1.66 $^{A, b}$

Note: Different uppercases represent significant statistical differences for each herb during the storage period; while different lowercases among the three herbs for the same storage day (Fisher (LSD), $p < 0.5$).

Dill had the highest content of vitamin C on the first day (Table 1) (Cătunescu et al., 2012b), similar to 116 to 186 mg/100 g FW previously reported (Lisiewska et al., 2003; Lisiewska et al., 2006), but 3 times higher than the content obtained by Galoburda et al. (2012).

Parsley contained less ascorbic acid, similar to other reports (Cătunescu et al., 2012b; Stan et al., 2014; Cătunescu et al., 2017), higher than 133 mg/100 g found in other studies (Daradkeh and Essa, 2016), while lower than 310 mg/100 g reported by Lisiewska et al. (2003) for Hamburg variety and 257 mg/100 g for plain leaf parsley.

Lovage had a content of vitamin C similar to parsley's (Cătunescu et al., 2012b).

Thus, the three minimally processed herbs are all a "good source" of vitamin C, as defined by the Food and Drug Administration (FDA), because one table spoonful (10 g) provides the 10-19% of the recommended dietary allowance (RDA)(e-CFR, 2016).

During the 12 days of storage the content of vitamin C decreased linearly by 18% for parsley ($r = -0.970$, $p < 0.05$), 8% for dill ($r = -0.998$, $p < 0.05$) and 3% for lovage ($r = -0.978$, $p < 0.05$). The linear regression of vitamin C content (y) and storage (x) are showed in equation 1 for parsley, equation 2 for dill and equation 3 for lovage.

$$y = -2.86 x + 179.56;\ R^2 = 0.94 \quad (1)$$
$$y = -1.45 x + 205.63;\ R^2 = 0.98 \quad (2)$$
$$y = -0.41 x + 174.03;\ R^2 = 0.96 \quad (3)$$

Similar, Howard et al. (1999) showed that the content of vitamin C decreases linearly during storage of vegetable at refrigeration temperatures. Lisiewska et al. (2003) observed that the dill stored at refrigeration temperatures gradually lost its vitamin C. Hydrosoluble vitamins – vitamin C included - are mainly lost because of water losses during handling or storage, temperature fluctuations and enzymatic oxidation (Mezeyová et al., 2016).

Thus, vitamin C, due to its low stability, is a good quality marker of herbs, both fresh and minimally processed during storage.

The content of vitamin C showed a significant correlation with sensory scores for taste, odor, aroma and overall quality (Cătunescu et al., 2012a) in the cases of dill and lovage (Table 2). These correlations can be attributed to the role that vitamin C can play as an aroma intensifier and as a general antioxidant. However, vitamin C is lost linearly with storage, as are other sensory attributes and correlation does not necessarily mean causation.

Table 2. Correlation of vitamin C content with some quality attributes of parsley, dill and lovage

Quality attribute	Pearson correlation coefficient (r)		
	parsley	dill	lovage
taste	0.45	0.94	**0.96**
odor	0.38	0.86	**0.98**
aroma	0.41	0.90	**1.00**
quality numbers	0.58	**0.98**	**0.99**
overall quality	0.54	**0.98**	**1.00**

Note: Values in bold are different from 0 with a significance level $\alpha = 5\%$.

Effect of cold storage on total polyphenols content. The content of total polyphenols was determined on methanolic extracts. Acidified pure methanol was showed to optimally solubilize phenols, compared with a 50% acetone solution or 70-80% methanol (Parry et al., 2006).

Lovage had the highest content of total polyphenols (Table 3).

Table 3. Total polyphenols content of minimally processed parsley, dill and lovage during a 12-days storage (mg GAE/100g fresh herbs)

Storage day	parsley	dill	lovage
1	$281.36 \pm 7.55^{C, b}$	$292.01 \pm 10.49^{A, b}$	$417.43 \pm 6.37^{B, a}$
5	$337.45 \pm 3.15^{A, b}$	$246.42 \pm 11.26^{B, c}$	$413.35 \pm 5.25^{B, a}$
8	$324.75 \pm 4.25^{B, b}$	$243.00 \pm 1.78^{B, c}$	$467.14 \pm 7.62^{A, a}$
12	$203.33 \pm 0.87^{D, c}$	$296.33 \pm 9.38^{A, b}$	$386.94 \pm 4.89^{C, a}$

Note: Different uppercases represent significant statistical differences for each herb during the storage period; while different lowercases among the three herbs for the same storage day (Fisher (LSD), $p < 0.5$).

Lisiewska et al. (2003) found a similar content of phenols in dill leaf 248 ± 9 mg/100 g FW.

The content of total polyphenols varies with the species, but also within the same group depending on external factors (pedo-climatic) and internal (intra-species variability), similar to other bioactive compounds (Bravo, 1998). Zheng and Wang (2001) found a slightly different content of phenols in the three herbs: 263 mg GAE/100 g FW for lovage, 112 mg GAE/100 g FW for parsley and 312 mg GAE/100 g FW for dill.

Al-Mamary (2002) reported 193.46 ± 4.22 mg GAE/100 g FW for parsley and established that the herbs from *Apiaceae* family were the richest in phenols. Zheng and Wang (2001) indicated, however, a very high content of phenols in oregano and marjoram (1,180 and 1 respectively, 165 mg GAE/100 g FW). But, parsley, lovage and dill are relatively rich in polyphenols when compared with other herbs known for their antioxidant activity such as: bay leaves, rosemary, thyme and lemon balm (Zheng and Wang, 2001).

Similar, the three herbs stand out when compared with other vegetables and fruit traditionally recognized as a "good source" of phenols. Bravo (1998) reported 100-2025 mg GAE/100 g FW for onions, 27-298 mg GAE/100 g FW apples, 140-1200 mg GAE/100 g FW for blackcurrant, 135-280 mg GAE/100 g FW for blackberries, 50-490 mg GAE/100 g FW grapes and 38-218 mg GAE/100 g FW strawberries.

Culinary herbs are, generally, rich in flavonoids, with much higher concentrations compared to other vegetables (Blasa et al., 2010; Daradkeh and Essa, 2016). Parsley contains apigenin, phenolic acids, especially caffeic acid, and reduced quantities of quercetin and luteolin (Hedges and Lister, 2007; Daradkeh and Essa, 2016). Jipa et al. (2008) indicated 225.93 mg of apigenin; 8.08 mg myricetin; 1.49 mg kaempferol; 0.33 mg luteolin and 1.24 mg quercetin per 100 g DW of parsley. Lovage is one of the richest herbs in quercetin (170 mg/100 g FW), but also contains kaempferol (7 mg/100 g FW); while dill contains quercetin (48-110 mg/100 g FW) kaempferol (16-24 mg/100 g FW) and isorhamnetin (5-72 mg /100 g FW) (Hedges and Lister, 2007).

The content of total polyphenols increased on the 5th day of storage, reaching the highest levels: it increased by 20% for parsley, 18.5% for dill and 12% for lovage when compared to the beginning of the study. But, it decreased on the 12th day by 28% for parsley and 7% for lovage, while remaining at approximately the level of the 1st day for dill.

The variation of total polyphenols during the 12 days of study followed the curve of a 2nd degree function. The curves of phenols content (y) and storage (x) are showed in equation 4 for parsley, equation 5 for dill and equation 6 for lovage.

$$y = -3.17x^2 + 34.31x + 249.13; R^2 = 0.99 \quad (4)$$
$$y = -1.77x^2 + 22.77x + 224.75; R^2 = 0.99 \quad (5)$$
$$y = -1.36x^2 + 13.85x + 410.71; R^2 = 0.71 \quad (6)$$

A similar variation was described for apples, beans, white grapes and celery. Schmitz-Eiberger and Matthes (2011) observed an increase in the content of phenols up to 34% during storage of apples, followed by a decrease, but the values fluctuated. Granito et al. (2008) reported a slight increase, by 7%, in the content of total phenols in beans up to the 90th day of storage, followed by a 76% decrease in the 150th day. Similar the content of phenols increased in white grapes and hawthorn by 24% and 59%, respectively, on the 9th day of storage and decreased subsequently up to the 17th day (Šamec and Piljac-Žegarac, 2011).

The increase of total polyphenols at the beginning of storage is generated by the physiological processes of the plant senescence processes. The aromatic amino acids, precursors of phenolic acids deaminate and the conjugated polyphenols hydrolyze resulting phenolic acid. Similarly, proteins and complex carbohydrates degrade, releasing phenolic compounds (Šamec and Piljac-Žegarac, 2011).

CONCLUSIONS

On the 1st day of storage dill had the highest content of vitamin C, followed by parsley and lovage. These values, above 170 mg/100 FW, indicated that herbs had a significantly higher content compared to other vegetables. During the 12 days of storage, vitamin C showed a substantial decrease during storage similar to other plant products stored at refrigeration temperature. It was reduced linearly, by 18% for parsley, 8% for lovage and 3% for dill.

Thus, vitamin C, due to its low stability, is a quality marker for herbs, both fresh and processed.

For dill and lovage, a positive correlation was observed between the content of vitamin C and taste, odor, flavor and overall quality ($r = [0.89\ldots0.99]$, $p < 0.05$).

The content of total polyphenols was evaluated for methanol extracts. Lovage had the highest content of phenols, followed by parsley and dill.

The three herbs had a content similar to other fruit and vegetables known for their polyphenols.

The content of total polyphenols increased on the 5th day of storage, reaching the maximum levels and later decreased until the end of the study. It was noted that the evolution of total phenols during storage at refrigeration temperature was given by a function of second degree.

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