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THE EFFECT OF POST-HARVEST TREATMENT AND STORAGE CONDITIONS ON VITAMIN C CONTENT IN TWO LEAFY PARSLEY CULTIVARS

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The objective of the study was to determine the content of vitamin C (L-ascorbic acid) in two leafy parsley (Petroselinum crispum Mill. Fuss) cultivars subjected to different post-harvest treatments. Ascorbic acid (AA), due to its instability, is one of the indicators of leafy vegetable freshness and quality. High content of this compound in vegetables is desired because of its bioactive properties. The cultivars differ in morphology of usable parts: 'Rialto' has flat lamina and 'Petra' triple-curled lamina. The plant material was obtained in 2015 and 2016 from experimental field in Warsaw-Wilanów. The leaves were washed directly after harvest: a) in tap water or b) in tap water with ozone added. Two methods of postharvest storage were applied: A) at the cold store and B) under simulated retail conditions. In the case of cold store method (A), the plants were tied in tufts and stored at the temperature of 0 °C and RH 90 % for 7, 14, 28 days in two variants: 1) in containers, where leaves petioles were immersed in water, and 2) in special bulk modified atmosphere packaging (MAP), dedicated to fresh herbs (Stepac, Israel). In the case of storage under simulated retail conditions (B), the leaves were kept for 48 hours at 10 °C, RH 30-40 % in two variants: 1) tufts wrapped in perforated PE film with petioles immersed in water, and 2) packed to retail MAPs, dedicated to leafy herbs (Stepac, Israel). Concentration of L-ascorbic acid in the leaves was determined spectrophotometrically, with the method based on reaction of Folin's phenol reagent in low pH. Fresh and stored 'Rialto' leaves were characterised by a higher concentration of AA than 'Petra' leaves (110 and 44 mg g⁻¹ f.w., respectively). Significant decrease of AA after ozone treatment was observed only for 'Rialto' directly after harvest. Storage length had significant influence on the content of AA in both cultivars. Decrease of AA content was observed during storage period. Average concentration of AA after 7 days of storage was 85 and 44 mg g⁻¹ f.w. for 'Rialto' and 'Petra', respectively. After 28 days of storage the concentration was 54% lower for 'Rialto' and 36% lower for 'Petra', compared to the initial content. Leaves after simulated retail conditions showed slightly lower AA content than those stored for 7 days in the cold room. Retail MAPs guaranteed significantly higher preservation of AA in both cultivars than wrapping in film with immersing in water. Washing in ozone-added water showed influence only on AA content in 'Rialto' leaves wrapped in film - they showed lower concentration of AA under simulated retail conditions. Our study showed that the content of AA in parsley leaves was cultivar dependent. Ozone treatment did not have negative effect on AA during the storage. Storage duration, rather than packaging methods, plays a key role in preservation of high content of AA. However, method of packaging is important under retail conditions, where temperature is higher and RH is much lower, than at a cold room.

Keywords: ascorbic acid, MAP, parsley, storage, ozone

INTRODUCTION

Many epidemiological studies prove that a high consumption of fresh herbs, vegetables and fruits protect against certain types of cancer and other important diseases. Leafy parsley (*Petroselinum crispum* (Mill.) Fuss) is aromatic herb, popular in the World. It originates from the Mediterranean regions of Europe and is used for garnishing and seasoning. The seed oil is used as a flavouring agent for meat and sausages. The parsley is also known in traditional medicine as an effective remedy against digestive disorders, nephritis, kidney trouble, high blood pressure, eye disorders. The effect relates to presence of biologically active compounds in the leaves in stems. Parsley is rich in secondary metabolites of a wide spectrum of biological activities, such as phenylpropanoid apiol, oleanolic acid, furanocoumarin isoimperatorin and oxypeucedanin. It is also rich in vitamin C (Lisiewska and Kmiecik, 1997; Sbai et al., 2016). Vitamin C, which is chemically L-ascorbic acid (AA) – 2,3-enediol-L-gulonic

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acid-g-lactone, is an essential, water-soluble vitamin. For nutritional purposes, vitamin C is the sum of ascorbic acid and dehydroascorbic acid (DHAA) ('total ascorbic acid'). The DHAA absorbed from the intestinal tract undergoes intracellular reduction (via NADPH- and glutathione-dependent reductases), rendering it biologically active (Bender, 2003; Wilson, 2005). Vitamin C is a cofactor in numerous physiological reactions in important biochemical processes in human body. These include post-translational hydroxylation of proline and lysine in collagen and other connective tissue proteins. The vitamin also plays role in collagen gene expression, synthesis of norepinephrine and adrenal hormones, activation of many peptide hormones, as well as synthesis of carnitine (Davies et al., 1991; Arrigoni and De Tullio, 2000; Johnston et al., 2007; Smirnoff, 1996). Besides these key functions, AA also acts as a cellular antioxidant. It facilitates intestinal absorption of iron and maintenance of plasma iron in a reduced form (Bender, 2003; Smirnoff, 1996). USDA 'National Nutrient Database for Standard Reference' (USDA, 2017) is one of official sources of dietary recommendations for vitamin C intake. The Recommended Daily Allowance (RDA) for vitamin C is 25 mg/day for children 4-8 years old, 75 mg/day for adult females, and 90 mg/day for adult men (Levine et al., 1995). Higher intake showed some health benefits for humans (Christen et al., 2010; Hemila, 2011; Honarbakhsh and Schachter, 2009; Jacob and Sotoudeh, 2002; Levine et al., 2009; Li and Schellhorn, 2007; Willcox et al., 2008). The basic natural sources of vitamin C are vegetables and fruits, including green leafy vegetables, like parsley (Eitenmiller et al., 2008).

Leafy aromatic plants, including leafy parsley, are prone to fast quality deterioration process. It results in tissue and cell integrity disruption, with a concomitant increase in enzymatic, respiratory and microbiological activity and therefore reduces shelf-life. As the result of the processes degradation of important for human biologically active compounds is observed (Watada et al., 1996; O'Beirn et al., 1999; Santos et al., 2014). This effect might be minimised by the use of adequate temperature management and modified atmosphere packaging (MAP). This technological method is used in postharvest preservation of many fruits and vegetables. MAP involves either actively or passively controlling or modifying the atmosphere surrounding the product within a package made of various types of polymeric films (Farber et al., 2003). In the case of parsley, some studies showed that the leaves kept in atmosphere composition of 10% O₂ and 10% CO₂ showed less decrease in chlorophyll (Yamauchi and Watada, 1993). Also parsley flavour and aroma were retained better in perforated film packages than in sealed ones (Manzano et al., 1995; Rosa et al., 2007). For storage of perishable vegetables and herbs special packaging films for MAPs were introduced by several producers, eg. Xtend film produced by Stepac, Israel (www.stepac.com/xtend). This film is used for special MAPs dedicated to different plant species, for storage in bulk on in retail. Food industry is also seeking for effective methods to ensure the safer food products, effective against food spoilage and pathogenic bacteria, yet harmless to humans. Ozone is a gas of strong antimicrobial action, due to potential oxidizing capacity and is used to disinfect water for drinking purposes in Europe (Guzel-Seydim et al., 2004). Ozone destroys micro-organisms (bacteria and fungi) by the oxidation of vital cellular components. It is important that at room temperature, ozone decomposes quickly to oxygen. There are some attempts to introduce ozone for postharvest treatment of several horticultural crops, like vegetables and fruits (Suslov, 2017). According to literature sources, ozone was added to water for washing of fresh produce with the concentrations varied from 0.1 ppm to 10 ppm, in relation to plant species (Hodges, 2003; Alexandre et al., 2011). Selma et al. (2007) reported that washing of lettuce leaves in water with ozone added in concentration of 1.6 ppm or 2.2 ppm for 1 min resulted in significant reduction of Shigella sonnei bacteria on the leaves. Positive effect of ozone treatment on lettuce microbial safety was confirmed also in Kim et al. (1999) report.

The aim of the study was to determine changes in the content of vitamin C (L-ascorbic acid) in two cultivars of leafy parsley, differed in leaves morphology, subjected to various post-harvest treatments.

MATERIAL AND METHODS

The storage experiment was performed in two successive growing seasons (2015 and 2016). The cultivars used in the study were 'Rialto' and 'Petra' (Bejo Zaden, Poland). The cultivars differ in morphology of usable parts: 'Rialto' is the cultivar with flat lamina and 'Petra' - with triple-curled lamina. The plant material was obtained from experimental field of Warsaw University of Life Sciences, located in Warsaw-Wilanów. The leaves were washed directly after harvest in: a) tap water or b) in tap water with ozone (O_3) added in the concentration of 5 ppm.

Two methods of postharvest storage were applied: A) in the cold store and B) in simulated retail conditions.

In the case of cold storage method (A), the plants were tied in tufts and stored at the temperature of 0°C and RH 90% for 7, 14, 28 days in two variants: 1) in containers, where leaves petioles were immersed in water, and 2) in bulk Modified Atmosphere Packagings (MAPs), dedicated to fresh herbs (Xtend film, Stepac, Israel).

In the case of retail conditions storage (B), the leaves were kept for 48 hours at 10°C, RH 30-40%, with access of light, in two variants: 1) tufts wrapped in perforated PE film sleeve, with petioles immersed in water (traditional method of parsley storage in retail), and 2) packed to retail Modified Atmosphere Packagings (MAPs), dedicated to leafy herbs (Xtend film, Stepac, Israel).

Ozone was continuously generated by ozone generator, efficiency of 3 g O_3 h⁻¹. In the generator, oxygen from the air passed through a corona discharge generator to produce ozone. Washing of the plant material in water with established ozone concentration was done for 1 minute in a container, capacity of 100 dm³, connected with ozone generator.

Concentration of AA in the plant leaves was determined spectrophotometrically, with the method based on reaction of Folin's phenol reagent in low pH (according to Jagota and Dani, 1982). For the analyses, representative samples of the plant material from each combination were taken. The analyses were carried out in four replicates from each experimental

combination, based on the means of the two years of the study. Data obtained were subjected to statistical analysis in StatisticaTM software, using two- or three-way ANOVA.

RESULTS AND DISCUSSION

Leafy parsley is described as a good source of bioactive, health-promoting compounds, including ascorbic acid (Eitenmiller et al., 2008, Sbai et al., 2016). Parsley cultivars used in the study differed in AA content directly after harvest and washing, using the two washing variants (Table 1). 'Rialto' was characterized by a higher AA content than 'Petra parsley (110 and 44 mg g⁻¹ f.w., respectively). The AA content in the plants was generally high and the results obtained by us are comparable to data reported for leafy parsley by other authors (Lisiewska and Kmiecik, 1997). Fresh and stored 'Rialto' leaves were characterised by significantly higher concentration of AA than 'Petra' leaves. It was found for both washing methods. However, for 'Rialto' parsley, ozone treated material showed a lower AA content than the untreated one. The negative effect of ozone on AA content for 'Rialto' parsley can be explained by different morphology of its leaves compared to 'Petra', which are flat and therefore more prone to gas exchange. Ozone, as a strong oxidant (Suslov, 2017) can oxidize AA in plant cells and therefore can influence AA degradation in parsley tissue observed in our study. AA is found in cell walls, as well as in cytosol, chloroplasts, mitochondria and vacuoles of plant cells. It is particularly sensitive to fluctuation of pH, temperature, water content in the tissue, oxygen and light. These conditions may stimulate enzymatic oxidation of ascorbic acid to dehydroascorbic acid (also considered as active as the vitamin C). The reaction is reversible, but the dehydroascorbic acid is further converted to compounds no longer having biological properties, such as 2,3-diketogulonic acid or L-threonic acid and oxalic acid (Green and Fry, 2004). According to Smirnoff (1996), ascorbate in the cell walls is the first line of plant defence against ozone. This activity may explain the decrease of AA concentration after ozone treatment observed for 'Rialto' leaves. The losses of AA in response to fumigation with ozone was reported also by Guri (1983) for Phaseolus vulgaris. However, since there is no information on the effect of washing method or treatment with ozone on chemical composition of leafy parsley, it is impossible to directly compare the results obtained by us with other literature data.

Washing method	Cultivar		Means for the treatments
	Rialto	Petra	
H ₂ O	123.9 a	44.6 c	84.2 A
$H_2O + O_3$	96.2 b	43.4 c	69.8 B
	Means for	or cultivars	
=	110.0 A	44.0 B	

Table 1. Ascorbic acid content in parsley leaves of both cultivars directly after harvest and washing (mg 100 g⁻¹ f.w.)

Values and means for factors which differ according to Tukey's HSD test at p=0.05 are marked with different letters.

Storage of parsley in the cold store conditions showed, in the case of 'Rialto' leaves, no significant influence of washing method on AA content (Table 2). Storage method (in container + water or in bulk MAPs) significantly influenced AA content in the leaves. The content was higher in the case of storage in MAPs. It was also evident that with prolonging storage period AA content decreased significantly. The tendency was characteristic for both washing methods and both storage methods. The highest AA content was found for ozone treated parsley, stored in the cold room for 7 days in container + water, and the lowest AA content for ozone treated parsley packed in bulk MAPs and stored for 28 days.

Table 2. Ascorbic acid content in 'Rialto' parsley leaves after storage in cold store conditions (mg 100 g⁻¹ f.w.)

		Storage periods (days)		
Washing method	7	14	28	Means for the
		Storage method		treatments
		In container + water		71.8 A
H ₂ O	81.1 b	57.2 d	65.4 c	67.9 a
$H_2O + O_3$	108.2 a	70.3 c	48.8 e	75.8 a
		In Xtend bulk MAPs		63.1 B
H ₂ O	71.1 c	68.8 c	48.7 e	62.9 a
$H_2O + O_3$	81.0 b	68.9 c	39.9 f	63.3 a
		Means for storage periods	6	
_	85.3 A	66.3 B	50.7 C	

Means for factors which differ according to Tukey's HSD test at p=0.05 are marked with different capital letters.

Values inside the table which differ according to Tukey's HSD test at p=0.05 are marked with different small letters.

In the case of 'Petra' parsley leaves it can be seen, based on the means for the treatments, that the washing with ozone-added water did not affect AA content (Table 3). With prolonging storage period AA content in the leaves decreased. The highest AA content was determined for the leaves after 7-day storage and the lowest for the leaves stored for 28 days in bulk MAPs and washed in ozone-added water. Average concentration of AA after 7 days of storage was 85 mg g^{-1} f.w. for 'Rialto' and 44 mg·g⁻¹ f.w. for 'Petra' leaves. After 28 days of storage the concentration was about 55 % and 35 % lower for 'Rialto' and 'Petra', respectively, in comparison to the freshly harvested leaves. The decrease of AA content during storage and influence of storage temperature on AA changes in plants was reported also by Lee and Kader

(2000).

	Storage periods (days)			 Means for the
Washing method	7	14	28	- treatments
		Storage method		- treatments
		In container + water		38.1 A
H ₂ O	41.3 ab	37.5 bc	30.5 c	36.4 a
$H_2O + O_3$	46.0 a	33.8 c	39.7 b	39.8 a
		In Xtend bulk MAPs		32.6 B
H ₂ O	45.1 a	29.1 c	25.9 cd	33.4 a
$H_2O + O_3$	43.6 a	35.0 bc	17.1 e	31.9 a
		Means for storage periods		
-	44.0 A	33.8 B	28.3 C	

Table 3. Ascorbic acid content in 'Petra' parsley leaves after storage in cold store conditions (mg 100 g^{-1} f.w.)

Means for factors which differ according to Tukey's HSD test at p=0.05 are marked with different capital letters. Values inside the table which differ according to Tukey's HSD test at p=0.05 are marked with different small letters.

In the case of storage at simulated retail conditions (10 °C), parsley leaves showed slightly lower AA content than those stored for 7 days in the cold room (0 °C) (Table 4 and 5). Retail MAPs guaranteed significantly higher preservation of AA in leaves of both cultivars than wrapping in film sleeve and immersing in water. Washing in ozone-treated water showed influence on AA concentration in 'Rialto' parsley immersed in water only. Also Gil et al. (1999) observed that vitamin C was better preserved in MAP-stored spinach than in those stored in ambient atmosphere. Similarly, modified atmosphere packaging (MAP) of broccoli florets resulted in better maintenance of AA, compared to broccoli stored under ambient atmosphere (Barth and Zhuang, 1996).

Table 4. Ascorbic acid content in 'Rialto' parsley leaves after storage in simulated retail conditions (mg 100 g⁻¹ f.w.)

	Method of storage		Means for the treatments
Washing method	In PE film sleeve, immersed in water	In Xtend retail MAPs	
H ₂ O	81.0 a	78.8 a	79.9 A
$H_2O + O_3$	63.6 b	82.1 a	72.8 B
	Means for stora	age methods	
	72.3 B	80.5 A	

Means for factors which differ according to Tukey's HSD test at p=0.05 are marked with different capital letters. Values inside the table which differ according to Tukey's HSD test at p=0.05 are marked with different small letters.

Table 5. Ascorbic acid content in 'Petra'	parsley leaves after storage in simulated	l retail conditions (mg 100 g^{-1} f.w.)
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	Method of s	Means for the treatments	
Washing method	In PE film sleeve, immersed in water	In Xtend retail MAPs	
H ₂ O	35.6 b	46.2 a	40.9 A
$H_2O + O_3$	43.7 ab	41.9 ab	42.8 A
	Means for storag	ge methods	
	39.7 B	44.1 A	_

Means for factors which differ according to Tukey's HSD test at p=0.05 are marked with different capital letters.

Values inside the table which differ according to Tukey's HSD test at p=0.05 are marked with different small letters.

The experiment showed that washing of plant material in ozonated water did not have negative effect on further loss of AA content during storage of parsley in the cold store. However, it is worth noting that in the case of retail conditions 'Rialto' leaves previously washed in ozonated water and not stored in MAPs were characterized by a lower content of AA. Certainly, storage duration, not packaging methods tested in the study influenced the content of AA during prolonged storage. However, method of packaging is more important in retail, where storage temperature is higher then in a cold store and RH is much lower.

CONCLUSION

Washing in ozonated water does not significantly effect vitamin C content in stored leaves of 'Rialto' and 'Petra' parsley in the case of cold store and in simulated retail conditions, as well. However, ozone treatment decreased AA content in flat-lamina leaves of 'Rialto' when they were analyzed directly after the treatment, before storage. Flat-lamina ('Rialto) and curled-lamina ('Petra') leafy parsley retain high content of AA if stored at cold store conditions up to 7 days in tufts immersed in water and in bulk MAPs (with or without previous washing in ozone-added water). After 14 days of storage in cold store conditions the loss of ascorbic acid is higher for 'Petra' parsley than for 'Rialto'. Leafy parsley stored under retail conditions, which are characterized by a higher temperature and lower relative humidity then cold store conditions, keep more ascorbic acid when stored in dedicated retail MAPs, than when stored with traditional method in tufts immersed in water. Further studies on different ozone concentrations are suggested, to establish optimal

concentration /treatment time combination for parsley.

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