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Nutritional and sensory quality during refrigerated storage of fresh-cut mints (*Mentha* × *piperita* and *M. spicata*)

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ABSTRACT

The effect of storage time on quality attributes of refrigerated fresh-cut mints (*Mentha* × *piperita* and *M. spicata*) was studied. Atmosphere composition, respiratory activity, weight loss, surface colour, total chlorophyll, carotenoids, browning potential, total phenols, flavonoids, radical-scavenging activity, ascorbic acid and essential oil yield and composition were analysed. Respiratory activity of peppermint and spearmint samples diminished moderately (42% and 28%, respectively) after 21 days at 0 °C. A slight modification of the internal atmosphere was achieved. Surface colour, chlorophyll, carotenoid and antioxidant compounds remained almost constant. The yield of essential oil did not change or it showed an apparent increase after 21 days at 0 °C, depending on plant growth stage. The characteristic flavour components of peppermint (menthone and menthol) increased, while the contents of the main constituents of spearmint essential oil showed minor variations after storage. The conditions assayed for packaging and storing fresh-cut mints were adequate to achieve a relatively long shelf life and they retained their antioxidant properties.

1. Introduction

Aromatic plants produce active compounds (phytochemicals having a pharmacological effect on living organisms) that consist partially of essential oils (Muñoz López de Bustamante, 2002). Frequently, aromatic herbs also present pharmacological activities which allow them to be considered as medicinal plants (Aburjai & Natsheh, 2003). Besides, a number of herbs used for their organoleptic properties can transfer to food and drinks certain scents, colours and flavours, making them more palatable, pleasing and delicious to smell and taste. These seasoning herbs (e.g. savory, coriander, cumin, mint, oregano, rosemary, sage, thyme) have a wide and growing field of application in the food industry, meat processing, canning, liquors elaboration and confectionery (Lubbe & Verpoorte, 2011). Culinary herbs have always been part of the diet as flavour agents and even as food preservatives.

Most aromatic plants produced are sold as dried herbs or are intended to yield essential oils. An interesting alternative is the marketing of fresh-cut herbs, packaged in bags or trays covered with plastic films of different permeabilities to CO_2, O_2 and water vapour (Murdock, 2002). These products hold high added value and should be handled in the same way as other fresh-cut vegetables. A need to evaluate how the nutritional and sensory quality attributes of fresh culinary herbs vary during storage arises from the previous considerations. Fresh herbs, properly packaged for sale, retain a greater proportion of the aroma than do the dried product and this is a clear advantage. Other factors that contribute to increase the use of fresh herbs are the replacement of salt by other seasonings and flavouring agents and the current consumption trends towards gourmet and ethnic foods (Cantwell & Reid, 1993; Sharma, 2008).

Different types of mints are included among the most widespread perennial aromatic spices. The genus *Mentha* belongs to the family Lamiaceae (Labiatae) (Tucker & Naczi, 2007) and many species have been traditionally used as medicinal and aromatic herbs (Lawrence, 2007). The most economically important species are *Mentha aquatica*, *Mentha canadensis*, *Mentha spicata* and their hybrids *Mentha* × *gracilis*, *Mentha* × *piperita* and *Mentha* × *villosonervata* (Tucker, 2007). According to McKay and Blumberg (2006) *M.* × *piperita* (peppermint) is one of the most widely consumed single ingredient herbal teas, or tisanes. Peppermint characteristic flavour is related to menthol and its isomers (isomenthol, neomenthol, and neoisomenthol), menthone, menthyl esters and

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piperitone (Lawrence, 2007). *M. spicata*, commonly known as spearmint or garden mint, is often used in European and Indian cuisines. Spearmint is popularly consumed in the form of mint chutney and added to several meat preparations as a flavour enhancer (Kanatt, Chander, & Sharma, 2007). The characteristic flavour of spearmint is related to carvone, dihydrocarvone, carveol, dihydrocarveol and limonene (Lawrence, 2007).

The objective of the present work was to assess the incidence of storage time on some physicochemical attributes and chemical components related to the postharvest quality of two species of *Mentha* (*M.* × *piperita* and *M. spicata*), packaged on trays covered with polyvinyl chloride film and refrigerated at 0 °C.

2. Materials and methods

2.1. Plant material

Mint plants (*M.* × *piperita* and *M. spicata*) were randomly harvested from a two year old crop, planted at the Experimental Station of the Facultad de Ciencias Agrarias y Forestales UNLP (La Plata, Buenos Aires, Argentina). Voucher samples were deposited in the collection of the Herbarium of the National University of La Plata: *M. piperita* voucher N° 9991 LPAG and *M. spicata* voucher N° 9992 LPAG. Plants were grown according to conventional agricultural practices, without application of supplementary irrigation. Plants approximately 30 cm in height were harvested. The collected material (herbaceous stems and leaves) was brought to the laboratory and processed immediately. Samples were washed with tap water and disinfected by immersion in cold chlorinated water (200 mg l^{-1} of active chlorine; pH 6–7; 1 min). Water excess was eliminated by forced air flow and samples were conditioned in transparent polystyrene trays (750 cm^3) containing about 30 g of product. Trays were covered with self-adhering polyvinyl chloride (PVC) film and stored for three weeks at 0 °C. Samples were withdrawn periodically to perform the corresponding analysis. In total, 48 trays for each species were prepared and stored.

2.2. Analyses

2.2.1. Composition of the atmosphere inside the packaging

A Shimadzu 6A gas chromatograph (Japan), fitted with a thermal conductivity detector, was used. Separation took place in an Alltech CTR1 column. Conditions were as follows: carrier gas helium, 0.5 ml s^{-1}; injector and detector temperature, 120 °C; column temperature, 30 °C. A calibration curve with different concentrations of CO_2 and O_2 was obtained. Determinations were performed in triplicate, measuring the atmospheric composition in three different trays for each species and each sampling point.

2.2.2. Respiratory activity

Samples of peppermint and spearmint (herbaceous stems and leaves) were weighed and placed inside sealed glass jars of 2 l capacity. The cumulative CO_2 was measured using an infrared sensor (Compuflow 8650 Indoor Air Quality Metre) inserted into the bottle. CO_2 concentration was plotted against time. From the slope of the line, CO_2 production was calculated and expressed in μl kg^{-1} s^{-1}.

2.2.3. Weight loss of trays

Trays containing fresh-cut mint samples were weighed at the beginning and end of the storage period. A precision balance (±0.01 g, OHAUS AV4101 Adventurer Pro Model) was used. Weight loss was expressed as percentage (%) referred to the initial weight.

2.2.4. Surface colour

Colour measurements were performed using a colourimetre Konica Minolta CR-400 (Japan) with a circular measuring area of 8 mm in diametre. The instrument was calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$ $y = 0.3192$). Readings were taken by placing the colourimetre head on the adaxial surface of the leaves. The coordinates L^*, a^*, b^* of the CIE scale were recorded and the functions hue [$h^\circ = 180 + \tan^{-1}$ (b^*/a^*)] and Chroma [$C = (a^{*2} + b^{*2})^{1/2}$] were analysed. Colour was measured on 20 different leaves of each species at each sampling point.

2.2.5. Total chlorophyll and carotenoid content

The determination of chlorophyll and total carotenoids were performed from intact tissues by extraction with dimethylformamide for 24 h at 4 °C and subsequent reading of the absorbance at 480, 646.8 and 663.8 nm, using a Beckman DU650 spectrophotometre (California, USA). The contents of chlorophyll and total carotenoids were calculated according to Wellburn (1994). Final results were expressed as g kg^{-1} fresh tissue. Extractions were performed in triplicate.

2.2.6. Browning potential, total phenols, flavonoids and radical-scavenging activity

Fresh and stored peppermint and spearmint samples were frozen in liquid nitrogen and crushed in a laboratory mill. Two grammes of the frozen ground tissues were homogenised in 10 ml of ethanol (96% w/w). The suspensions were centrifuged at 9600×*g* for 10 min and supernatants were recovered. The extracts were obtained in duplicate for each species and sampling point. Browning potential (Loaiza-Velarde, Tomás-Barberá, & Saltveit, 1997), total phenols (Singleton & Rossi, 1965), flavonoids (Kim, Jeong, & Lee, 2003) and radical-scavenging activity (Brand-Williams, Cuvelier, & Berset, 1995) were determined. Browning potential was expressed as absorbance units (AU) g^{-1} fresh tissue; total phenols and flavonoids as mg catechin g^{-1} fresh tissue; and radical-scavenging activity (RSA) as the reciprocal of the effective mean concentration EC_{50}. EC_{50} was defined as the mass (μg) of tissue required to reduce $DPPH^\bullet$ concentration to half its initial value.

2.2.7. Ascorbic acid

Ascorbic acid content was quantified by means of the enzymatic kit K-ASCO 02/06 (Megazyme© International Ireland Limited). Peppermint and spearmint samples corresponding to the beginning (day 0) and end (day 21) of the storage period were frozen in liquid nitrogen and crushed in a laboratory mill. Two-gramme samples were homogenised and extracted with 6 ml of 1 M potassium phosphate buffer (pH = 3.5). Suspensions were centrifuged at 9600×*g* for 10 min. Supernatants were recovered and filtered. Aliquots of 1.0 ml of the extracts were used to perform the assay procedure accompanying the kit. Extractions were performed in duplicate. Absorbance readings were made at 578 nm.

2.2.8. Essential oil yield and composition

The extraction of essential oils was performed by hydrodistillation, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). Exactly weighed samples (200 g) of fresh plant material were placed inside the flask with water and distillation proceeded for 4 h. The essential oil yield (ml kg^{-1} dry matter) was calculated, considering the dry matter content of samples on the day of the extraction. Dry matter content was determined in an oven at 40 °C until constant weight was achieved. Quantification of essential oil content and dry weight of samples were performed in triplicate.

The volatile distillates were collected over anhydrous sodium sulphate and refrigerated (8 °C) until the time of analysis. GC–MS analyses of the composition of the essential oils were carried out

using a Shimadzu QP 5050 apparatus which was equipped with MS reference libraries (Adams, 2001; Mc Lafferty & Stauffer, 1991). Analyses were carried out using two different capillary columns with associated chromatographic conditions. The first system employed a Mega SE-52 cross-linked fused-silica capillary column (25 m × 0.25 mm i.d.) coated with 5% phenyl-polymethylsiloxane (0.25 μm phase thickness). The oven temperature was programmed at 60 °C for 8 min, rising to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min; the injector temperature was 250 °C; the carrier gas was helium at 122.2 kPa (51.6 cm/s); the injection mode was split, with a split ratio of 1:40; the sample volume injected was 0.2 μl; the interface temperature was 250 °C and the acquisition mass range was 40–400 m/z.

In the second system, a BP 20 (SGE, Ringwood, Australia) bonded fused-silica capillary column (25 m × 0.25 mm i.d.), coated with polyethylene glycol (0.25 μm phase thickness), was employed; the oven temperature was programmed at 40 °C for 8 min, rising to 180 °C at 3 °C/min, then to 230 °C at 20 °C/min; the injector temperature was 250 °C; the carrier gas was hydrogen at 92.6 kPa (55.9 cm/s); the injection mode was split, with a split ratio of 1:40; the sample volume injected was 0.2 μl; the interface temperature was 250 °C and the acquisition mass range was 40–400 m/z.

2.3. Experimental design and statistical analysis

A factorial design was used, with two factors: the species and the storage time. Complete storage experiments were performed twice. Analysis of variance (ANOVA) and comparison of means with the Fisher's least significant difference (LSD) test were conducted, at a significance level $p = 0.05$.

3. Results and discussion

3.1. Composition of the atmosphere inside the packaging

Fig. 1a shows variations observed in the atmosphere composition inside the packages containing mint samples. For both mint species, an increase ($p < 0.05$) in the CO_2 concentration and a decrease ($p < 0.05$) in O_2 content were observed after one day of storage. CO_2 and O_2 contents at the end of storage reached 1–2% and 19–20%, respectively. Storing mint samples at 0 °C caused a low production of CO_2 from the respiratory activity of the packaged product that did not allow a greater accumulation of this gas inside the trays, added to a relatively high permeability of the film used. Similarly, when analysing the composition of the internal atmosphere in trays containing fresh-cut chives covered with PVC and stored at 0 °C for 17 days, Viña and Cerimele (2009) found that CO_2 concentration increased significantly on the second day and then remained constant until the end of the storage.

3.2. Respiratory activity

According to Cantwell and Reid (1993), the respiration rate of fresh herbs is relatively high, within the range 14–83 μl of CO_2 kg^{-1} s^{-1}. Results showed that the respiratory activity of fresh-cut *M. piperita* and *M. spicata* was initially high and it was 1.6 times higher in *M. spicata* than in *M. piperita* (Table 1). Respiration rate decreased 1.7 (*M. piperita*) and 1.4 times (*M. spicata*), after cold storage. This decrease in respiratory activity could have contributed, in part, to reduce the speed of reactions leading to tissue damage.

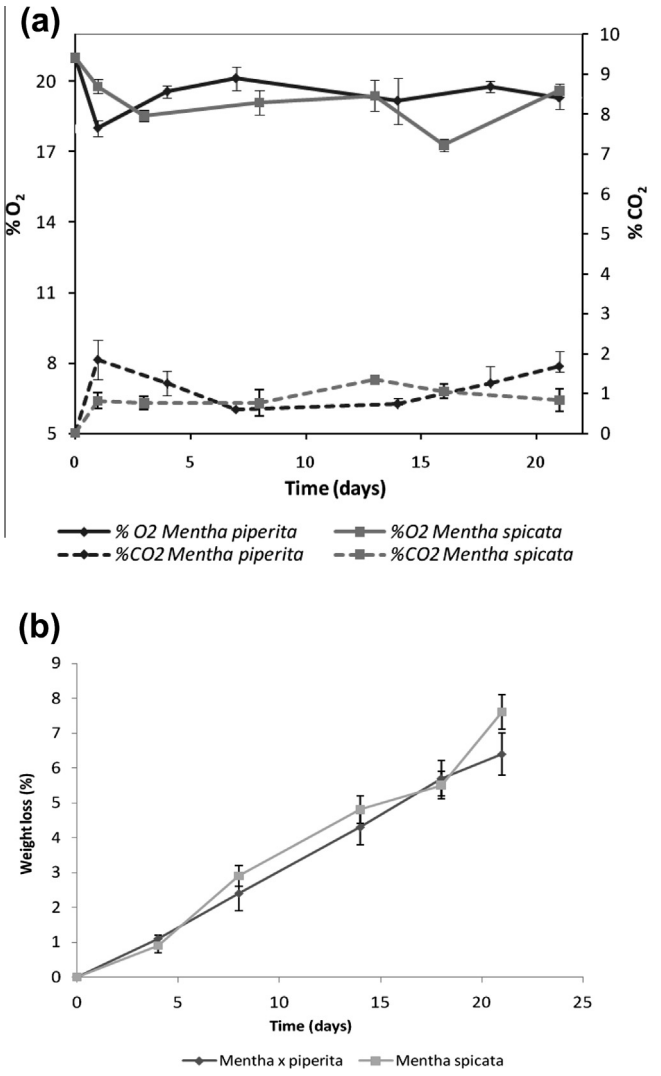

Fig. 1. (a) Internal atmosphere composition (% O_2 and % CO_2); (b) Weight loss (%) of fresh-cut *Mentha* × *piperita* and *M. spicata* trays stored at 0 °C for 21 days.

3.3. Weight loss of trays

Weight loss increased linearly (Fig. 1b) with storage time, for both *M. piperita* ($R^2 = 0.998$) and *M. spicata* ($R^2 = 0.997$). Weight loss at the end of the assayed period ranged from 6–8%, for both species. Hruschka and Wang (1979) have described visible wilting symptoms in non-packed mint stored at 0 °C, when mean water loss reached 18%. The packaging and storage conditions selected in this work reduced (almost three times) the water loss value, even after 21 days in the chamber. According to Cantwell and Reid (1993), dehydration rate of *M.* × *piperita* and *M. spicata* samples corresponded to 2.1 and 1.8 ml g^{-1} day^{-1}, respectively.

3.4. Surface colour

Colour change is a factor limiting the shelf life of fresh vegetables and is caused mainly by the degradation of pigments and the incidence of enzymatic browning (Cantwell & Reid, 1993). In green vegetables, the decrease in hue angle is due to the degradation of chlorophyll pigments from bright green to olive green and finally to yellow (Sothornvit & Kiatchanapaibul, 2009). With respect to the initial colour of mint samples, there were no significant differences ($p > 0.05$) in L^*, Chroma and hue values between

Table 1
Respiratory activity (μl of CO_2 kg^{-1} s^{-1}), pigments (g kg^{-1} fresh tissue) and ascorbic acid content of fresh-cut mints, at the beginning and end of the storage period.

Time at 0 °C (days)	Respiratory activity (μl of CO_2 kg^{-1} s^{-1})		Pigment content (g kg^{-1} fresh tissue)						Ascorbic acid (μg kg^{-1} fresh tissue)	
			Chlorophyll a		*Chlorophyll b*		*Carotenoids*			
	M. piperita	*M. spicata*	*M. piperita*	*M. spicata*	*M. piperita*	*M. spicata*	*M. piperita*	*M. spicata*	*M. piperita*	*M. spicata*
0	50.6 ± 2.2[a]	83.1 ± 3.1[a]	2.10 ± 0.10[a]	2.27 ± 0.10[a]	0.57 ± 0.03[a]	0.56 ± 0.03[a]	0.50 ± 0.02[a]	0.60 ± 0.01[a]	140 ± 10[a]	480 ± 30[a]
21	29.4 ± 1.4[b]	59.4 ± 2.5[b]	2.11 ± 0.07[a]	2.70 ± 0.11[b]	0.48 ± 0.04[b]	0.61 ± 0.03[a]	0.55 ± 0.01[b]	0.69 ± 0.02[b]	100 ± 10[b]	370 ± 20[b]

Note: Values followed by different letters within a column correspond to statistically significant differences ($p < 0.05$).

M. piperita (L^* = 39.4 ± 1.3; Chroma = 20.4 ± 2.1; hue = 129.5 ± 3.6) and *M. spicata* (L^* = 37.2 ± 1.9; Chroma = 17.2 ± 2.3; hue = 128.1 ± 5.2). L^* values measured in this work for *M. piperita* and *M. spicata* were slightly above those registered by Therdthai and Zhou (2009) in *M. cordifolia* (L^* = 35.4) but they were lower than the L^* values reported by Hsu, Simonne, Jitareerat, and Marshall (2010) in *M. piperita*.

During the storage, no significant variations were observed in L^*, Chroma and hue values for both species of mint (data not shown). Thus, the recommended temperature (0 °C) and the packaging system used in this work maintained the initial colour of fresh-cut *M. piperita* and *M. spicata*. Taking into account the yellowing index scale described for *M. longifolia* by Kenigsbuch, Chalupowicz, Aharon, Maurer, and Aharoni (2007), *M. piperita* and *M. spicata* samples received a yellow index equal to 1.0, which corresponds to fresh green leaves with *L* (lightness) readings ranging up to 51.09; *a* co-ordinate readings (green to red) were up to 11.84 and *b* scale readings (from blue to yellow) up to 14.89.

According to Cantwell and Reid (1993), only 25% of mint samples stored at 0 °C for 3 weeks were marketable due to a noticeable decrease in visual quality. Viña and Cerimele (2009) reported that fresh-cut chives stored for 14 days, showed a significant hue decrease that accounted for 9% and 15% of samples stored at 0 and 4 °C respectively. Chive samples kept at 4 °C were not marketable at this point because of the perceptible yellowing of leaves.

Able, Wong, Amikha Prasad, and O'Hare (2005) pointed out that, at 2 °C, physiological damage (primarily in the form of wilting) was the primary cause of the decline in general appearance of detached pak choy leaves. Likewise, the authors reported that the hue angle did not decrease at this temperature. Detached pak choy leaves, stored at 2 °C, had a hue angle of 123.7 ± 0.5 at the end of their storage life (≈27 days).

3.5. Total chlorophyll and carotenoid content

At the beginning of the storage period, *M. spicata* samples showed significantly higher content of chlorophyll *a* and total carotenoids than did *M. piperita* (Table 1). Chlorophyll *a* and *b* levels measured in this work were noticeably higher than those reported by Hsu et al. (2010). For both *M. piperita* and *M. spicata*, the initial content of chlorophyll *a* did not decrease significantly during storage (Table 1). The chlorophyll *b* content decreased slightly in *M.* × *piperita* and remained constant in *M. spicata*. These observations would be consistent with the conservation of colour that was verified during refrigerated storage. According to Cantwell and Reid (1993), there is a relationship between respiratory rate and chlorophyll degradation.

Carotenoid content showed an apparent increase during refrigerated conservation of mint samples, although it could be due to sample variability.

The lower metabolic activity induced at 0 °C may have contributed to preserve the initial colour and pigments content of the product, at least over 21 days.

3.6. Total phenols, browning potential, total flavonoids and radical-scavenging activity (RSA)

The initial content of total phenols was higher in *M. spicata* and approximately 2 times greater than that of *M. piperita*. An increase of total phenolic content in both species of mint was observed during the storage at 0 °C (Fig. 2a). In *M. piperita*, total phenol content reached its maximum at 3–8 days with an increase of 62%. At the end of the storage, total phenol levels showed no significant difference with respect to the day of harvest (Fig. 2a). In *M. spicata*, total phenol content presented a maximum at day 18 (51% higher than the level measured at the beginning of the storage) (Fig. 2a). Total phenol content at the end of storage was 43% higher than the initial one.

Referring to other fresh-cut products, in minimally processed lettuce, escarole and rocket salad, Degl'Innocenti et al. (2007) pointed out that the content of phenols did not show important changes during 3 days at 4 °C. Viña and Chaves (2006) pointed out that total phenol content in fresh-cut celery did not change significantly during 28 days of storage at 0 °C. In contrast, for chives stored at 0 °C for 13 days, total phenolic content remained constant until day 6, when there was an increase of 12% over the initial content (Viña & Cerimele, 2009). In minimally processed carrots stored for 12 days at 15 °C, Basilio Heredia and Cisneros- Zevallos (2009) reported that the intensity of the mechanical damage exerted on the product (whole, sliced or grated carrots) played a decisive role in the activation of the synthesis and the accumulation of phenolic antioxidant compounds.

As shown in Fig. 2a, the variations registered in browning potential of fresh-cut mints corresponded to the evolution of total phenol content during storage. The browning potential of *M. piperita* showed a slight increase on the third day of storage (27% higher than the browning potential measured at harvest) and it decreased from day 8 on reaching levels below the initial one (22% lower than on the day of harvest). The browning potential of *M. spicata*, which was significantly higher than that of *M. piperita*, increased from day 3 and showed its maximum at 18 days of storage (1.7 times higher than the initial value) (Fig. 2a). At the end of the storage, potential browning remained 32% above the value measured at the beginning of the assayed period. Viña and Cerimele (2009) observed a significant increase in browning potential of fresh-cut chives after 7 days of storage at 0 °C, and this increase continued until the end of storage (21 days).

As shown in Fig. 2b, the flavonoid content at day 0 represented almost all of the phenolic compounds in both species of mint tested and it was higher (2.6 times) in *M. spicata*. Flavonoid content increased during the first days of storage (Fig. 2b). In *M. piperita*, total flavonoid content reached a maximum at 3–8 days of storage, being 59% higher than the initial content. At the end of storage, the flavonoid content was 45% below the starting value. Although results showed that the initial content of total phenols was represented mostly by flavonoids, this phenomenon was not verified during storage. This might indicate that, in *M. piperita*, other types of phenols, such as simple phenols, also contributed to total phenol content towards the end of storage.

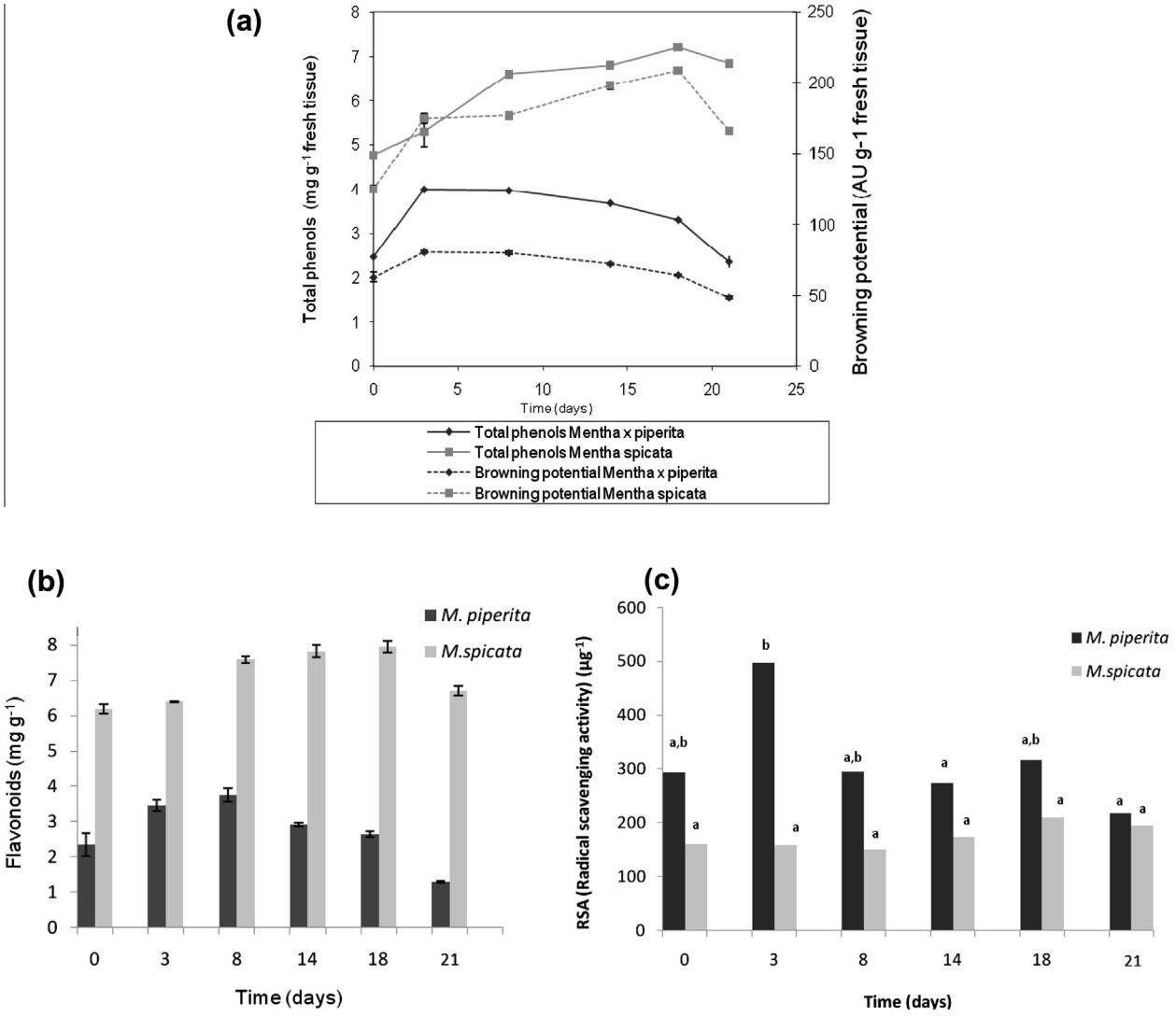

Fig. 2. (a) Total phenols (mg g^{-1} fresh tissue) and browning potential (AU μg^{-1} fresh tissue) AU: Absorbance Units at 320 nm; (b) Total flavonoids (mg g^{-1} fresh tissue); (c) Radical-scavenging activity (RSA) (μg^{-1}) of fresh-cut *Mentha* × *piperita* and *M. spicata* stored at 0 °C for 21 days.

In *M. spicata*, the maximum level of flavonoids was reached at 14–18 days of storage, 28% higher than the initial content. At the end of storage, the level of total flavonoids remained 8% higher than the value registered at the day of harvest (Fig. 2b). For *M. spicata* samples, flavonoid content accounted for almost the whole of the phenol content throughout the storage.

Radical-scavenging activity (RSA) results are shown in Fig. 2c. The RSA values of fresh mint samples were 161 and 293 μg^{-1} for *M. spicata* and *M. piperita*, respectively.

M. spicata showed no significant changes in RSA over time. On the other hand, *M. piperita* showed an apparent increase on RSA at day 3, to finally reach RSA values similar to the initial ones.

While total phenol and flavonoid contents (Fig. 2a and b) were significantly higher in *M. spicata* than in *M. piperita* samples, RSA (Fig. 2c) was higher in the latter species at most sampling points. The different composition of peppermint and spearmint essential oils might be a factor affecting this observation.

It has been mentioned that various extracts of *M. piperita* exhibited a significant antioxidant activity and its essential oil showed about half of the antioxidant power exhibited by the antioxidant BHT used as reference (Singh, Shushni, & Asma Belkheir, 2011). Likewise, Mimica-Dukić, Bozin, Soković, Mihajlović, and Matavulj (2003) reported that the essential oils of *M. aquatica*, *M. longifolia* and *M. piperita* were able to reduce the DPPH radical into its neutral form, and that this activity was dependent on concentration. However, only the essential oil of *M. piperita* reduced the initial concentration of DPPH by 50%, showing also the highest OH radical scavenger activity and reducing (by 24%) its generation in the Fenton reaction (Mimica-Dukić et al., 2003). Moreover, Yang, Jeon, Lee, Shim, and Lee (2010) reported that the essential oil of peppermint (*M. piperita*) had the highest ABTS radical-scavenger activity, in comparison with the essential oils of lavender, rosemary, lemon, grapefruit and frankincense (*Boswellia carteri*).

3.7. Ascorbic acid

The *M. spicata* ascorbic acid level was approximately 3.5 times higher than that of *M. piperita* (Table 1). Capecka, Mareczek, and Leja (2005) reported an ascorbic acid content equal to 52.6 mg/100 g in *M. piperita*. Singh, Kawatra, and Sehgal (2001) pointed out that ascorbic acid content in *M. spicata* was 23.9 mg/100 g. Both values were significantly higher than those found in the present study. Differences in ascorbic acid content of mint may be

Table 2
Essential oil yield (ml kg^{-1} dry basis) of *M. piperita* and *M. spicata*, freshly harvested and stored at 0 °C for 21 days.

Time at 0 °C (days)	Essential oil yield (ml kg^{-1} dry basis)			
	Mentha × *piperita*		*Mentha spicata*	
	First experiment (November 2009)	Second experiment (March 2010)	First experiment (November 2009)	Second experiment (December 2009)
0	17.3[a]	31.4[c]	15.1[a]	25.9[c]
21	21.3[b]	31.4[c]	22.4[b]	32.1[d]
$LSD_{0.05}$	2.1	1.8	2.5	3.1

Note: Values followed by different letters correspond to statistically significant differences ($p < 0.05$).

attributed to different sources of plant material, stages of maturity and/or analytical methods.

Table 1 shows that ascorbic acid content decreased significantly during refrigerated storage in both species of mint analysed.

Regarding the evolution of ascorbic acid content during storage of fresh-cut products, results reported in the literature vary with the product. For example, Viña and Chaves (2006) observed no changes in the ascorbic acid content of minimally processed celery stored at 0 °C for 28 days, while Viña and Cerimele (2009) found that ascorbic acid content increased significantly in fresh-cut chives at 13 days of storage at 0 °C. Similarly, Degl'Innocenti et al. (2007) reported that, in fresh-cut lettuce and escarole leaves, ascorbic acid content increased during storage, particularly in lettuce. Ansorena, Marcovich, and Roura (2011) working with minimally processed heat-treated broccoli indicated that the reduced ascorbic acid content of samples decreased with the storage time at 5 °C, regardless of the use of edible coating.

3.8. Essential oil yield and composition

The essential oil yield ranged between 15 and 32 ml kg^{-1} (dry basis), depending on mint species (Table 2). Chauhan et al. (2009) reported an essential oil yield of 0.57% (wet basis) in *M. spicata*, using the same extraction method as the one used in the present work. According to Singh et al. (2011), the yield of *M. piperita* essential oil was about 0.64%, based on the dry weight of plant material. Variations in oil content and composition may be attributed to factors related to ecotype, phenophases and the environment, including temperature, relative humidity, irradiance and photoperiod (Chauhan et al., 2009; Fahlen, Walander, & Wennersten, 1997). The yield and quantitative composition of the essential oils of many aromatic plants are greatly influenced by the genotype and agronomic conditions, such as harvesting time, plant age and crop density (Chauhan et al., 2009; Marotti, Piccaglia, Giovanelli, Deans, & Eaglesham, 1994).

The harvest of plant materials that were the subject of this research did not always coincide with the moment indicated as the optimal growth stage for obtaining essential oil on a commercial scale. For achieving a higher essential oil yield, mint plants should be harvested at the flowering stage. Since obtaining the highest essential oil yield was not the aim of this work and the plant parts used for producing fresh-cut herbs were the foliaceous stems, the harvest of mint samples was done in stages prior to full bloom.

As shown in Table 2, an apparent increase in essential oil yield between the beginning and end of storage was obtained for both species. For *M. piperita*, the increase was 23% in the first experiment, while no increase in the second one was observed (Table 2). For *M. spicata*, the increase was 48% in the first experience and 24% in the second. These differences may be due to variations in the degree of plant development and senescence, among others. The increase of essential oil yield during the storage at 0 °C might be

Table 3
Essential oil composition (%) of *M. piperita*, freshly harvested and stored at 0 °C for 21 days.

Day 0		Day 21		LRIs SE 52	LRIs BP 20
Component	%	Component	%		
β-Pinene	0.12	β-Pinene	0.19	978	1110
Sabiene	0.07	Sabiene	0.11	973	1122
β-Myrcene	0.15	β-Myrcene	0.29	989	1161
α-Terpinolene	0.03	α-Terpinolene	0.01	978	1301
3-Octanol	0.27	3-Octanol	0.16	994	1380
Limonene	0.65	Limonene	0.23	1017	1178
1,8-Cineol	2.47	1,8-Cineol	3.34	1030	1198
cis-β-Ocimene	0.22	cis-β-Ocimene	0.37	1048	1250
trans-β-Ocimene	0.05	trans-β-Ocimene	0.11	1087	1282
γ-Terpinene	0.04	γ-Terpinene	0.13	1024	1270
p-Cimene	0.02	p-Cimene	0.03	1105	
3-Hexen-1-ol	0.05	3-Hexen-1-ol	0.01	1151	1465
Octen-3–1	–	Octen-3–1	0.01	990	
Menthone	46.8	Menthone	48.7	1146	1462
Menthofuran	2.79	Menthofuran	2.69	1164	1482
Isomenthone	5.67	Isomenthone	5.64	1159	1420
trans-Pinocamphone	0.18	*trans*-Pinocamphone	0.03	1162	1523
Germacrene D-4-ol	0.18	Germacrene D-4-ol	0.13	1574	2057
Menthyl acetate	0.22	Menthyl acetate	0.18	1296	1555
neo-Menthol	–	neo-Menthol	0.17	1161	1600
Isopulegone	1.03	Isopulegone	0.28	1167	1533
β-Elemene	0.54	β-Elemene	0.45	1390	1591
(*E*)-Caryophyllene	1.13	(*E*)-Caryophyllene	1.15	1420	1563
Pulegone	3.25	Pulegone	0.15	1241	1637
Menthol	21.6	Menthol	23.5	1177	1630
α-Terpineol	0.53	α-Terpineol	0.44	1190	1694
Piperitone	2.10	Piperitone	2.44	1247	1697
Bicyclogermacrene	0.28	Bicyclogermacrene	0.28	1494	1735
β-Copaene	1.20	β-Copaene	1.45	1433	1580
trans-Nerolidol	0.21	trans-Nerolidol	0.2	1561	2036
Carvacrol	0.03	Carvacrol	0.07	1300	2211

Table 4
Essential oil composition (%) of *M. spicata*, freshly harvested and stored at 0 °C for 21 days.

Day 0		Day 21		LRIs SE 52	LRIs BP 20
Component	%	Component	%		
trans-β-Ocimene	0.25	trans-β-Ocimene	0.20	928	1037
β-Pinene	0.25	β-Pinene	0.20	978	1110
Sabiene	0.16	Sabiene	0.14	973	1122
β-Myrcene	8.90	β-Myrcene	8.67	989	1161
α-Terpinolene	0.07	α-Terpinolene	0.04	978	1301
Limonene	15.2	Limonene	16.5	1017	1178
cis-β-Ocimene	0.89	cis-β-Ocimene	0.82	1048	1250
Myrcenol	0.01	Myrcenol	0.07	1113	1585
Limonene oxide trans	0.03	Limonene oxide trans	0.02	1138	1462
trans-Pinocarveol	0.04	*trans*-Pinocarveol	0.02	1140	1661
3-Hexen-1-ol	0.01	3-Hexen-1-ol	0.01	1151	1465
Isomenthone	6.87	Isomenthone	7.23	1159	1484
trans-β-Ocimene	0.37	*trans*-β-Ocimene	0.31	1087	1282
trans-Pinocamphone	2.37	*trans*-Pinocamphone	1.17	1162	1523
Menthofuran	1.68	Menthofuran	6.17	1164	1482
Menthol	1.86	Menthol	1.25	1177	1630
Dihydrocarveol	0.83	Dihydrocarveol	0.99	1195	1707
Dihydrocarvone	1.62	Dihydrocarvone	0.90	1201	1623
trans-Carveol	0.34	*trans*-Carveol	0.76	1217	1836
cis-Carveol	0.45	*cis*-Carveol	0.06	1227	1854
Carvone	41.4	Carvone	41.3	1242	1734
Piperitone	0.18	Piperitone	0.10	1247	1697
(*E*)-Caryophyllene	3.37	(*E*)-Caryophyllene	2.07	1420	1563
Germacrene D	0.32	Germacrene D	0.11	1481	1708
β-Selinene	0.06	β-Selinene	0.02	1486	1717
Germacrene D-4-ol	0.80	Germacrene D-4-ol	0.14	1574	2057

due to an effect of tissue dehydration and/or membrane modification during the conservation, which could have favoured a better extraction of essential oils from the morphological and anatomical structures that act as reservoirs (i.e. glandular trichomes).

The composition of the essential oils of the two mint species are quite different. For *M. piperita*, 33 components were identified at harvest, corresponding to 92% of the total components detected. The major groups were identified: oxygenated monoterpenes (86.7%), sesquiterpenes (3.15%), monoterpenes (1.37%), oxygenated sesquiterpenes (0.39%) and other components (0.36%). The monoterpenes were the major constituents.

Individual components present in greater proportion in the oil of *M. piperita* were: menthone (46.8%), menthol (21.6%), isomenthone (5.67%), pulegone (3.25%), menthofuran (2.79%), 1,8-cineole (2.47%), piperitone (2.10%) and β-copaene (1.20%) (Table 3). Compounds responsible for the characteristic flavour are: menthol, menthone, piperitone and menthyl esters (the latter present at low concentrations in this case) (Lawrence, 2007).

Ozel and Ozguven (2002) characterised the essential oil of three cultivars of peppermint (*M. piperita* L. cv. Mitcham, *M. piperita* L. cv. Prilubskaja and *M. piperita* L. cv. Eskißehir) and found that the major components were menthol (28.5–33.5%), menthone + menthofuran (28.5–43.9%) and 1,8-cineole (3.7–9.3%) in oils obtained by steam distillation from materials harvested prior to flowering or during flowering.

Von Schantz and Norri (1968) compared the compositions of essential oils of Ukrainian *M. piperita* harvested at different developmental stages and found that, in plants harvested before flowering, the main components were menthol (42.8%), menthone (26.2%) and menthyl acetate (6.3%). The main components of plants harvested at the end of flowering were menthol (42.5%), menthyl acetate (18%) and limonene (8.3%).

For *M. spicata*, 25 components were identified at the beginning of the storage corresponding to 88.3% of the total components detected (Table 4). The major groups identified were oxygenated monoterpenes (57.8%), monoterpenes (25.9%), sesquiterpene hydrocarbons (3.76%) and oxygenated sesquiterpenes (0.81%). Other constituents represented 0.01%.

The main components of freshly harvested *M. spicata* essential oil were carvone (41.5%), limonene (15.2%) and myrcene (8.93%) (Table 4). Those responsible for the characteristic flavour are: carvone, dihydrocarvone, limonene, carveol, and dihydrocarveol; these two last compounds were found in low concentrations. Chauhan et al. (2009) characterised the essential oil of *M. spicata* harvested during flowering in the north-western region of India and found that the major components were carvone (76.6%), limonene (9.6%) and 1,8-cineol (1.9%) in oils obtained by hydrodistillation.

Differences between the composition of oils obtained in this work and those reported in the literature (Chauhan et al., 2009; Ozel & Ozguven, 2002; Von Schantz & Norrie, 1968) could be explained by variations in genotype and agronomic factors, such as climate and soil conditions, harvest time, crop age and plant density, as well as the methods of extraction of the essential oils.

Regarding the variations observed after cold storage, the major components of *M. piperita* oil after 21 days at 0 °C were the same as in the freshly harvested plant material, but their percentages were slightly different (Table 3). The main compounds responsible for the flavour of *M. piperita* varied significantly during refrigerated storage: menthone increased 3.2% after 21 days in the chamber compared to the initial level; menthol showed a noticeable increase: 8.3% of the initial level in the same period (Table 3). Piperitone showed a slight increase, while menthofuran content remained almost constant (Table 3). It should be noted that menthofuran is not a characteristic flavour component of this species, but an indicator of the maturation and/or senescence degree of mint.

The modifications in the main constituents of *M. spicata* essential oil contents showed minor variations after storage, as shown in Table 4. Since carvone has been identified as the main responsible compound for the aroma of "spearmint" (De Carvalho

& Da Fonseca, 2006), the minimal loss observed in this component during storage probably implies that the sensory quality of this herb will not be modified under the conditions studied.

4. Conclusions

The results of the present work showed that the chosen conditioning and conservation system moderately reduced the respiratory activity of *M. piperita* and *M. spicata* samples and slightly modified the internal atmosphere of the packages. Surface colour, chlorophyll and carotenoid contents, as well as antioxidant compounds, remained almost constant for 21 days at 0 °C. For both species, the amount of essential oil (expressed on a dry basis) did not change or showed an apparent increase during storage, depending on plant growth stage as can be seen when comparing results from different harvest moments (Table 2). In *M. piperita*, menthone and menthol (compounds that contribute to the characteristic flavour) accounted for 68.5% of the constituents of the essential oil. In *M. spicata*, carvone and limonene (major components that contribute to the particular aroma) accounted together for 56.5% of the compounds detected. During 21 days at 0 °C, the characteristic flavour components of peppermint (menthone and menthol) increased, while the content of the main constituents of spearmint essential oil showed minor variations after storage. Thus, the conditions assayed for the packaging and storage of fresh-cut mints were adequate to maintain their colour, pigment content and antioxidant properties. Results concerning essential oil composition should be complemented with future research about terpenoid metabolism; thus useful information about the product design could be achieved.

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