

QUALITY CHANGES IN FRESH CHIVES (*ALLIUM* *SCHOENOPRASUM* L.) DURING REFRIGERATED STORAGE

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ABSTRACT

This study investigates quality changes in fresh chives stored at 0 and 4C during 21 and 14 days, respectively. The product was packaged in trays and covered with polyvinyl chloride films. Weight loss, surface color, total chlorophyll, carotenoids, reducing sugars and browning potential were evaluated in order to determine shelf life at each temperature. In addition, internal atmosphere composition, total phenolics, radical scavenging activity and ascorbic acid content were measured for samples stored at 0C. Results showed that surface color change and losses of green pigments and reducing sugar content could be delayed for a week by storing fresh chives at 0C. The increase in browning potential was of lower magnitude at 0C than at 4C. High quality levels and marketability of fresh chives could be maintained for 2 weeks at 0C.

PRACTICAL APPLICATIONS

The marketing of packaged and refrigerated, value-added culinary herbs is a choice of increasing interest for vegetables processors. However, data on changes in the chemical composition and antioxidative properties of fresh

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herbs during storage are scarce. This study provides information on how quality attributes of fresh chives are affected under different conditions of postharvest handling and storage.

INTRODUCTION

Chive (*Allium schoenoprasum* L.) is a Central European spice plant belonging to the Liliaceae family. From an agricultural point of view, chives are morphologically well adapted to dry and sunny habitats because of their tubiform leaves that offer a reduced transpiring surface and their small bulbs, which act as water reservoirs (Egert and Tevini 2002). Chive leaves are traditionally used after being dried or, alternately, are stored frozen. The first procedure considerably reduces the characteristic aroma (Kmieciak and Lisiewska 1999), while freezing often affects texture and color. Therefore, the marketing of value-added fresh chives packaged in plastic trays and stored at refrigeration temperatures is another way of processing of increasing interest.

Several species of the *Allium* genus such as onion, garlic and leek have been used both in human diet and folk medicine since ancient times (Tsiaganis *et al.* 2006). The specific organosulfur compounds present in these species have been extensively studied for their chemopreventive potential against cancer (Le Bon and Siess 2000).

Epidemiological studies have shown that a higher intake of *Allium* products is associated with a reduced risk of several types of cancers (Sengupta *et al.* 2004). Garlic, onion, leeks and chives have been reported to reduce the incidence of stomach and colorectal cancers. Evidence for a protective effect against malignant neoplastic diseases at other sites, including the breast, is still insufficient, according to Bianchini and Vainio (2001).

Mechanisms proposed to explain the cancer-preventive effects of *Allium* species include inhibition of mutagenesis, modulation of enzyme activities, inhibition of DNA adducts formation, free-radical scavenging activities and effects on cell proliferation and tumor growth (Sengupta *et al.* 2004).

In *Allium schoenoprasum*, Štajner *et al.* (2004) have found that leaves have the highest antioxidant activity in comparison with bulbs and stalks because of the high activity of enzymes related to the antioxidant system and the high levels of antioxidants (flavonoids, vitamin C and carotenoids). Nevertheless, despite this extensive body of research, available information on changes in the chemical composition of chives during storage is still scarce (Kmieciak and Lisiewska 1999).

When it comes to presentation, quality characteristics of fresh culinary herbs, such as chives, are largely visual and include appearance of freshness, uniformity of size, form and color and lack of defects (damaged or yellowed

leaves, decay, insect damage, wilting) (Cantwell and Reid 2006). Characteristic flavor is essential for culinary herbs quality as well. Because essential oils and aroma generally decrease during storage, the formal quantitation of the effects of storage on herb quality is crucial. The objective of this study was to evaluate quality changes in packaged and refrigerated chives. We have focused on fresh chives stored at 0 and 4C for 21 and 14 days, respectively.

MATERIALS AND METHODS

Plant Material

Chive plants (*A. schoenoprasum* L.) were grown at the Experimental Station "Julio Hirschhorn" of the Universidad Nacional de La Plata Agronomy College (Los Hornos, Buenos Aires, Argentina). Its geographic location is 34°52' S, 57°58' W, its height above mean sea level is 15 m and its average annual precipitation is 1,018 mm.

The crop was 2 years old. Row spacing was 0.70 m, and in-row distance was 0.25 m. Plants were grown without supplementary irrigation.

Plants about 30 cm in height were harvested manually, both in April and in November 2005. The harvested material was brought to the laboratory and processed immediately. Leaves showing evidence of damage or disease symptoms were discarded, and any soil was removed by dry cleaning. Leaves were cut (approximately 20 cm long) with a sharp, stainless steel knife and placed on plastic trays (23 × 11 × 4 cm) covered with self-adhering polyvinyl chloride film (thickness: 10 µm; O₂ permeability: 11,232 cm³/m²/atm/day; CO₂ permeability: 48,552 cm³/m²/atm/day; water vapor permeability: 40 g/m²/day).

Determinations for Assessing Shelf Life of Fresh Chives Stored at 0 and 4C

Trays containing approximately 50 g of product were stored for 3 and 2 weeks at 0 and 4C, respectively. Samples were withdrawn for analysis at 0, 7, 14 and 21 days.

Weight loss of trays was determined with a digital balance (± 0.01 g) at the beginning and end of the storage period. Relative weight loss was calculated with respect to the initial weight, and results were expressed as percentage (%).

Surface color measurements were carried out by using a Minolta CR 300 colorimeter (Osaka, Japan) with an 8-mm-diameter measuring area. The instrument was calibrated with a standard white plate ($Y = 93.2$, $x = 0.3133$,

$y = 0.3192$). L^* (lightness), a^* and b^* coordinates of the CIE scale were recorded. Hue angle ($h = \tan^{-1}[b^*/a^*]$) was calculated.

For total chlorophyll and carotenoid content analysis, leaves were frozen in liquid N_2 and crushed in a laboratory mill (Janke & Kunkel Ika Labortechnik A10, Staufen, Germany). Samples (0.5 g) were extracted with 5 mL of cold acetone. Total chlorophyll and carotenoid content were determined according to Lichtenthaler (1987). Absorbance was measured at 470, 644.8 and 661.6 nm. Final results were expressed as mg/100 g fresh tissue.

Extraction of soluble sugars was performed on 0.3–0.5 g of frozen and crushed material, with 7 mL of ethanol 96% wt/wt at room temperature. The mixture was centrifuged at $11,000\times g$ for 10 min. Reducing sugars content was determined spectrophotometrically at 520 nm by using a modification of the Somogyi–Nelson method (Southgate 1976). Glucose was used as standard. Results were expressed as g glucose/100 g fresh tissue.

Browning potential was calculated from reading the absorbance at 320 nm on the extracts performed with ethanol 96% wt/wt (Loaiza-Velarde *et al.* 1997). Final results were expressed as absorbance units/g fresh tissue.

Determinations Carried Out in Samples Stored at 0C for 14 Days

Once the shelf life of the product at the optimal temperature was established, atmosphere composition and antioxidant activity of fresh chives were determined. In this case, samples were withdrawn for analysis at 0, 3, 6, 9 and 14 days.

The composition of internal atmosphere inside packages was measured with a Shimadzu gas chromatograph model GC-6APTF (Kyoto, Japan) fitted with a thermal conductivity detector. An Alltech CTR1 (Deerfield, IL) column was employed. The carrier gas was helium, at a flow rate of 30 mL/min. The injector and detector temperatures were both 120C. The column was held at 30C. A calibration curve was constructed based on different CO_2 and O_2 concentrations. Analysis of the internal atmosphere was performed at 0, 1, 2, 3, 6, 9, 13, and 17 days of storage. Final results were expressed as mL/100 mL.

Total phenolics were quantitated on the ethanolic extracts, employing the Folin–Ciocalteu reagent (Swain and Hillis 1959). Absorbance readings were taken at 760 nm. Catechin was used as standard, and final results were expressed as mg catechin/100 g fresh tissue.

Ascorbic acid content was determined, with a modified version of the method proposed by Wimalasiri and Wills (1983). Samples were extracted with 5 mL aqueous solution of 3% citric acid. A Waters Model 6,000A (Milford, MA) high-performance liquid chromatograph was used. A C18 column was employed (particle diameter: 5 μm ; internal diameter: 4.6 mm; length: 25 cm). The mobile phase was 0.2 M KH_2PO_4 (pH = 2.4). Flow rate

was 1 mL/min. Detection was carried out with an ultraviolet (UV) detector at 254 nm. For identification and quantitation, a standard ascorbic acid solution was employed. Final results were expressed as mg ascorbic acid/100 g fresh tissue.

The radical scavenging activity of the ethanolic extracts was determined by reaction with the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) (Brand-Williams *et al.* 1995). Concentration of the extracts was varied in the reaction mixtures by adding 0, 10, 20, 40, 60, 80 or 100 μ L of each of them to a 2 mL of DPPH[•] in methanol solution (25 ppm), completing a final volume of 2.1 mL with methanol. The reaction was allowed to progress, and absorbance was measured at 515 nm after a constant value was reached. Then, DPPH[•] was calculated through a calibration straight line obtained in a range of concentrations of this compound. Finally, the remaining DPPH[•] concentration was plotted as a function of the extract volume in the reaction mixture to calculate EC₅₀ (effective mean concentration) for each sampling point. Final results were expressed as μ mol DPPH[•]/100 g fresh tissue.

Statistical Analysis

Two replications of the experiments were performed. Each determination was carried out at least twice. Results were subjected to analysis of variance. Sources of variation were temperature and storage time. Comparison of means was conducted with the Fisher's least significant difference test at a significance level $P = 0.05$. Least significant difference values are provided in all figures captions and tables.

RESULTS AND DISCUSSION

Moisture loss appeared as one of the primary parameters affecting fresh chives quality. Weight loss of trays increased significantly during storage time at both 0 and 4C (Table 1). In all sampling points, weight loss of trays was significantly higher at 4C than at 0C. For both assayed temperatures, the linear regression analysis showed a statistically significant relationship between weight loss and storage time. At 0C ($r = 0.999$, $P \ll 0.01$), the weight loss rate was 0.55% per day, whereas, at 4C ($r = 0.993$, $P \ll 0.01$), the weight loss was 0.87% per day. Likewise, Tsouvaltzis *et al.* (2006) found that cut leek (*Allium porrum* L.) stalks lost fresh weight linearly with storage time at 4C ($r = 0.940$, $P < 0.0001$) to as much as 2.64% of initial fresh weight, on day 9 of storage.

Weight loss values registered in our experiments after 14 days of storage were higher than the ones reported by Aharoni *et al.* (1989) in chives packaged in perforated polyethylene bags and stored for 5 days at 6C plus 2 days at 12C.

TABLE 1.
WEIGHT LOSS (%) OF TRAYS CONTAINING FRESH
CHIVES LEAVES STORED AT 0 OR 4C FOR 21 DAYS

Time (days)	Weight loss (%)	
	0C	4C
0	0.00 ^{ax}	0.00 ^{ax}
7	3.84 ^{bx}	4.70 ^{by}
14	7.70 ^{cx}	11.75 ^{cy}
21	11.79 ^{dx}	20.07 ^{dy}
LSD _{0.05}	0.43	

Values within each column followed by different letters (a–d) and within each row (x–y) indicate significant differences ($P < 0.05$). LSD, least significant difference.

TABLE 2.
HUE AND L^* VALUES OF THE CIE SCALE IN FRESH
CHIVES STORED AT 0 OR 4C FOR 21 DAYS

Time (days)	Hue		L^*	
	0C	4C	0C	4C
0	135.2 ^{oax}	135.2 ^{oax}	38.2 ^{ax}	38.2 ^{ax}
7	128.2 ^{obx}	127.9 ^{obx}	43.8 ^{bx}	43.4 ^{bx}
14	123.2 ^{ocx}	114.7 ^{ody}	48.1 ^{cx}	50.5 ^{cx}
21	114.2 ^{od}	–	55.7 ^d	–
LSD _{0.05}	3.4		2.7	

Values within each column (a–d) and within each row (x–y) followed by different letters indicate significant differences ($P < 0.05$). LSD, least significant difference.

Surface color evolution of fresh chives was characterized by means of the color angle (h) (Table 2). After 14 days of storage, h decreased significantly by 9% and 15% for samples stored at 0 and 4C, respectively. Samples maintained at 4C were not marketable at this point because of noticeable yellowing of leaves. After 21 days at 0C, h reached a value similar to the one found at 4C after 14 days of storage. Thus, storing chives at 0C delayed the loss in color by approximately 1 week.

The variation of L^* during the storage of fresh chives is shown in Table 2, where a significant increase can be observed for samples stored at both 0 and 4C. At 0C, the increase of L^* correlated inversely with the variation in h of refrigerated fresh chives (Pearson's correlation coefficient = -0.928).

At the beginning of the experiments, the total chlorophyll content of fresh chives was 73 mg/100 g fresh tissue. Kmiecik and Lisiewska (1999) reported

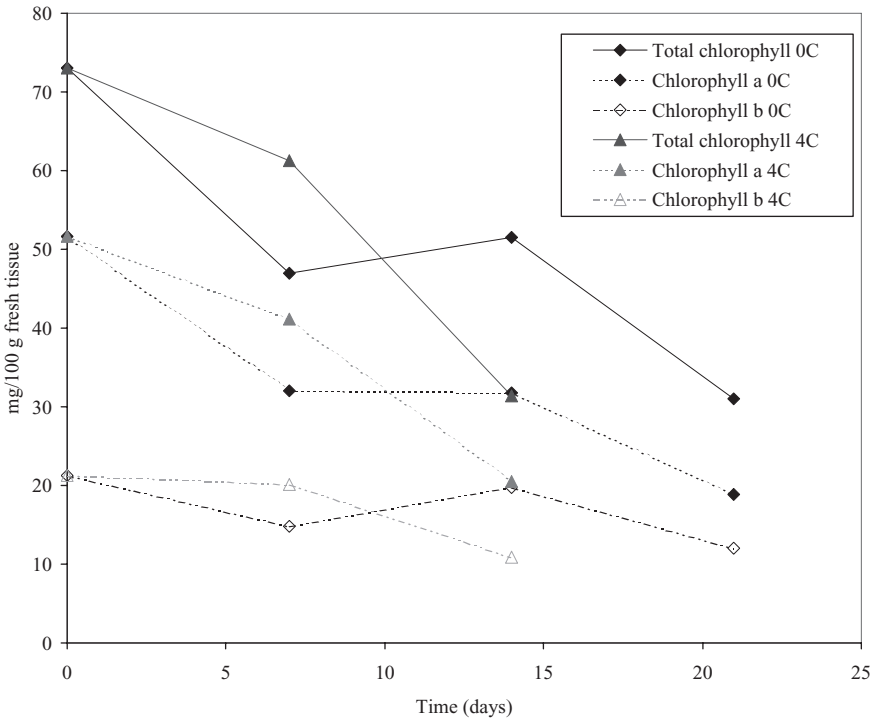


FIG. 1. TOTAL CHLOROPHYLL, CHLOROPHYLL *a* AND CHLOROPHYLL *b* CONTENT (mg/100 g FRESH TISSUE) OF CHIVES STORED DURING 21 OR 14 DAYS AT 0 OR 4C, RESPECTIVELY
 LSD_{0.05} total chlorophyll = 5.2; LSD_{0.05} chlorophyll *a* = 5.3; LSD_{0.05} chlorophyll *b* = 1.9.
 LSD, least significant difference.

a higher chlorophyll (*a* plus *b*) content in fresh chive leaves, which corresponded to 121 mg/100 g of fresh matter.

After 21 days at 0C, total chlorophyll levels diminished significantly, reaching a value that corresponded to 42% of the initial one. The evolution of chlorophyll *a* was very similar to the trend showed by total chlorophyll content, whereas chlorophyll *b* levels remained constant during 21 days at 0C (Fig. 1). When samples were kept at 4C, total chlorophyll content decreased by 43% with respect to the starting level, after 14 days of storage (Fig. 1). Chlorophyll *a* evolved in the same way as total chlorophyll did. Chlorophyll *b* remained constant during the first week at 4C and then diminished significantly (day 14). Results indicate that total chlorophyll and chlorophyll *a* degradation could be delayed approximately by a week by storing samples at 0C. At the beginning of the storage, the ratio between total carotenoids and

TABLE 3.
TOTAL CAROTENOID CONTENT (mg/100 g FRESH TISSUE)
OF FRESH CHIVES STORED AT 0 OR 4C FOR 21 OR
14 DAYS

Time (days)	Total carotenoid content (mg/100 g fresh tissue)	
	0C	4C
0	16.8 ^{abx}	16.8 ^{abx}
7	14.8 ^{cx}	18.4 ^{by}
14	18.7 ^{ax}	15.4 ^{by}
21	15.6 ^{bc}	—
LSD _{0.05}	1.9	

Values within each column followed by different letters (a–c) and within each row (x–y) indicate significant differences ($P < 0.05$). LSD, least significant difference.

total chlorophyll content was 0.23, slightly below the overall stoichiometry of total carotenoid to total chlorophyll (a plus b) (Bowyer and Leegod 1997). Total carotenoid content of fresh chives showed slight variations during refrigerated storage (Table 3).

Initial reducing sugars content of fresh chives was 3.03 g glucose/100 g fresh tissue, which is consistent with the levels reported by Kmiecik and Lisiewska (1999). When samples were maintained at 0C, reducing sugars content decreased up to 46% of the starting value after 21 days, while samples stored at 4C lost 48% of reducing sugars after 14 days of storage (Fig. 2). Hong *et al.* (2000) reported that soluble sugar concentrations in the white stems bases of green onions (*Allium cepa* × *Allium fistulosum*) decreased by 30% of the initial content when they were stored at 5C for 7 days.

Browning potential of fresh chives rose up significantly at both 0 and 4C, after 7 days of storage (Fig. 2). From then on, this parameter remained constant for samples stored at 0C but continued increasing in samples kept at 4C.

According to the results concerning weight loss, surface color, pigments, reducing sugars and browning potential during the refrigerated storage of fresh chives, the shelf life of the product at 0C was set at 14 days. Storing fresh chives at 4C shortened the shelf life of the product approximately by a week in comparison with the evolution of attributes observed at 0C.

Regarding internal atmosphere composition of samples stored at 0C, Fig. 3 shows that CO₂ concentration increased significantly on the second day of storage. Then, CO₂ levels remained stable up to the end of storage. On the other hand, O₂ consumption was not detectable probably because of the high O₂ permeability rate of the film used. Figure 3 shows that O₂ concentration inside packages remained constant for more than 2 weeks at 0C. Aharoni *et al.*

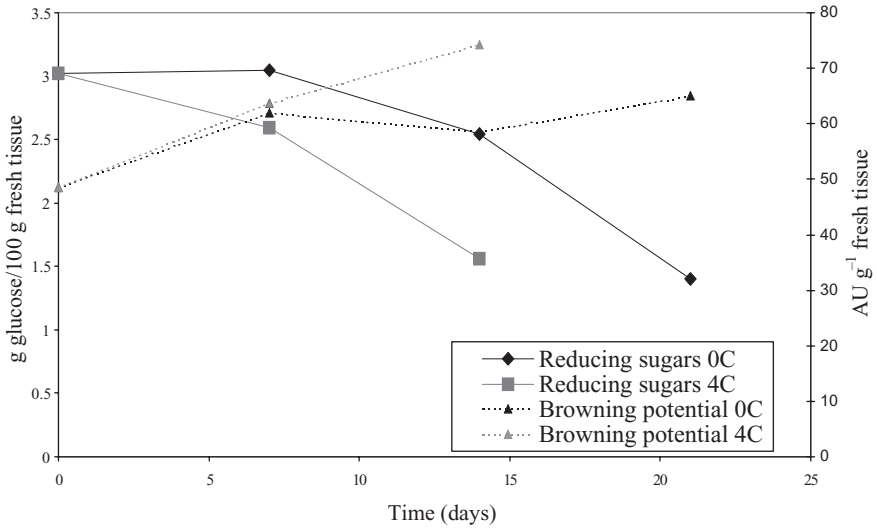


FIG. 2. REDUCING SUGAR CONTENT (g GLUCOSE/100 g FRESH TISSUE) AND BROWNING POTENTIAL (ABSORBANCE UNITS/g FRESH TISSUE) OF CHIVES STORED DURING 21 OR 14 DAYS AT 0 OR 4C, RESPECTIVELY
 LSD_{0.05} reducing sugars = 0.3; LSD_{0.05} browning potential = 8.5.
 LSD, least significant difference.

(1988) pointed out that green herbs susceptible to yellowing and decay were well conserved when they were packaged in nonperforated polyethylene liners. This was attributed to the elevation of CO₂ concentration (to 1–5%) within the packages. In the same way, Aharoni *et al.* (1989) found that packaging chives in a sealed polyethylene-lined carton allowed to the maximum reduction of yellowing and decay after 5 days at 6C plus 2 days at 12C. The authors also mentioned that the accumulation of CO₂ (5.7%) rather than the decrease in O₂ (12%) inside the packages could have been the reason for the retardation of senescence.

Changes in total phenolics content are shown in Table 4. The values remained constant up to day 6, when total phenolics concentration showed a significant increase. At this point, the reached level was 12% higher than the initial one. At day 13, values did not significantly differ from the initial or from the highest one. Total phenolics content found in chive leaves was intermediate between those reported for other *Allium* vegetables, such as shallots (114.7 mg gallic acid equivalents/100 g of sample = 177.1 mg catechin/100 g sample) and *Vidalia* onion (16.8 mg gallic acid equivalents/100 g of sample = 25.9 mg catechin/100 g of sample) (Yang *et al.* 2004).

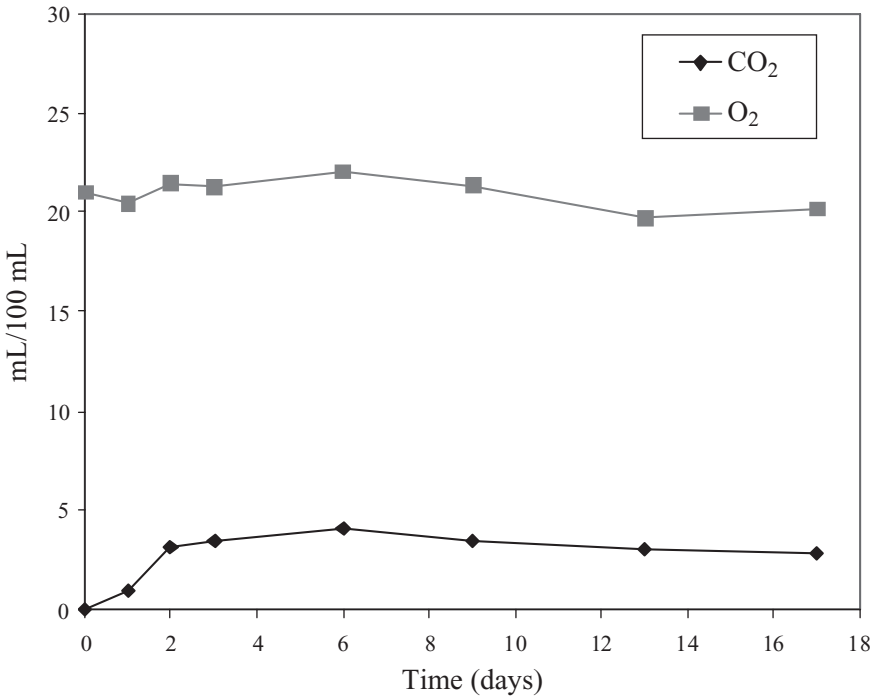


FIG. 3. CO₂ AND O₂ CONTENT (mL/100 mL) INSIDE PACKAGES OF FRESH CHIVES STORED AT 0C

LSD_{0.05} CO₂ = 1.6; LSD_{0.05} O₂ = 2.3.

LSD, least significant difference.

Initial ascorbic acid content of fresh chives (18.4 mg/100 g fresh tissue) diminished significantly after 3 days of storage at 0C (Table 4). From then on, values remained constant up to the end of the assayed period. The total average diminution of ascorbic acid content during 13 days at 0C represented 30% with respect to the initial value. Kmiecik and Lisiewska (1999) have reported 133 mg vitamin C/100 g fresh matter in nonblanched chives, being 96% of this value the percentage of L-ascorbic acid. Štajner *et al.* (2004) have mentioned that the content of vitamin C in the leaves of *A. schoenoprasum* was 0.12 ± 0.03 mg/mg protein.

Radical scavenging activity of fresh chives was 327 μ mol DPPH'/100 g fresh tissue. The values remained constant during the first 3 days and then showed a slight trend to increase between days 6 and 13 of the refrigerated storage (Table 4). Nuutila *et al.* (2003) have investigated the antioxidant activities of *Allium* vegetables extracts as well as several chemical components. The

TABLE 4.
TOTAL PHENOLICS (mg CATECHIN/100 g FRESH TISSUE), ASCORBIC ACID (mg/100 g FRESH TISSUE) AND RSA ($\mu\text{mol DPPH}/100 \text{ g FRESH TISSUE}$) OF FRESH CHIVES STORED AT 0C FOR 13 DAYS

Time (days)	Total phenols (mg catechin/100 g fresh tissue)	Ascorbic acid (mg/100 g fresh tissue)	RSA ($\mu\text{mol DPPH}/100 \text{ g fresh tissue}$)
0	84.4 ^{bc}	18.4 ^a	327.2 ^{ab}
3	79.1 ^c	11.2 ^b	278.0 ^b
6	94.3 ^a	12.6 ^b	360.2 ^a
9	84.8 ^{bc}	13.2 ^b	347.1 ^a
13	92.3 ^{ab}	14.8 ^{ab}	396.1 ^a
LSD _{0.05}	9.3	4.7	77.4

Values within each column followed by different letters indicate significant differences ($P < 0.05$). LSD, least significant difference; RSA, radical scavenging activity.

authors have found kaempferol as a polyphenolic constituent of chives leaves, being one of the compounds contributing to the antioxidant activity of this herb.

CONCLUSIONS

Results found in this work show that fresh chives' shelf life can be extended by at least a week when they are stored at 0C as opposed to a temperature of 4C, which is not substantially higher. Weight loss of trays, surface color degradation, green pigments and reducing sugar loss could be delayed and browning potential can be reduced by keeping the product at 0C. As weight loss values were high at both 0 and 4C, other plastic films of lower water vapor permeability rates than polyvinyl chloride film should be assayed in future experiences. The analysis of total phenolics content and radical scavenging activity of samples kept at 0C did not show clear evidence of deleterious changes, even after 13 days of refrigerated storage.

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