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Postharvest Quality Losses of Butter Lettuce as Affected by Leaf Age and Temperature

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ABSTRACT

Whole lettuce plants were used to evaluate the effect of leaf age on ascorbic acid (AA), chlorophyll (C), microbial quality (M), colour and overall visual quality of fresh butter lettuce exposed at three isothermal environmental conditions: 0-2, 10-12 and 20-22°C during first 24 hours after harvest in order to describe quality changes in the product during transport from the field to the distribution center. Relative humidity was the optimal (97-99%). Temperature, leaf age and time significantly affected the quality parameters under study. Even though lettuces were stored under optimal temperature (0-2°C), significant C decreases were observed in outer leaves, increasing the loss at higher temperatures. The highest losses took place in the first 6 h, and then the rate of loss was significantly lower. Although the inner leaves presented changes in redness/greenness colour parameter, no significant changes in C were detected in these leaves. Increments in microbial counts occurred, and resulted higher in outer leaves respect to mid or inner ones. Increments were greater at higher temperature. Ascorbic acid significantly decreased at the three temperatures. After 6 h of storage, the AA losses were of 50% at 10-12 or 20-22°C, and of 30% at 0-2°C.

Keywords: ascorbic acid, butter lettuce, chlorophyll, colour, OVQ, microbiological quality

INTRODUCTION

Fresh vegetables constitute a fully recognized source for minerals, vitamins and dietary fiber (Negi *et al.* 2000). The quality of fresh vegetables at harvest primarily depends upon cultivars, growing conditions, preharvest cultural practices and maturity at harvest among others (Weston *et al.* 1997; Kader 2002). Fresh vegetables are highly perishable products and its shelf life is mainly affected by handling procedures, temperature, and relative humidity (Watada 1996, 1997; Kader 2002). Rapid management from farm to table, and at optimal conditions, is a desirable feature because it minimizes economical and quality losses during postharvest. If conditions are inadequate (such as poor handling or high temperature during transportation) losses could reach 50% of total production (Talukder *et al.* 2003; Nunes *et al.* 2009).

Effects of temperature over quality detriment of crops have been extensively studied and they are well known. In fact, storage temperature is considered to be the critical factor of shelf life (Watada 1997; Paull 1999; Zanoni *et al.* 2007) being temperature deviations the main reason for quality losses. In this way, effective temperature management is the most important and simplest procedure for delaying product deterioration (Nunes *et al.* 2004). Temperature has a big effect on the metabolic rate of the product. When the temperature of the product rises, the rate of physiological processes such as respiration and transpiration result accelerated. As a consequence, temperature increases by the heat generated in these processes. Thus, shelf life of the product results reduced. However, few researches have been developed to investigate the first few hours after harvest. Moreira *et al.* (2006) studied the effect of abusive temperatures after harvest over lettuce leaves and found that first hours after harvest are crucial for vegetable shelf life because quality losses in this earlier stages could not been recovered (Moreira *et al.* 2006). Jedermann *et al.* (2007) proposed that the effect of short exposure of some few hours to inadequate conditions (too high or too low temperature, to dry or to moist) are sufficient to favor quality losses in the product.

Lettuce is one of the most popular leafy vegetables in the world. The lettuce head is an assemblage of heterogenic morphological leaves that are packed together over the growing point of the plant. Its formation results from the accumulation of young leaves under the layers of leaves covering the growing point (Wien 1997). This space leaf distribution constitutes an interesting natural model that allows evaluating the response of both different degree of leaf exposition to environment and tissue development. Several studies have been demonstrated that leaf distribution affects leaf composition as well as its metabolic activity (Siomos *et al.* 2002; Del Nobile *et al.* 2006; Agüero *et al.* 2008a, 2008b).

In Argentine farms, lettuce bins are usually exposed to inadequate and uncontrolled field conditions (usually high temperature and low relative humidity) while it is waiting to be transported from the field to distribution centers. High temperatures usually result from exposure to either direct sunlight, hot air in the field or heat treatments used for the eradication of pests. Product exposed to sun light could rapidly raise a temperature 4 a 6°C more than air temperature (Thompson *et al.* 2001). In the field, the combination between the heat of the sun and the respiration of the produce provokes the heat up of the produce, reducing its postharvest life. So the knowledge of the events occurring within the plant when temperature is uncontrolled during first hours after harvest is of fundamental importance to improve and optimize lettuce management. The response of lettuce tissue to temperature could be affected by the leaf

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age. Studies evaluating the effect of abusive temperatures management and leaf distribution on quality indices are very scarce.

The objective of the present research was to describe the response of different lettuce leaves (outer, mid and inner) to the exposure at three isothermal conditions (0-2, 10-12 and 20-22°C) during the first hours after harvest. Leaf behavior was evaluated through several quality indicators: a) total chlorophyll content and b) colour changes as greenness indices, c) total microbial counts and as microbiological descriptor, and d) ascorbic acid retention as nutritional index.

MATERIALS AND METHODS

Plant material and sample preparation

Heads of greenhouse butter lettuce (*Lactuca sativa* var. 'Lores') were grown in Sierra de los Padres, Mar del Plata, Argentina; and were harvested at optimal maturity after reaching a marketable size (approximately 24-30 leaves per head, corresponding to a weight of 500 ± 60 g). Once harvested, lettuce heads were immediately transported to the laboratory (20 km, approximately) maintaining temperature and relative humidity conditions at optimal levels (0-2°C and 97-99% RH, respectively). Once arrived to laboratory, six whole plants were analyzed to obtain the initial value for each quality parameter. These experimental data represented the zero time of storage in each of the quality indicators analyzed.

Plants were not subjected to any preconditioning operation; they were just put in environmental chambers (SCT, Pharma, Argentina) at 0-2, 10-12 and 20-22°C, maintaining the relative humidity levels in 97-99%. Temperatures of 10-12 and 20-22°C were chosen to simulate abusive refrigerated and room warm temperatures, respectively. Quality indicators evolution in samples at abusive temperatures was compared to the optimal temperature of lettuce management (0-2°C). Sampling was carried out at 0, 3, 6 and 24 h.

At each sampling time, 8 plants were taken from each storage chamber. Four plants were used to assess chlorophyll and ascorbic acid contents, and the other four to analyze microbial counts, sensorial quality and colour. All parameters (with exception of sensorial quality) were measured in three different zones of the lettuce head called external (outer and older leaves), middle (mid leaves) and internal zone (inner and younger leaves). For each lettuce plant, zones were delimited visually, applying an organoleptic criterion, according to which the internal zone was compact with yellow leaves and the middle and external zones corresponded to non-compact leaves of green and dark green colour, respectively. Each zone had a mean of approximately 6-9 leaves (Agüero *et al.* 2008a). Three independent experimental runs were done.

Total chlorophyll content

The total chlorophyll (TC) content of each zone was determined following the methodology described by Moreira *et al.* (2003). All leaves in each zone were homogenized with a commercial blender (Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) and two samples (1 g each) were taken from each homogenate. Each sample was then homogenized with 19 ml of a cold solution 18: 1 propanone: ammonium hydroxide (0.1N). This homogenate was filtered through sintered glass and water was removed from the filtrate with anhydrous sodium sulfate. Absorbance of the filtrate at 660.0 and 642.5 nm was measured with a UV 1601 PC UV-visible spectrophotometer (Shimadzu Corp., Japan). TC was calculated applying the formula $TC = 7.12A_{660} + 16.8\ A_{642.5}$ in which TC is the total chlorophyll concentration (mg L^{-1}) and A_{660} and $A_{642.5}$ are the absorbance at the corresponding wavelengths. TC is reported as mg of chlorophyll/100 g fresh weight.

Total microbial counts

The enumeration of total microorganisms was performed in each lettuce zone. Ten grams of leaves were macerated in a buffer solution (PO_4K_3, pH = 7.2). Microbial counts were carried out using Plate Count Agar (PCA) incubated at 32-35°C for 48-72 h (ICMSF 1983). The culture media was from Britania (Buenos Aires, Argentina). Studies were performed by duplicate. Microbiological counts were expressed as log CFU/g of fresh weight.

Ascorbic acid content

Acorbic acid (AA) content was determined in each lettuce zone following the titrimetric method described by Roura *et al.* (2003). Ground lettuce leaves (20 g) were extracted with 100 mL of metaphosphoric acid solution (60 $g.Kg^{-1}$) for 3 min using a commercial blender (Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) with an homogenizer speed of 3500 to 7000 rpm. The homogenate was made up to 250 mL with 30 $g.Kg^{-1}$ metaphosphoric acid and filtered through Whatman n° 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0°C. Three aliquots (5 mL each) of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid contents were reported as mg/100 g of fresh weight (Moreira *et al.* 2003).

Colour

Colour determination was carried out using a Minolta colorimeter CR 300 Series (Osaka, Japan) with an 8 mm diameter measuring area. The colour of lettuce zones was measured by L, a^* and b^* chromaticity co-ordinates of the CIELab^* scale. The instrument was calibrated with a standard white plate (L = 93.97, a^* = -21.85 and b^* = 1.21). Colour was measured in all leaves integrating each lettuce zone. Ten different points were at least measured in each leaf. Mean values for L, a^* and b^* parameters were calculated for each leaf, and then for each zone.

Sensorial evaluation

Immediately after lettuces were removed from storage conditions, a panel composed by 9 trained judges proceeded to the assessment. Said judges were 30 to 55 years of age and belonged to the Food Engineering Group (UNMDP). They all had sensorial assessment experience in leafy vegetables. The coded (3 digit) samples were presented one at the time in random order to the judges who sat at a round table and made independent evaluations. The judges were asked to evaluate overall visual quality of whole lettuce heads taking into account the following characteristics: color (shade and uniformity), texture, browning and presence/absence of defects. A 9-point scale was used, where 9 = excellent, 5 = acceptable and 1 = poor (Kader 2002).

Statistical analysis

Results reported in this paper, are lsmean values (least square mean, means estimators by the method of least squares) together with their standard deviations (Kuehl 2001).

Data were analyzed using SAS, software version 8.0 (SAS Inc. 1999). PROC GLM (General linear model procedure) was used for the analysis of variance (ANOVA). For all parameters, except sensorial quality, the factors employed as sources of variation were: TIME (time after harvest), PLANT WITHIN TIME, ZONE (zone of the plant), TEMPERATURE and interactions: TIME-ZONE; TIME-TEMPERATURE and TIME-ZONE-TEMPERATURE. Differences between zones, temperatures and time were determined by the Tukey-Kramer multiple comparison test ($p < 0.05$). PROC UNIVARIATE was used to validate ANOVA assumptions (Kuehl 2001). For sensorial quality data, the factor ZONE was not taken into account in order that overall visual quality was evaluated in the whole lettuce plant. For this parameter, the factors employed as sources of variation were: TIME (time after harvest), PLANT WITHIN TIME, TEMPERATURE and interaction: TIME-TEMPERATURE. Differences between temperatures and time were determined by the Tukey-Kramer multiple comparison test ($p < 0.05$). PROC UNIVARIATE was used to validate ANOVA assumptions (Kuehl 2001).

RESULTS AND DISCUSSION

Total chlorophyll content

Initial chlorophyll content (TC) in harvested lettuce was: 51.74 ± 1.93, 22.13 ± 2.11 and 10.60 ± 1.76 (mg of chlorophyll/100 g fresh weight) for external, middle and internal zones, respectively. Great differences ($p < 0.0001$) in chlorophyll content were detected among lettuce zones, being the content of the external zone 5 times higher than that of the internal one. The higher exposure of outer leaves to sun light respect inner leaves could be responsible for the differences in chlorophyll concentrations between zones. Agüero *et al.* (2008a), working with butterhead lettuce, reported lower TC values in all lettuce zones than those informed in the present work. However, they found similar relations between TC contents in each zone, being values in external zone higher than internal one.

The exposure of almost all vegetables to inadequate temperatures (higher than the optimal recommended for each case) brings about certain degradation on chlorophyll pigments (King *et al.* 2001; Yin *et al.* 2007; Agüero *et al.* 2008a; Zhang *et al.* 2008). ANOVA applied to chlorophyll data showed a significant interaction between ZONE and TIME factors considered in the analysis. This fact implies that each zone have a particular behavior during postharvest. In this way, while the external zone exhibited chlorophyll pigments degradation throughout 24 h at any assayed temperature, the middle and internal zones showed no TC changes during such period. **Fig. 1** depicts the evolution of TC in external zone during 24 h after harvest. The chlorophyll degradation of external zone was more pronounced at higher temperatures (12, 20 and 36% at 0-2, 10-12 and 20-22°C, respectively). Differences in chlorophyll degradation between zones could be related to the higher exposure of outer leaves to environmental factors such as light and oxygen, which could fasten pigment deterioration. It is assumed that during postharvest life the leaf pigments undergo degradation that leads to leaf discoloration. Ferrante *et al.* (2007) reported that TC and carotenoids contents start to decline a few days after harvest and this phenomenon has been observed in many leafy vegetables. In the present research, it was detected TC degradation at 3 h after harvest. After this period, the rate of pigment loss was significantly lower. As a response to harvest stress, when water and nutrient supply was cut off, outer leaves (the most mature leaves of lettuce head, and leaves that are also near its physiological senescence) could trigger different physiological responses. Cran *et al.* (1974) found different responses in TC between mature and young spinach leaves when they are exposed to light or darkness during 7 days. During natural leaf senescence, nutrients are usually mobilized from the leaf to be used in other parts of the plant. At harvest, senescence process is induced artificially as a result of the removal of nutrient supplies (Page *et al.* 2001). As a rapid response, the degradation of TC was evident in outer leaves, which started showing signs of chlorophyll losses.

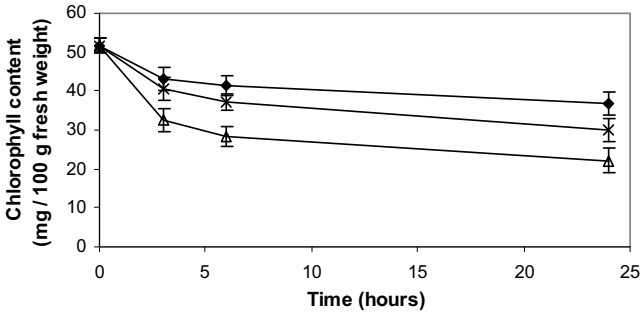

Fig. 1 Chlorophyll content evolution in external zone of lettuce heads during 24 h of exposure to isothermal conditions: (△) 20°C; (×) 10°C; (♦) 0°C.

Table 1 *a** value in external (E), middle (M) and internal (I) zones of lettuce heads during 24 h of exposition to three different isothermal conditions (0-2, 10-12 and 20-22ºC).

Time (h)	Zone	Initial *a** values		
0	E	-19.73 ± 0.83		
	M	-19.25 ± 1.28		
	I	-16.30 ± 1.25		
		***a** values*		
		0-2ºC	**10-12ºC**	**20-22ºC**
3	E	-19.37 ± 0.32	-19.35 ± 0.56	-18.89 ± 0.24
	M	-19.49 ± 1.52	-18.85 ± 0.56	-18.90 ± 1.07
	I	-15.89 ± 0.88	-13.47 ± 3.86	-12.88 ± 1.52
6	E	-19.51 ± 1.09	-18.13 ± 2.37	-18.93 ± 0.03
	M	-18.43 ± 0.90	-16.84 ± 0.21	-18.03 ± 1.61
	I	-12.86 ± 1.04	-12.49 ± 0.58	-12.43 ± 2.41
24	E	-19.32 ± 0.34	-19.07 ± 0.35	-18.87 ± 0.06
	M	-19.14 ± 0.16	-17.94 ± 2.26	-17.44 ± 0.40
	I	-15.26 ± 0.76	-12.22 ± 1.86	-11.06 ± 2.04

Colour

Colour is an important sensorial attribute and a critical factor affecting quality (Rico *et al.* 2008). Several colour systems have been developed for colorimetric measurement, being all of them mathematically convertible. However, the Hunter Lab colour space was selected based on its documented adequacy for theoretically quantifying colour changes in green vegetables (Gnanasekharan *et al.* 1992).

Lightness parameter (*L**) in lettuce at harvest showed significant differences among zones. The highest *L** value was observed in internal zone (76.06 ± 2.00), decreasing towards the external one (65.71 ± 3.38 and 59.13 ± 1.95 for middle and external zones, respectively). The exposure of lettuce heads to different temperature conditions during 24 h did not introduce significant changes in *L** parameter as a function of time or temperature (data not shown). Other authors (Ihl *et al.* 2003; Han *et al.* 2004; Martínez-Romero *et al.* 2008) also found for lettuce no significant changes in L* parameter throughout storage time.

Redness/greenness parameter (*a**) in lettuce at harvest resulted in a negative value in all zones indicating the predominance of green colour in the product. Results showed significant differences between *a** values in each zone at harvest, being more negative (greener) in external (-19.73 ± 0.83) and middle (-19.25 ± 1.28) zones (without significant differences between them) than in internal one (-16.30 ± 1.25). Green colour of inner leaves is less intense and this fact was reflected in *a** values obtained for each zone. **Table 1** presents *a** values obtained during sampling time in lettuce exposed to different temperature conditions. ANOVA applied to *a** data showed no significant interaction between factors considered in the analysis (neither triple nor doubles). However, each factor individually resulted significant ($p < 0.0001$ for ZONE, $p = 0.0064$ for TEMPERATURE, and $p = 0.0006$ for TIME). For the ZONE factor, the significant differences detected between zones at harvest were maintained as time advanced at the three evaluated temperatures; i.e. at each sampling time and at the three assayed temperatures, outer and mid leaves resulted more green (lower a* values) than inner ones. For TEMPERATURE factor, samples exposed to the highest temperature registered the lowest absolute value of *a** parameter during the sampling time in the three lettuce zones. Finally, for TIME factor, significant decreases in the absolute value of *a** (less green) were observed during 24 h in the three zones and the three assayed temperatures and the highest decrease was observed in internal zone at 20-22ºC. Other authors associated decreases in absolute value of *a** with chlorophyll degradation (Ihl *et al.* 2003; Castañer *et al.* 2006; Rico *et al.* 2008). However, in the present work, decreases in chlorophyll concentration were not detected in internal zone while changes in *a** parameter were detected. Del Nobile *et al.* (2006), studying the effect of film packaging on quality and shelf life of lettuce, found the highest

Table 2 *b** value in external (E), middle (M) and internal (I) zones of lettuce heads during 24 h of exposition to three different isothermal conditions (0-2, 10-12 and 20-22ºC).

Time (h)	Zone	Initial *b** values		
0	E	34.64 ± 0.88		
	M	37.26 ± 0.85		
	I	40.50 ± 0.78		
		***b** values**		
		0-2ºC	**10-12ºC**	**20-22ºC**
3	E	35.05 ± 0.05	34.81 ± 0.22	34.03 ± 1.83
	M	38.25 ± 0.75	38.29 ± 1.24	35.73 ± 2.41
	I	42.18 ± 3.49	39.18 ± 4.46	38.27 ± 0.40
6	E	36.45 ± 0.54	33.77 ± 3.35	33.72 ± 0.09
	M	37.34 ± 2.53	34.27 ± 1.82	34.42 ± 4.47
	I	42.31 ± 2.85	39.08 ± 5.83	36.92 ± 2.95
24	E	34.19 ± 0.56	34.31 ± 0.02	33.69 ± 0.52
	M	36.96 ± 1.93	34.21 ± 2.79	33.07 ± 1.53
	I	46.09 ± 0.56	37.92 ± 6.32	33.24 ± 6.14

changes of *a** parameter in inner leaves and associated this result to the highest susceptibility of this leaves to browning.

Blueness/yellowness parameter (*b**) in lettuce at harvest resulted positive in all zones indicating the predominance of yellow in this scale. Significant differences between zones were found in *b** values at harvest, being higher (more yellow) in inner leaves (40.50 ± 0.78) than in mid (37.26 ± 0.85) and outer leaves (34.64 ± 0.88). *b** values obtained for lettuce zones during time of exposure to different temperatures are presented in **Table 2**. ANOVA applied to *b** data showed neither double nor triple interactions among the factors considered in the analysis. Furthermore, TIME factor resulted non significant indicating that no changes were registered in *b** values as time advanced at any analyzed temperature and zone. ZONE and TEMPERATURE factors resulted significant ($p < 0.0001$, and $p = 0.0021$, respectively). For the ZONE factor, significant differences in *b** values detected among lettuce zones at harvest were maintained as time advanced at the three evaluated temperatures. For the TEMPERATURE factor, it was found that samples exposed to high temperatures registered lowest values of *b** parameter.

Total microbial counts

The largest population in green vegetables is the mesophilic microflora (Watada 1996). Numerous studies have been carried out analyzing the effect of temperature on the microbial load and evolution in different vegetable products (Thompson *et al.* 2001; Moreira *et al.* 2006). In the present research, the leaf age was taken into account as an additional factor that could affect initial microbial load as well as its evolution during the first hours postharvest.

Initial microbial counts for external, middle and internal zones were not significantly different (5.95 ± 0.10, 5.83 ± 0.11 and 5.64 ± 0.15 log CFU/g, respectively). Aycicek *et al.* (2006) reported that outer leaves of cos and iceberg-lettuce samples had higher bacterial loads than inner leaves. Jacques (1996) reported that the size and composition of microbial populations are different under the influence of biotic and abiotic factors related to the microorganisms themselves (nutritional resource utilization, abilities to compete for space, resistance or production of toxic compounds), to the host (its genotype, the age and the position of the leaves), and to the environmental conditions (micro- and macro-climate, activity of vectors and pathogens, application of pesticides and other chemicals). Consequently, for butter lettuce var. 'Lores' cultivated under greenhouse, the fact that there are no differences in the initial microbial counts among the different lettuce zone could express that microorganisms found similar preharvest environmental and nutritional conditions in each zone.

Fig. 2 shows total increments in microbial counts for each lettuce zone at each temperature ($LOG(N_t/N_0)$).

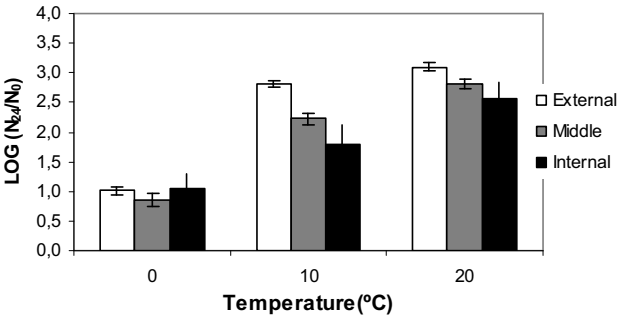

Fig. 2 Microbial counts change ($LOG(N_{24}/N_0)$) in external, middle and internal zone of lettuce heads after 24 h of exposure to isothermal conditions.

ANOVA applied to microbial data yielded significant interactions between factors ZONE-TIME, and TEMPERATURE-TIME ($p = 0.0012$ and $p < 0.0001$, respectively), thereby denoting that microbial counts evolution during 24 h differs taking into account the zone and the temperature. In the three lettuce zones increments in microbial counts occurred during the first 24 h of exposure to different temperatures, being higher in external zone with respect to middle and internal ones and resulting greater when temperature was 20°C instead of 10 or 0°C. Moreover, when lettuce was stored at optimal conditions, an increase of 1 log was detected in the three lettuce zones during 24 of exposure without significant differences between zones. Plants exposed at 10°C, presented higher microbial counts than lettuces at optimal temperature (0-2ºC), and significant differences between zones were detected. Microbial counts in external zone reached almost 3 log while for internal zone increments were lower than 2 log. Middle zone, at 10°C showed an intermediate behavior reaching an increment of 2.3 log. So, at 10°C, both factors considered in the analysis (time and leaf age) affected the microbial behavior. Finally, when temperature was 20°C the behavior of microbial count was similar to that observed at 10°C. In this way, microbial counts were higher in external than in middle and internal zones, reaching values increments higher than 3 log in external zone. When temperature was close to optimum temperature for mesophilic bacteria growth, the native microflora found more favorable substrate for development in outer leaves than in other zone of the lettuce head. This could be attributed to the fact that external zone is shaped by more senescent tissue that is more subject to bacterial growth than young tissue without mechanical damage and associated plant cell leakage (King *et al.* 1991).

Ascorbic acid content

Initial ascorbic acid (AA) contents were 9.55 ± 0.54, 11.35 ± 0.68 and 9.52 ± 0.88 mg/100 g of fresh weight, in external, middle and internal zones, respectively. These values are in the order of that found by other authors. Roura *et al.* (2003) and Moreira *et al.* (2006) reported initial mean values for AA contents in Romaine lettuce leaves of 8.3 ± 1.0 and 9.4 ± 1.6 mg/100 g of fresh weight, respectively. In addition, Moreira *et al.* (2006) reported a great variability in the initial AA contents of fresh lettuce leaves (Romaine lettuce) ranging between 6.0 and 16.6 mg/100 g of fresh weight. Differences in initial AA content between zones could be clearly related to leaf age. In this way, external zone (composed by the oldest leaves of the plant) presented lower ascorbic acid content than middle zone. Respect inner leaves (the youngest tissue in the plant) the low content in AA could be attributed to differences in the maturity state of the leaves. Other authors also reported for other horticultural crops differences in AA content between unripe and ripe stages (Audisio *et al.* 1995; Lee *et al.* 2000; Roura *et al.* 2001).

Ascorbic acid is one of the most sensitive vitamins in

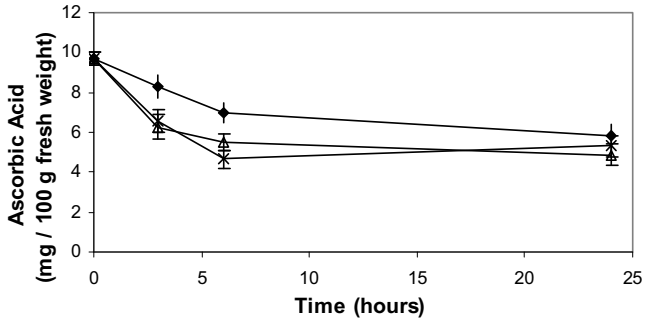

Fig. 3 LSMEANS for Ascorbic acid content evolution in the three zones together during 24 h of exposure to isothermal conditions: (△) 20°C; (×) 10°C; (♦) 0°C.

foods. Numerous factors affect its degradation, mainly temperature (Agüero *et al.* 2008c), salt and sugar concentrations (Davey *et al.* 2000), pH (Huelin *et al.* 1971), oxygen (Lee *et al.* 2000), enzymes (Fennema 1993), among others. ANOVA applied to AA data did not show any significant interaction between ZONE, TIME AND TEMPERATURE. TEMPERATURE and TIME factors resulted significant ($p = 0.0068$ and $p < 0.0001$, respectively), while ZONE factor was not significant, indicating that the three zones evolved similarly, without significant differences between them, i.e. at each temperature and at each sampling point, the three zones presented similar AA values. For this reason, the three zones were considered together as a pool of data to compare effect of temperature and time. **Fig. 3** shows the evolution of AA in the lettuce head (considering external, middle and internal zones together) during 24 h at each temperature. The highest AA contents corresponded to lettuces exposed to 0°C during the entire sampling period. The behavior of AA at 10 and 20°C did not present significant differences between them but they were lower than values at 0°C. The TIME factor analysis showed losses of about 50% in the first 6 h at 10 or 20°C, while 30% of losses at 0°C in this time. At the end of the 24 h of sampling, losses reached 40% (at 0°C) and 50% (at 10 and 20°C). Losses of AA at optimal temperature (0-2°C) could be associated to stress induced by harvest (Davey *et al.* 2000). Lee *et al.* 1982 reported losses of over 20% of ascorbic acid in peas in the immediate post-harvest period (1 h). Moreira *et al.* (2006) analyzing changes in ascorbic acid content in Romaine lettuce heads reported losses of about 30% at 24 h of storage and 18% for samples stored at 8 and 0°C, respectively. In the present research, losses of about 30% of AA content at 6 h were observed even though optimal temperature (0°C) was used. Tulio *et al.* (2002) analyzing the effects of storage temperatures on the postharvest quality of jute leaves, reported that the decreases in AA were low at temperature around 1°C but high at temperatures between 20 and 30°C. These facts are in agreement with results found in the present work. Some authors (Foyer *et al.* 1983; Izumi *et al.* 1997) have linked AA losses with chlorophyll degradation, because chloroplasts contain about 30-40% of the total AA within green vegetable cells. Hence, destruction of chloroplasts leads to AA losses. In the present research, mid and inner leaves showed decreases in AA in the first 24 h after harvest, but chlorophyll content remained constant in these leaves during this period therefore, these two quality parameters are not associated, and changes in AA may be due to causes such as temperature, harvesting stress, among others.

Sensorial quality

There are different quality components of lettuce, such as a fresh looking appearance, bright green color, crispness, and mainly absence of browning. It is generally accepted that storage time introduces some degradation in the appearance of lettuce heads, characterized mainly by loss of texture, discoloration extension and development of browning. However, little is known about the impact of abusive temperature conditions during the first hours after harvest on the sensorial quality of lettuce.

Table 3 Overall visual quality of lettuce head during the exposure to three different isothermal conditions (0-2°C, 10-12°C and 20-22°C).

Time (h)	Initial OVQ		
0	9 ± 0.1		
	OVQ		
	0-2°C	**10-12°C**	**20-22°C**
3	8.9 ± 0.1	8.9 ± 0.2	8.9 ± 0.1
6	8.9 ± 0.1	8.8 ± 0.1	8.7 ± 0.3
24	8.5 ± 0.5	8.1 ± 0.3	7.5 ± 0.6

Table 3 shows changes in overall visual quality of lettuce head during 24 h of the exposure to different temperatures. ANOVA applied to OVQ data yielded a significant interaction TEMPERATURE-TIME ($p < 0.01$). This fact implies that OVQ scores evolved differently as time advances during 24 h of exposition to different temperatures. In this way, panelists did not detect significant changes in OVQ of lettuce heads exposed to optimal temperature. When lettuce heads were exposed to abusive temperatures, panelists detected significant decreases in OVQ at 24 h of exposure ($p = 0.0021$ and $p < 0.0001$, respectively). Main differences were registered at 24 h in texture quality of lettuce heads exposed to 10 and 20°C. Panelists detected moderate losses of turgidity and texture. Another phenomenon detected by panelists was the degree of browning developed in the base of the lettuce due to the cut made during picking. At 24 h of exposure, lettuces at 10-12 and 20-22°C presented cut bases with moderate and objectionable browning to severe browning (rusty-brown), respectively. At optimal exposed temperature, only slight and not objectionable browning in the cut base was detected. After 24 h of storage the best quality attributes were observed in lettuces exposed to optimal temperature immediately after harvest.

CONCLUSION

Leaf age had a significant effect on the initial greenness and nutritional indices of butterhead lettuce. Older and senescent leaves (external zone) showed higher chlorophyll concentration and lower L^* (lightness parameter) and a^* values (redness/greenness parameter), than younger leaves characterized by clearest colour (high L^* and a^* values and low chlorophyll content). The distribution of ascorbic acid in the lettuce leaves at harvest showed a different pattern. The mid leaves were those that yielded the highest ascorbic acid content. Microbial count in lettuce at harvest was not affected by leaf age, showing the three lettuce zones, similar values of this parameter at harvest.

Detriment in butterhead lettuce initial quality begins in the first hours after harvest and it was affected by both temperature and leaf age. Important nutritional losses occurred even though lettuces were exposed to optimal temperature (0-2°C); however, losses were more important at higher abusive temperature. Leaf age did not affect the degradation of ascorbic acid and the three lettuce zones showed similar degradation pattern only affected by temperature. Chlorophyll losses were detected at 3 h of storage at the three exposure temperatures and were only evident in outer leaves, presumably as a physiological response of mature leaves to artificial senescence induced by harvest. Mid and inner leaves (younger than outer leaves) did not show any change in its chlorophyll contents during 24 h of exposure at different temperatures. From a bacteriological point of view, 24 h of exposure to both 10 and 20°C dangerously increased the number of microbial counts diminishing the microbiological quality of the product. Leaf age had a significant effect on microbial counts when temperature was 10 or 20°C, showing the older leaves the highest incre-

ments in microbial counts. In general terms, evolution of quality parameters was affected by temperature, time and degree of tissue development.

Maintaining the correct temperature from the first postharvest hours is an important factor in minimizing quality loss. The knowledge of the effect of leaf age on the evolution of quality indices is of fundamental importance for producers because they can take decisions based on this differential behavior related to the degree of development of tissue. In this way they could use different lettuce leaves for alternative uses as fresh consumption, minimally processed products, among others.

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