Article

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Freshly characterization and storability of mini head lettuces at optimal and abusive temperatures

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Abstract

Selection of lettuce varieties less sensitive to quality deterioration and more tolerant to abusive temperatures during handling, transportation, and storage is essential to minimize economical and quality losses that affect both producers and consumers. This work was focused on the quality changes of four baby head lettuces (*Lactuca sativa* L.), two butter (red and green) and two oak-leaf (red and green) types, during storage at 0 °C and 10 °C for 10 days. Lettuce quality was determined by measuring bioactive content (ascorbic acid, total phenolics), physicochemical (total chlorophyll, browning potential), and microbiological indices. At harvest, red varieties presented lower browning potential and higher bioactive compounds but no differences were observed in microbial populations. During storage, ascorbic acid underwent first order degradation for all varieties, with a degradation rate at 10 °C twice faster than at 0 °C. At 0 °C, only the red oak-leaf lettuce exhibited chlorophyll degradation, while at 10 °C all varieties presented degradation. No changes were observed in total phenolics and browning potential of butter lettuces during storage at both temperatures. Microbial population counts were significant affected by the storage temperature. Red butter baby lettuce presented slightly better bioactive content and microbiological characteristics and then better storability.

Keywords

Phenolics, chlorophylls, cold storage, food quality

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INTRODUCTION

Nowadays, lettuce is one of the most commonly consumed leafy vegetables and is a basic ingredient of many salads (Ferreres et al., 1997). Although iceberg and butterhead lettuces are predominantly used for prepared salads (Agüero et al., 2013; Martínez-Sánchez et al., 2012), consumer demand for softer leaves with variation in taste, shape, and color has encouraged the development of new lettuce products of baby size (Martínez-Sánchez et al., 2012). In this sense, restaurants and some grocery stores are highlighting the vast lettuce offerings.

In addition to pre-packaged salad choices, consumers have now the option of finding miniature lettuce heads packaged directly from the field into a simple package. These baby lettuces have higher percentage of usable product and are easier and faster to process than normal whole lettuce heads

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(Martínez-Sánchez et al., 2012). Moreover, baby heads offer the grower the ability to produce a marketable crop in less space, since their small size allows for denser spacing, and less time than full heads. Additionally, consumers feel that packaged mini lettuce heads can add variety and be a fresh alternative to processed salad mixes.

It is known that the shelf-life of the fresh produce is affected by the quality of the raw material, which needs to be excellent (Watada et al., 1996), and the regulation of temperature and relative humidity of air near the produce during the early postharvest (Agüero et al., 2013). Effective temperature management is, in fact, the simplest and most important procedure for delaying product deterioration (Nunes et al., 2004). Abusive storage temperatures cause undesirable changes in the sensory characteristics of fresh vegetables that impact on the visual quality and decrease the product acceptability. Added to that, inadequate temperature of storage affects the nutritional value, reducing mainly the ascorbic acid content, and favors microorganisms' proliferation to levels that may exceed tolerable limits (Francis et al., 1999). Although storage temperatures close to 0–2 °C are recommended, fresh vegetables may be prepared, shipped, and stored at 5 °C and, sometimes at temperatures as high as 10 °C (Watada et al., 1996). In this regard, the selection of lettuce varieties less sensitive to quality deterioration and more tolerant to abusive temperature during handling and supply chain processes is essential in order to minimize the deterioration that affects the quality of the fresh produce.

Although the demand for baby lettuce heads is on the increase, as consumers, chefs, and grocers take note of the excellent benefits in terms of convenience, freshness, flavor, and quality that this product offers, few researches were carried out about the behavior of these lettuce varieties under different conditions of storage temperature. The purpose of the present work was to analyze the changes in the bioactive content, and the microbiological and physicochemical quality in four varieties of baby lettuces kept at two different temperatures (0–2 °C and 8–10 °C) during 10 days.

MATERIALS AND METHODS

Plant material and sample preparation

Baby head lettuces, corresponding to mature heads of four genetically small lettuce varieties developed to produce miniature of *Lactuca sativa* L, two butter type: red and green ("Renoir RZ" and "Sartre RZ", respectively) and two oak-leaf type: red and green ("Krysthine RZ" and "Versai RZ", respectively) were studied. All baby lettuce varieties were grown and harvested in Sierra de los Padres, Mar del Plata, Argentine. Greenhouse lettuces were cultivated by applying "mulch" technology with a black plastic film separating each plant from the soil. The four lettuce varieties were exposed to the same light regimens, temperature (29/14 °C, day/night), and water irrigation (drip irrigation with an increase volume during the cultivation period from 4–8 L/m^2 to 8–20 L/m^2). Macro and micronutrients (N, P, K, Ca, Mg, among others) were supplied with irrigation water. One hundred and five lettuces from each variety were hand harvested 80 days after transplanting after reaching a marketable size (between 140 and 170 g, which corresponded to 19–20 leaves per head for oak-leaf types and 55–60 leaves per head for butter varieties). Once harvested, mini lettuce heads were immediately transported in a refrigerated container to the laboratory and fifteen whole plants of each variety were analyzed within 1 h after harvest; five were used for total phenolic and total chlorophyll contents quantification, five for reduced ascorbic acid and browning potential assessment and five for microbiological studies. These experimental data represented the zero time of storage in each of the quality indicators.

The other 90 plants of each variety were put in polyethylene bags (with an O_2 permeability 600 cm^3/(m^{2}d), CO_2 permeability 4000 cm^3/(m^{2}d), and water vapor permeability 4 g/(m^{2}d); $P = 101{,}325$ Pa, $T = 25$ °C), placing three plants from each variety per bag (28 × 55 cm^2, useful volume: 4 L). Bags were hermetically sealed and stored in two environmental chambers (SCT, Pharma, Argentina) at 0–2 °C and 8–10 °C, both at 97–99% of relative humidity, with 15 bags per variety at each temperature condition. Temperatures of 0–2 °C and 8–10 °C were chosen to simulate optimal and abusive refrigerated temperatures, respectively. Sampling was carried out at day 0, 2, 7, and 10 of storage. Oxygen and carbon dioxide concentrations within the bags were in the range 18.5–20.5% and 0.1–0.5%, respectively, so the film material was not a barrier to these gases. At each sampling time (except zero time), five bags from each variety were taken from each storage condition. From each bag, one plant was used to assess total phenolic and total chlorophyll contents, the second plant was used to analyze reduced ascorbic acid content and browning potential and the last plant was used for the microbiological studies. In this way for each variety of baby head lettuce, each quality index was valued by fivefold.

Two independent experimental runs were performed, separated from each other for 20 days.

Reduced ascorbic acid content

Reduced ascorbic acid content (AA) was determined following the titrimetric assay described by

Roura et al. (2001). Samples (20 g) from each lettuce variety and storage condition were extracted with 100 mL of metaphosphoric acid solution (60 g/kg) for 3 min using a commercial blender (Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) with a homogenizer speed of 3500 to 7000 r/min. The homogenate was made up to 250 mL with 30 g/kg metaphosphoric acid and filtered through Whatman N° 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0 °C. 10 mL of the filtrate were titrated with 2,6-dichloroindophenol. Ascorbic acid content was calculated as

$$AA = \frac{V_F \times V_T \times F}{Wt \times V_A} \times 100 \quad (1)$$

where AA is mg of ascorbic acid/100 g of fresh weight (FW), V_F is the volume of homogenate after filtration (mL), V_T is the average volume of indophenol reagent used in the titration of the sample (mL), F is mg ascorbic acid equivalents to 1.0 mL indophenol standard solution, Wt is the weight of sample (g), V_S the volume of sample titrated (mL) (Pelletier, 1985). Determinations were performed by triplicate.

Total chlorophyll content

Total chlorophyll (TC) in each lettuce variety and storage condition was determined according to Roura et al. (2001). Lettuce leaves 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 (0.1 mol/L). 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 Corporation, Japan). TC was calculated as: TC (mg/L) = $7.12 \times A_{660} + 16.8 \times A_{642.5}$, where TC is the total chlorophyll concentration (mg/L) and A_{660} and $A_{642.5}$ are the absorbances at the corresponding wavelengths. TC was expressed as mg chlorophyll/100 g of fresh weight (FW).

Total phenolic content

Total phenolic content (TPC) was determined spectrophotometrically using the Folin-Ciocalteu reagent (FCR) according to the methodology proposed by Viacava et al. (2014). Lettuce leaves from each variety and storage condition were processed individually in a commercial blender (Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) and 2 g were homogenized with 6 mL of ethanol during 3 h at 0 °C in an orbital shaker. The mixture was centrifuged at 10,000 r/min for 15 min. An extract sample (0.2 mL) was added to 1 mL of FCR (diluted 1/10). After 3 min of incubation at room temperature, 0.8 mL of 75 g/L Na_2CO_3 solution was added and the reaction mixture was incubated for 2 h at the same temperature. The absorbance was measured at 765 nm and total phenolic content was calculated using gallic acid (GA) as standard. Results were expressed as mg GA/100 g fresh weight (FW).

Browning potential

Overall browning potential (BP) was measured as the absorbance of an aqueous extract of lettuce leaves at 320 nm (Pereyra et al., 2005). Briefly, 1 g of leaf tissue from each lettuce variety and storage condition was homogenized with 20 mL of distilled water using a tissue homogenizer (Braun, Kronberg, Germany) with a speed of 3500–7000 r/min. The homogenate was filtered through Whatman N° 42 filter paper. The cloudy supernatant was centrifuged at 10,000 r/min for 15 min. The absorbance of a supernatant aliquot was measured with a spectrophotometer at 320 nm.

Microbiological studies

The enumeration and differentiation of microorganisms and particular microbial groups were performed using the following culture media and conditions: mesophilic aerobic bacteria on plate count agar (PCA) incubated at 35 °C for 24 to 48 h (ICMSF, 1983); psychrotrophic bacteria on the same medium incubated at 5 °C for 3 to 4 days (ICMSF, 1983; Mossel and Moreno García, 1985); *Enterobacteriaceae* in Mac Conkey agar incubated at 37 °C for 24 h. Molds and yeast were counted in yeast–glucose–chloranphenicol (YGC) medium incubated at 25 °C for 5 days (ICMSF, 1983). Microbial counts were performed in duplicate, at harvest and during storage at 0 and 10 °C. All culture mediums were from Britania, Buenos Aires, Argentina.

Statistical analysis

Statistical ANOVA ($p < 0.05$) analysis was performed using R, software version 2.12 (R Development Core Team, 2011). Results reported in this paper are LSMEAN values (least square mean, estimators of means by the method of least squares) together with their standard deviations (Kuehl, 2001). For each temperature of storage, the factors employed as sources of variation were DAY (storage time), VARIETY, and DAY–VARIETY interaction. Differences between varieties and days of storage were determined by the Tukey–Kramer multiple comparisons test ($p < 0.05$).

RESULTS AND DISCUSSION

Characterization of baby lettuces at harvest

Table 1 shows AA, TC, and TPC together with the BP index for the four varieties of baby head lettuces. The latter index gives an estimate of the browning susceptibility of the raw material. Significant differences ($p < 0.05$) in the content of AA were initially detected with regards to the color leaves. In agreement with results reported by Llorach et al. (2008), we found that both red-leafed varieties showed higher content of AA than green-leafed varieties. Nevertheless, AA content depends also of other factors, like different types of raw materials according to genotypes but not associated with the leaf color of the lettuce. In this sense, Martínez-Sánchez et al. (2012) found no differences in AA content between green and red baby-sized lettuces of types Green Leaf and Red Leaf. Moreira et al. (2006) reported a great variability in the initial AA content of fresh Romaine lettuce leaves (6.0 to 16.6 mg/100g FW), which could reflect the influence of various factors such as lettuce variety, weather conditions, cultural practices, maturity at harvest, harvesting method and postharvest handling conditions (Lee and Kader, 2000).

As shown in Table 1, significant differences ($p < 0.05$) in chlorophyll content at harvest among genotypes were obtained. Despite the visual appearance, green-leafed lettuces had significantly lower chlorophyll content than red-leafed ones. Anthocyanins were reported to be one of the primary phenols in red lettuce leaf tissue (Caldwell, 2003). It is possible that anthocyanins present only in the red cultivars of lettuce plants may mask the color of chlorophyll. These findings are in agreement with those of Ozgen and Sekerci (2011), who informed that red cultivars of lettuce exhibited higher chlorophyll content than green ones.

Total phenolic content (TPC) was significantly ($p < 0.05$) influenced by genotypes at harvest. The oak-leaf varieties (both red and green leaves) showed significantly higher phenolic content than butter varieties (Table 1). In this last baby head lettuce genotype, the red cultivar presented a higher TPC with respect to the green cultivar. It is generally recognized that genotypes of red-leafed lettuce generally have higher TPC than green-leafed ones (Liu et al., 2007; Llorach et al., 2008; Nicolle et al., 2004) which might be attributed to the higher anthocyanin content of the red-pigmented lettuces (Caldwell, 2003).

The control of browning from harvest to consumer is critical for minimizing product loss (Martin-Diana et al., 2005). Enzymatic browning generally results in loss of nutritional quality. Moreover, also organoleptic quality changes, such as darkening, softening, and off-flavor development, can take place. Browning potential of the green cultivars was significantly higher respect to the red cultivars (Table 1). Our findings are in concordance with those of Martínez-Sánchez et al. (2012), who informed that browning at the cut edge was significantly higher in green leaf than in red leaf baby lettuces. Degl'Innocenti et al. (2005) also reported that red lettuce varieties tend to be more resistant to browning symptoms. Although the degree of browning depends on phenolic content and polyphenol oxidase (PPO) activity (Zawistowski et al., 1991), it was not found a direct relationship between phenolics content and the susceptibility of the tissue to develop browning. Our hypothesis is that browning could be related to the presence of some specific polyphenols and not total phenolic content. In this sense, Mai and Glomb (2013) neither found a relationship between browning and total phenolic content in lettuce, and explained that Folin–Ciocalteu method was develop to assess all of phenolic compounds present in the sample, even those that are not substrates of PPO. Low browning potential

Table 1. Nutritional and quality indicators at harvest in four varieties of baby head lettuces ($n = 5$)

Variety	AA[A]	TCC[B]	TPC[C]	BP[D]
Red butter (cv. ''Renoir RZ'')	22.07 ± 2.57 a	30.60 ± 2.02 a	24.50 ± 2.13 b	0.37 ± 0.09ab
Green butter (cv. ''Sartre RZ'')	15.63 ± 1.00 b	20.81 ± 1.64 c	15.76 ± 1.63 c	0.46 ± 0.06 a
Red oak-leaf (cv. ''Krysthine RZ'')	21.75 ± 1.84 a	27.90 ± 0.61ab	30.59 ± 3.53 a	0.30 ± 0.09 b
Green oak-leaf (cv. ''Versai RZ'')	17.52 ± 0.74 b	24.70 ± 3.47bc	29.36 ± 0.96 a	0.50 ± 0.04 a

[A]Ascorbic acid content reported as mg AA/100 g FW ± standard deviation.
[B]Total chlorophyll content reported as mg chlorophyll/100 g FW ± standard deviation.
[C]Total phenolic content reported as mg GA/100 g FW ± standard deviation.
[D]Values are units of absorbance at 320 nm.
Mean values with different letter within the same column are significantly different ($p < 0.05$).

constitutes a specific requirement for the selection of lettuce varieties. In this sense, compared to the green varieties, the red varieties presented lower browning potential and higher concentration of bioactive compounds as ascorbic acid, chlorophyll, and phenolics.

With respect to microbiological counts, initially, no significant differences were observed between the four varieties in the different microbial populations. For mesophilic bacteria, counts were around 5.2 log. Martínez-Sánchez et al. (2012) reported lower initial mesophilic counts (3–4 log) for baby lettuce, both red and green leaves. The concurring variability associated to biological material, differences in lettuce varieties, climatic and soil conditions as well as good production practices in the field could be responsible for the differences in the initial counts of the unwashed raw material between researches. The same behavior was found for yeast and molds; while in the present study log counts around 4.5–5.5 log CFU/g were obtained, Martínez-Sánchez et al. (2012) reported values around 3–3.5 log CFU/g for the same microbial population. For enterobacteria population initial counts varied between 4.9 and 5.9 log CFU/g, while initial counts of psychrotrophic bacteria were around 2.3 log for the four lettuce varieties. Many factors can contribute to microbial contamination throughout production of fresh produce. These include contaminated irrigation, the use of manure for fertilization, and poor worker hygiene, among others (NSW, 2009).

Changes in baby head lettuces quality during the storage at 0 and 10 °C

Reduced ascorbic acid content. A significant decrease ($p < 0.05$) in the AA content of the leaves during storage at both optimal and abusive temperatures was observed regardless of the variety. For both temperature conditions, ANOVA applied to AA data did not show any significant interaction between VARIETY and DAY factors, indicating that the four baby head lettuces evolved similarly during storage. However, at each temperature analyzed, VARIETY and DAY factors were significant ($p < 0.05$) and then, the main effects of VARIETY and DAY factors were analyzed per separate.

With regards to DAY factor, AA degradation was modeled by first order kinetics for all situations (varieties and storage conditions), since the natural logarithm of the ratio between AA and initial value (AA_0) against time fell on a straight line. Using the AA data of all baby head lettuce varieties as a pool, AA degradation in the four varieties was modeled by $\mathrm{LN}(AA/AA_0) = -0.0473*t - 0.0143$ ($R^2 = 0.9819$, $n = 87$) for lettuces stored at optimal temperature, and by $\mathrm{LN}(AA/AA_0) = -0.0896*t + 0.0017$ ($R^2 = 0.9854$, $n = 84$) for lettuces stored at abusive temperature (Figure 1), where AA is the ascorbic acid content at t time and AA_0 is the initial ascorbic acid content. It was possible to observe that the slope of the straight tendency line for samples stored at 10 °C was significantly different ($p < 0.05$) from the slope of the tendency line for samples stored at 0 °C. Thus, ascorbic acid degradation rate in all varieties of baby head lettuce was almost twice faster when they were stored at 10 °C than at 0 °C. Moreira et al. (2006), analyzing the effects of storage temperature on the postharvest quality of lettuce leaves, reported that the ascorbic acid content declined gradually at 0 °C, with an increase in the degradation rate around 2.7 and 2.8 when the temperatures of storage were 8 °C and 15 °C, respectively. In contrast, Spinardi and Ferrante (2012) reported that ascorbic acid of baby leaf lettuce significantly decreased during storage at 4 °C and 10 °C, but the authors did not observed differences between the two storage temperatures.

In order to evaluate VARIETY factor, Table 2 presents the AA content of each baby head lettuce variety, calculated as an average through the storage for each temperature assayed. At both temperatures, green butter baby lettuce presented the lower ascorbic acid content during the entire sampling period. AA of oak-leaf (both red and green) and red butter lettuces were not statistically different among themselves, with a mean of 17.79 ± 3.79 mg AA/100 g FW at 0 °C, and 15.94 ± 5.60 mg AA/100 g FW at 10 °C.

Leafy vegetables lose ascorbic acid in postharvest operations and it has been attributed to temperature, water loss (Kader, 1999) and harvesting stress (Davey et al., 2000). Baby head lettuces were stored at the same relative humidity (96–98%) and changes in fresh weight were minimal (less of 1%), which would not suffice to explain the losses in ascorbic acid. Thus, the loss of ascorbic acid in the present study could be attributed to tissue structural changes due to biological deterioration factors (senescence and microbial activity as shown in Figure 5) and its role as scavenging agent, exacerbated in abusive temperature conditions (Smirnoff, 1996).

Total chlorophyll content. The storage of almost all vegetables brings about certain degradation of chlorophyll pigments. At 0 °C, only the red oak-leaf lettuce exhibited chlorophyll degradation, with losses of around 26% during the storage (Figure 2(a)). No changes in chlorophyll content were observed in the other three lettuce varieties during storage at optimal temperature. However, significant chlorophyll degradation was detected during storage at 10 °C in all lettuces except for the green oak-leaf variety (Figure 2(b)). This last variety showed the greatest stability in chlorophyll

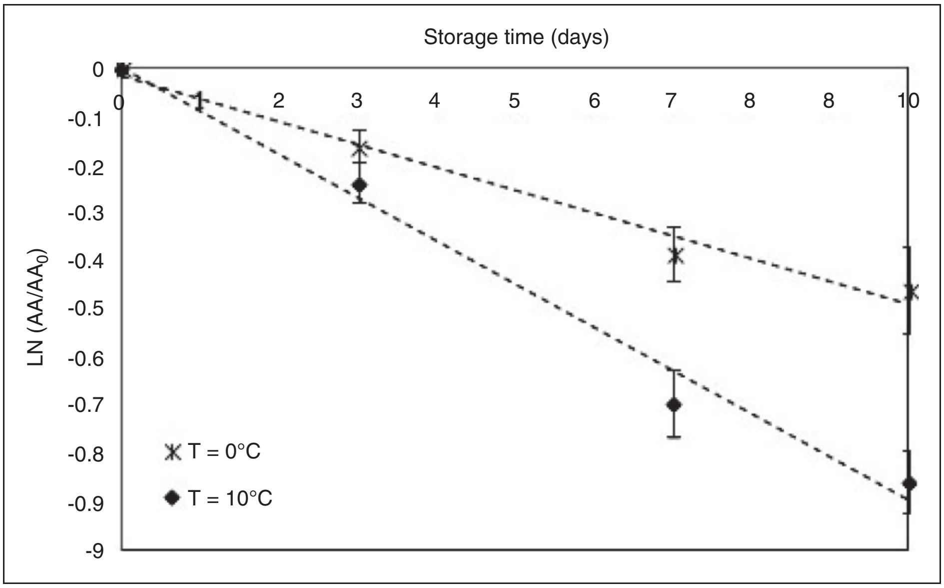

Figure 1. Ascorbic acid kinetic degradation in baby head lettuces stored at optimal and abusive tempratures. Lines for the pooled data of the four varieties of lettuce at each condition were LN $(AA/AA_0) = -\ 0.0473{*}t - 0.0143$, $R^2 = 0.9819$, $n = 87$ and LN $(AA/AA_0) = -\ 0.0896{*}t + 0.0017$, $R^2 = 0.9854$, $n = 84$, respectively.

Table 2. Ascorbic acid content in four varieties of baby head lettuce during optimal (0–2 °C) and abusive storage condition (8–10 °C) ($n = 5$)

Variety	Ascorbic acid content[A]	
	$T = 0$–2 °C	$T = 8$–10 °C
Red butter (cv. ''Renoir RZ'')	18.93 ± 3.23 a	17.14 ± 5.49 a
Green butter (cv. ''Sartre RZ'')	13.26 ± 3.05 b	12.07 ± 4.15 b
Red oak-leaf (cv. ''Krysthine RZ'')	18.10 ± 4.18 a	16.36 ± 5.97 a
Green oak-leaf (cv. ''Versai RZ'')	16.34 ± 3.98 a	14.31 ± 5.33 a

[A]Ascorbic acid content reported as mg AA/100 g FW ± standard deviation.
Mean values with different letter within the same column are significantly different ($p < 0.05$).

content among the others at both storage temperatures. Unlike, red oak-leaf showed the highest chlorophyll loss with a value of 12.72 ± 1.25 mg TC/100 g FW at the end of the storage period at 10 °C. This decrease represents a pigment loss of 53%, which is twice that found at 0 °C of storage temperature. On the other hand, green and red butter lettuces showed a loss of chlorophyll content of around 25% during storage at 10 °C, with final values of 15.72 ± 3.40 and 22.91 ± 1.81 mg TC/100 g FW, respectively. Ferrante and Maggiore (2007) reported chlorophyll losses of 22% and 35% during 15 days of storage of *Valeriana* lettuce leaves at 4 °C and 10 °C, respectively. Similarly, lower chlorophyll contents were measured in lettuce stored at 8 °C compared to 4 °C, with a greater reduction observed for chlorophyll *b* compared to chlorophyll *a* (Baldassarre et al., 2011). In contrast, Spinardi and Ferrante (2012) reported that total chlorophyll and carotenoids of baby leaf lettuce did not change during storage at 4 °C or 10 °C. Environmental factors such as light, temperature, humidity, oxygen, and ethylene (Watada et al., 1990; Yamauchi and Watada, 1991), and inner plant factors such as chlorophyllase and magnesium dechelactase activity (Jacob-Wilk et al., 1999; Shioi et al., 1996) are responsible for the loss of chlorophyll pigments during vegetable storage.

Total phenolic content. At both temperatures of storage, statistical analysis showed a significant interaction ($p < 0.05$) between factors considered in the analysis (VARIETY and DAY). This means that polyphenols in each baby head lettuce variety evolved differently during storage.

Total phenolic content in butter lettuces remained unchanged during storage at optimal or abusive temperatures (Figure 3(a) and (b)), with mean values of 24.94 ± 3.03 mg GA/100 g FW and 17.23 ± 2.40 mg GA/100 g FW (calculated as an average of temperatures and days of storage) for red and green cultivars, respectively. These results demonstrate the great stability of phenolics in these genotypes during storage. Despite its stability, green butter lettuce always

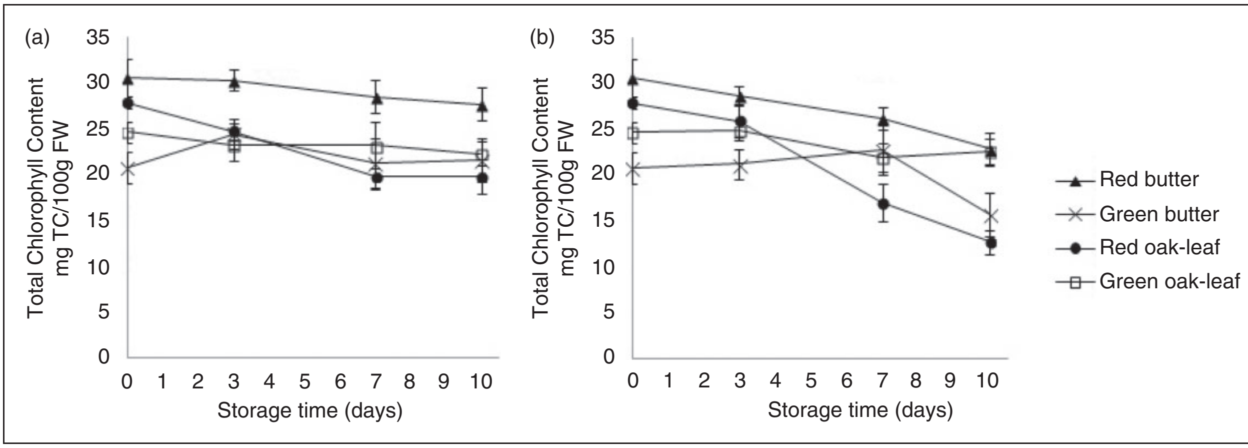

Figure 2. Total chlorophyll content evolution in four varieties of baby head lettuce during optimal (a) and abusive (b) storage condition. Values are mean ± s.d., $n = 5$.

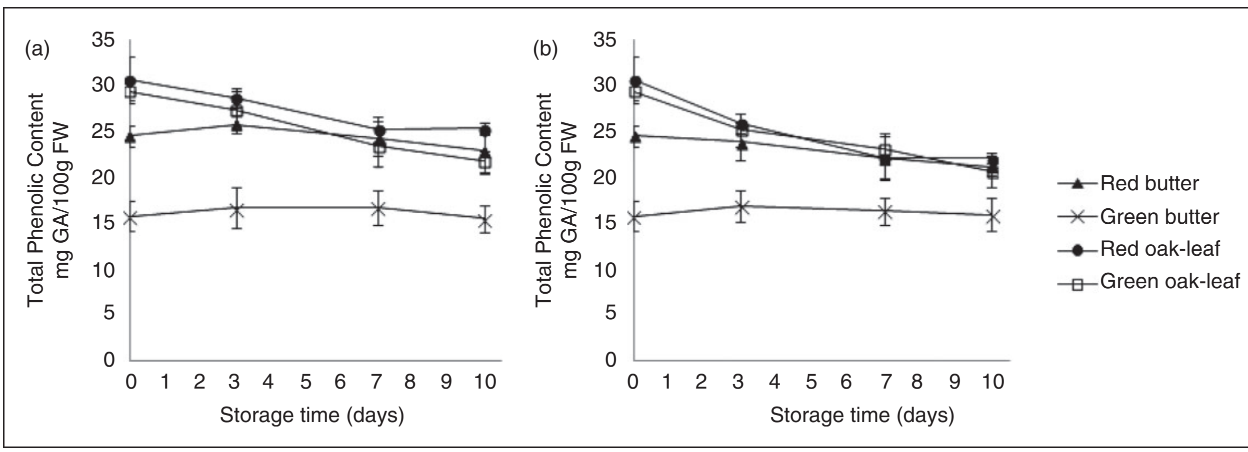

Figure 3. Total phenolic content evolution in four varieties of baby head lettuce during optimal (a) and abusive (b) storage condition. Values are mean ± s.d., $n = 5$.

presented lower total phenolic content than the other three lettuce varieties.

Green oak-leaf lettuce showed changes of total phenolics with respect to storage time but not with temperature. For this variety, total phenolics decreased rapidly during the entire storage period, with mean values of 29.36 ± 0.96 mg GA/100 g FW at day 0 and 21.16 ± 4.47 mg GA/100 g FW at day 10. In the case of red oak-leaf lettuce, total phenolic content did not statistically change when stored at 0 °C (mean value of 27.19 ± 6.54 mg GA/100 g FW), while a significant decrease after 10 days of storage at 10 °C was observed (30.59 ± 3.53 mg GA/100 g FW at day 0 and 22.07 ± 0.57 mg GA/100 g FW at day 10). Some authors also reported losses of phenolic compounds during the storage of lettuce (DuPont et al., 2000; Gil et al., 1998). In this sense, DuPont et al. (2000) reported losses of flavonol glycosides in whole heads of lettuces stored in icebank cooler (1 °C, relative humidity of 98%) for 7 days. These losses were of 16.3% and 34.1% for green oak-leaf and red oak-leaf lettuces. These authors associated flavonoid losses to demalonation of quercetin glycosides. Gil et al. (1998) also showed some losses in both malonated cyanidin and quercetin conjugates of lollo rosso stored in modified atmosphere packaging and suggested that demalonation led first to glucosides with further degradation to acetyl derivatives. Horowitz and Asent (1989) also stated that flavonoid malonylglycosides can lose carbon dioxide to give corresponding flavonoid acetylglycosides. Therefore, decreases of phenolics in the present investigation could be due to the hydrolysis of the malonyl group in flavonoid glycosides, compounds that are well known to be present in lettuce tissue (Llorach et al., 2008; Nicolle et al., 2004; Viacava et al., 2014). Additionally, it is widely known that a range of phenolics may be degraded by the enzyme PPO, especially after minimal processing, and several authors have studied the implication of this enzyme in lettuce (Ferreres et al., 1997; Friedman, 1996; Mai and Glomb, 2013; Zawistowski et al., 1991). Thus, although in the present study lettuces were not subjected to cutting procedures before storage, an enzymatic oxidation of phenolic compounds could also take place, and this could explain the decrease in the total phenolic content of green and red oak-leaf lettuces.

Phenolic compounds are generally stable during cold storage (<4 °C) of most leafy vegetables, but performance during storage is variable depending on variety and species. According to our results, it is possible to observe that genotype is the primary factor contributing to variation in phenolics content, as has been also suggested by other authors (Martínez-Sánchez et al., 2012).

Browning potential. Figure 4(a) and (b) illustrates the evolution of browning potential of baby head lettuces during storage at 0 and 10 °C, respectively. Through the ANOVA analysis of browning potential data, a significant interaction ($p < 0.05$) was found between factors (VARIETY and DAY) considered in the analysis for both storage temperature, meaning that each variety developed a typical behavior. However, this differential behavior was not influenced by the temperature of storage. At both storage temperatures, green and red butter varieties showed a slight decrease of browning potential during the first 7 days of storage, followed by an increase at the end of storage reaching final values similar than those found at harvest. Instead, the browning potential of green and red oak-leaf lettuces (at both storage temperatures) increased throughout the storage with final values 0.38 and 0.42 units of absorbance (average of both temperatures) higher than those observed at the beginning, respectively.

Results presented in this section evidenced a relationship between total phenolic content and browning potential, contrary to that mention for lettuces at harvest. While total phenolic content and browning potential did not change during storage in green and red butter lettuces, total phenolics and browning potential followed an opposite tendency in red and green oak-leaf stored varieties. In this sense, while total phenolics decreased in these last varieties during storage, browning potential increased. Browning of the lettuce tissue is caused by the action of the enzyme PPO, which catalyzes the oxidation of phenolic compounds to o-quinones. Then the o-quinones condense with other compounds and form brown polymers responsible of browning (Tomás-Barberán et al., 1997). Therefore, results found in this investigation could be related to an enzymatic oxidation of phenolic compounds with a consequent increase in the browning index (Friedman, 1996).

Microbiological indices. For both storage temperatures, mesophilic aerobic bacteria, psychrotrophic bacteria and enterobacteria counts (log CFU/g) of the four baby head lettuce varieties increased during storage (Figure 5(a) to (f)), without significant differences among varieties. While mesophilic bacteria and enterobacteria population gradually increased at both storage temperature, psychrotrophic bacteria underwent a sharply increase during the first 3 days of storage, followed by a stationary phase in the four baby head lettuce varieties (except the green butter lettuce, which showed a higher increase of psychrotrophic counts between the 7 and 10 days of storage at 10 °C). For mesophilic and phychrotrophic bacteria, the microbial counts were significantly higher at abusive temperature for the four lettuce varieties. However, in the case of

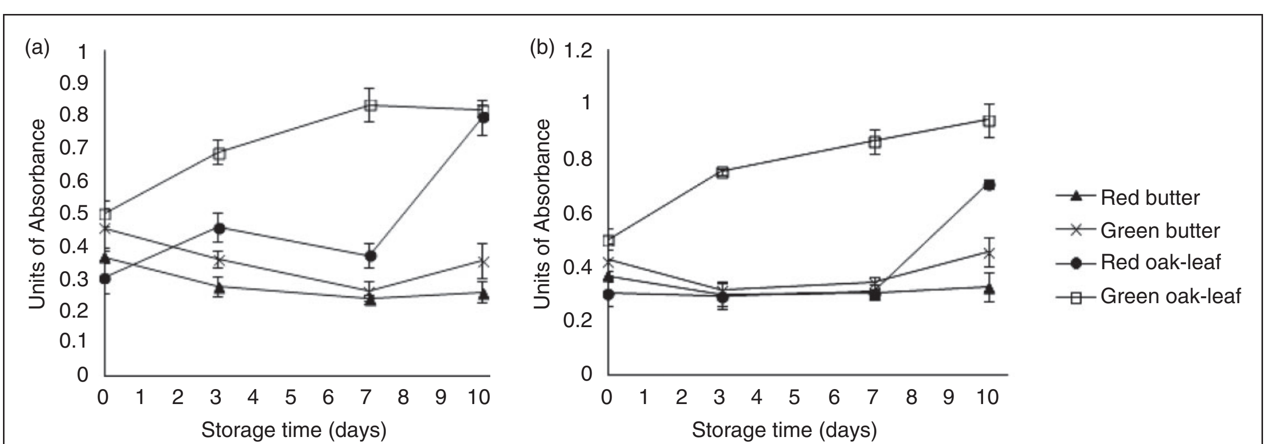

Figure 4. Browning potential content evolution in four varieties of baby head lettuce during optimal (a) and abusive (b) storage condition. Values are mean ± s.d., $n = 5$.

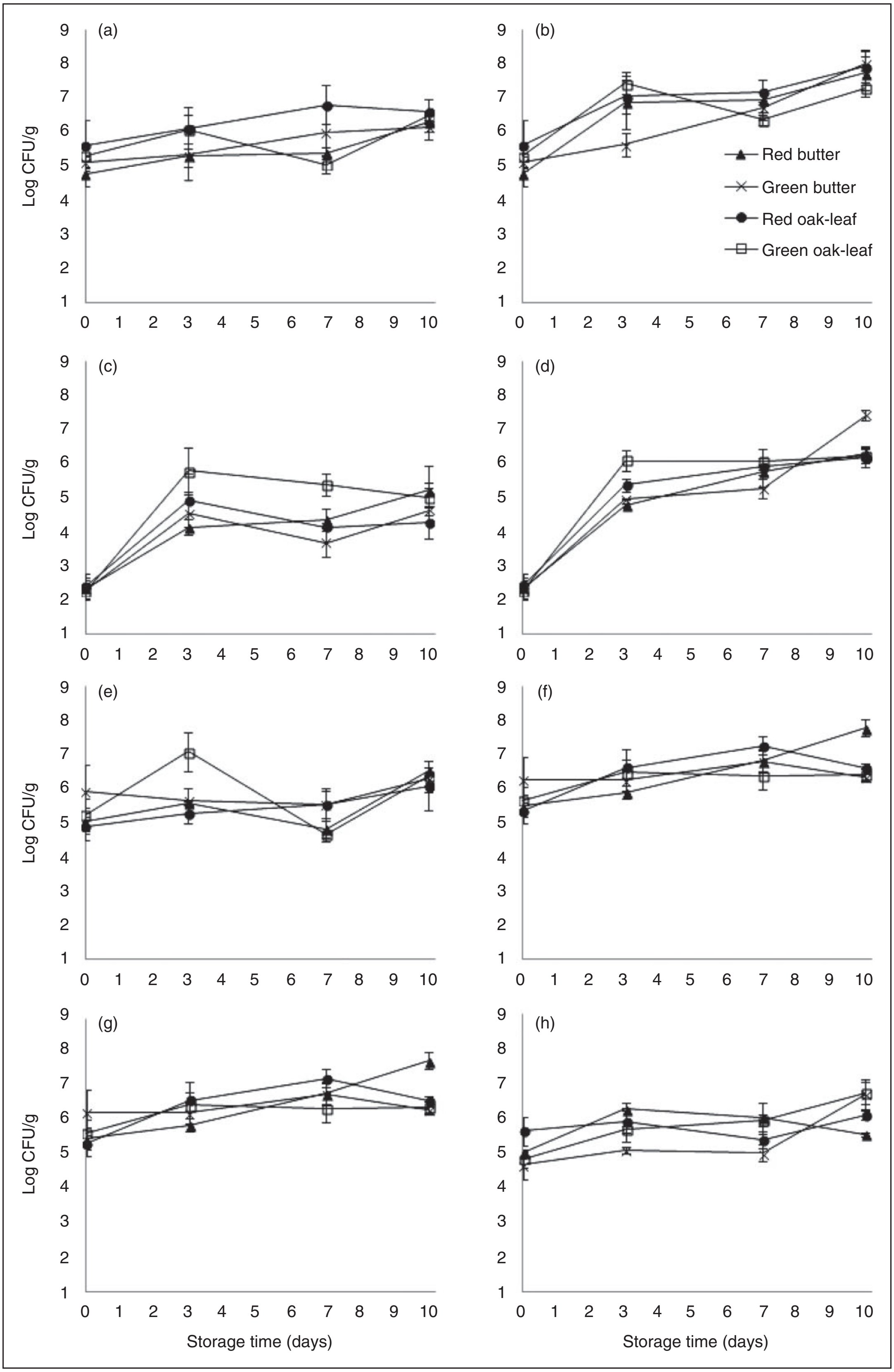

Figure 5. Microbial population counts in baby head lettuces stored at optimal (a, c, e, g) and abusive temperature (b, d, f, h). (a) and (b) mesophilic bacteria, (c) and (d) psychrotrophic bacteria, (e) and (f) enterobacteria, (g) and (h) yeast and molds. Values are mean ± s.d., $n = 5$.

enterobacteria population this difference was not observed, except for the red butter lettuce, which ended the storage period at 10 °C with counts 1.2 log higher than at 0 °C.

At optimal temperature, yeast and mold counts in the four lettuce varieties underwent a slightly decrease during the first 7 days of storage. Thereafter, yeast and mold counts increased, reaching values only 0.23–0.82 log higher than initial counts. Moreover, yeast and mold counts observed at 10 days of optimal storage temperature were in a similar range for the four lettuce varieties. On the other hand, yeast and mold counts in lettuces stored at abusive temperature showed different patterns in their evolution according to the variety analyzed (Figure 5(g) and (h)). Green oak-leaf and green butter lettuces showed an increase of about 2 log orders over the storage time. Instead, red oak-leaf and red butter lettuces kept the values constant of this microbial group during the storage at 10 °C (Figure 5(h)), ending the storage period with lower counts than the green varieties.

Martínez-Sánchez et al. (2012) also reported a similar tendency in mesophilic bacteria counts in samples of green and red baby-leaf lettuces stored during 16 days in passive modified atmosphere packaging. Although the authors utilized different storage conditions (4 °C during the first 3 days and 7 °C for the rest of the storage), they also observed a significant mesophilic bacteria increase. These authors also reported that yeast and mold counts of baby-leaf lettuces remained almost unchanged during the storage.

CONCLUSION

Analyzing the quality indicators for the freshly characterization, it could be concluded that both red-leafed varieties presented higher initial ascorbic acid and chlorophyll contents and lower browning potential. In fact, the red oak-leaf variety also presented higher total phenolic content.

Along storage, the bioactive content (ascorbic acid and total phenolic contents) and the physicochemical indices (total chlorophyll content and browning potential) were more appropriate to assess the effect of variety and storage temperature on baby lettuce quality retention, than the microbiological index. The results of the study demonstrated that the red butter baby lettuce presented higher bioactive content, better microbiological characteristics and greater stability in their quality indicators.

Leafy vegetables more resistant to temperature changes and less sensitive to enzymatic browning during storage ensure an extended shelf life. Additionally, produce that stores well will be more appealing to consumers than vegetables with visible quality decay. In this sense, the red butter baby lettuce showed good storability and could better accomplish both producer and consumer expectations in terms of convenience, freshness, and quality.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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