Early Postharvest Time Period Affects Quality of Butterhead Lettuce Packed in Crates

M. V. Agüero,1,2 G. E. Viacava,1,2 A. G. Ponce,1,2 and S. I. Roura1,2

1Grupo de Investigación en Ingeniería en Alimentos, Facultad de Ingeniería, Universidad Nacional de Mar del Plata, Mar del Plata, Argentina
2Consejo Nacional de Investigaciones Científicas y Técnicas - CONICET, Ciudad Autónoma de Buenos Aires, Argentina

The period between when butterhead lettuce (Lactuca sativa var. L) heads are harvested and stored in cold rooms can be up to 24 h. During this period, environmental conditions are uncontrolled, and heads could undergo deterioration that has not been previously quantified. Quality changes of lettuce heads packed in crates and exposed to optimal or suboptimal conditions during the 24 h after harvest were evaluated. The effect of placement within crates (lower, middle, and upper layer) was assessed. Proximity of heads within crates created a barrier to transfer of heat and humidity. This resulted in negative responses for plants under optimal conditions because there was a delay in temperature reduction and a deterioration in quality. At suboptimal conditions the response was positive because respiration and transpiration allowed development of a saturated environment around heads, delaying loss of quality. This effect was most obvious in heads from middle and lower layers, which presented the highest overall visual quality and water content. Use of crates caused extensive mechanical damage on heads, making it necessary to discard outer leaves, leading to 10 and 35% weight losses under optimal and suboptimal conditions, respectively. Initial quality rapidly decreases in the first day after harvest. It is necessary for technologies to be upgraded to reduce quality and economic losses.

**Keywords** Lactuca sativa, Argentina, Discards, Optimal conditions, Quality change, Suboptimal conditions, Weight loss.
Butterhead lettuce \((\textit{Lactuca sativa} \text{ var. Lores})\) is one of the most consumed leafy vegetables in the world. In many European countries and North America, vacuum cooling is the standard treatment used to maintain head quality (McDonald and Sun, 2000). This cooling method allows a quick removal of field heat by reducing tissue temperature from 25 to 1°C in 30 min. Beneficial effects of vacuum cooling on shelf life of iceberg lettuce \((\textit{Lactuca sativa} \text{ L.})\) have been studied (He and Li, 2008; Ozturk and Ozturk, 2009). However, this procedure does not constitute a common practice for butterhead lettuce in Argentina and other developing countries, where lettuce heads are packed in crates immediately after harvest and transported to distribution centers without any conditioning. The period between harvest and storage in a cold room is about 24 h, including time in the field, transportation, and delay at the distribution center. This period is known as “early postharvest,” and the importance of these first hours after harvest on vegetable shelf life is known (Agüero et al., 2011a; Moreira et al., 2006; Watada et al., 1996).

The environment surrounding fresh vegetables has a significant effect on shelf life of vegetables (Paull, 1999). Temperature (T) and relative humidity (RH) of air near the produce are main factors involved in postharvest preservation because they affect organoleptic quality (Nunes et al., 2009), which impacts consumer acceptability. The temperature effect on vegetable quality is well known (Paull, 1999). Temperature reduction is the simplest way to delay deterioration (through decreases in metabolic reaction rates) and retain fresh product appearance (Nunes et al., 2009; Paull, 1999). Relative humidity also directly impacts produce quality during storage (Nguyen et al., 2007). Water loss from vegetables is driven by the water gradient between the internal vegetable space and the surrounding air. Assuming that air inside the vegetable tissue is saturated (Paull, 1999), the water vapor pressure deficit (WVPD; the difference between the actual vapor pressure and the saturation vapor pressure; Hertog et al., 2004) could be used to quantify the water gradient. When produce is exposed to high WVPD, water and weight losses (WL), wilting, and other deleterious effects occur (Nguyen et al., 2007). Because water loss is a superficial phenomenon (Mahajan et al., 2008), it is a serious problem for lettuce, and for all leafy vegetables, because they have a high surface-to-volume ratio.

Although use of wooden crates, without precooling, is widespread and constitutes a general management practice between producers and distributors, there are few studies examining the impact of this type of handling on lettuce quality (Mondino et al., 2007). There is a worldwide tendency to revise the handling during commercialization of agri-food products (Ahumada and Villalobos, 2009). Quality changes occurring during distribution and sale of lettuce are potential areas for further research (Nunes et al., 2009).
The objective of the present work was to study the effects of exposure of lettuce heads packed in wooden crates during the first 24 h after harvest to environmental conditions on physiological, nutritional, microbiological, physicochemical, and sensory quality indices. The effect of placement of lettuce head in the crate was assessed. In the, effect of crate storage was compared to uncrated lettuce heads.

MATERIALS AND METHODS

Plant Material and Sample Preparation

Heads of butterhead lettuce were grown in greenhouses in Sierra de los Padres, Mar del Plata, Argentina. Lettuce heads were harvested after reaching a marketable size (≈24–30 leaves per head) and immediately transported to the laboratory, where eight units were analyzed in the first hour after harvest to evaluate initial lettuce quality status. Other lettuce heads were individually weighed and placed in wooden crates following the protocol used in commercial practice. A first layer of six heads (lower layer) was placed at the bottom of the crate with the cut end upwards. An additional six heads, forming the middle layer, were placed above the first one following the same orientation. Finally, nine heads, with the same previous orientation, formed the upper layer (Figure 1).

Two environmental chambers (SCT, Pharma, Buenos Aires, Argentina) were used to create the test conditions: 0–2°C and 95%–97% RH (a condition or optimal) and another was 20–22°C and 60%–62% RH (b condition or suboptimal). Temperature and relative humidity were recorded in crates at lower, middle, and upper layers to determine the evolution of these variables in the air around lettuce heads. Two data acquisition systems were used: Testostor 175-H1 (Testo, Buenos Aires, Argentina) and EasyLog (Lascar Electronics, Buenos Aires, Argentina). Two crates were exposed to optimal conditions, and two were used for suboptimal conditions during 24 h, the period designated as early postharvest. After this period, lettuce heads were removed from

![Figure 1](image-url): Schematic representation of crate assembly with (A) six lettuce heads in the lower layer, (B) six lettuce heads in the middle layer, and (C) nine lettuce heads in the upper layer.
crates and individually weighed. Lettuce heads from each layer were used to evaluate two simultaneous effects: environmental conditions during the early postharvest period (optimal or suboptimal) and placement of lettuce heads within crates.

An additional six heads were not crated and were exposed to each environmental condition to evaluate quality changes for comparison to crated heads. The experiment was replicated three times.

**Weight Loss**

Weight loss was determined in all lettuce heads after the early postharvest period. Each head was weighed after being harvested when it arrived at the laboratory \(W_0\) and then after 24 h \(W_{24}\). Weight loss (WL) of each individual head was calculated (Agüero et al., 2011c).

After \(W_{24}\) data were obtained, broken and/or deteriorated leaves were removed from lettuce heads and an additional weight was taken \(W_{24w}\), weight after waste disposal) and weight loss after discards were made \(WL_d\) was calculated considering this new weight.

The WL and \(WL_d\) were expressed as a percentage with respect to fresh initial weight. WL and \(WL_d\) mean values were calculated for each layer. Mean values were also calculated for individually exposed lettuce heads.

**Quality Indices**

Head lettuce quality was evaluated with the following indices: physiological (water status: water content [WC], relative water content [RWC], free water [FW], bound water [BW], and free water–to–total water ratio [FW/TW]); microbiological (mesophilic bacteria count, MBC); nutritional (reduced ascorbic acid content, AA); physicochemical (total chlorophyll content, TC), and sensory (overall visual quality, OVQ).

Three heads from each layer were used to evaluate physiological indices and three heads were assigned to nutritional, microbiological, physicochemical, and sensory indices. For uncrated exposed lettuce heads, the same number of units was used for determination of quality indices.

To determine plant water status, all leaves of each head were used as described by Viacava et al. (2011). Briefly, two rectangles \(15 \text{ cm}^2\) were cut from each leaf using a stainless steel cutter. The first rectangle, cut from the central apical area, was used for RWC and WC determinations. The other rectangle was cut 1 cm below the first, avoiding the midrib, and was used for FW, BW and FW/TW determinations.

For RWC and WC, each rectangle from each leaf was weighed individually to obtain fresh mass (FM). Each rectangle was then placed in a humidified chamber consisting of a 10-L plastic box containing 5 L of distilled water
and provided with a plastic hermetic cover to prevent moisture exchange. Rectangles were maintained for 20 h at 4°C in darkness. Next, rectangles were individually blotted with absorbent tissue paper and weighed to obtain turgid mass (TM). Finally, rectangles were dried for 24 h at 80°C in a forced air oven to determine dried mass (DM). Values of FM, TM, and DM were used to calculate RWC and WC (Viacava et al., 2011). After RWC and WC were determined, the percentage change with respect to values at harvest was calculated. RWC and WC are the percentage change after 24 h with respect to values at harvest.

The other rectangles obtained from leaves were weighed to obtain FM and placed in 15-mL Falcon tubes for freezing in liquid nitrogen. Next, the rectangles were thawed; the leaf tissue was spread out and air-dried on a table for 30 min, weighed to obtain air dry value (AD), oven-dried for 24 h at 80°C, and weighed (DM). Values of FM, AD, and DM were used to calculate TW, FW, and BW per unit of dried mass (Viacava et al., 2011).

Data were pooled and the mean value was obtained for each lettuce head and for each layer of the crate (lower, middle, and upper). The FW/TW values were calculated as the relation between these two indices. Once FW, BW, and FW/TW were determined, the percentage change with respect to values found at harvest was calculated. The indices are the percentage change after 24 h with respect to values found at harvest.

To evaluate other quality indices, three lettuce heads were assessed by a panel for organoleptic quality, and the heads were cut transversally in 2-cm portions and mixed, taking two samples from each lettuce head.

Total chlorophyll content (TC) was determined using the methodology of Moreira et al. (2005). Briefly, lettuce portions 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 homogenized with 19 mL of a cold 18:1 propanone : ammonium hydroxide solution (0.1 mol L⁻¹). The 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 read with a UV 1601 PC ultraviolet-visible spectrophotometer (Shimadzu Corporation, Tokyo, Japan). Total chlorophyll concentration (CC) was calculated as

\[
CC \text{ (mg} \cdot \text{L}^{-1}) = 7.12 \cdot A_{660} + 16.8 \cdot A_{642.5},
\]

where \(A_{660}\) and \(A_{642.5}\) are absorbances at the corresponding wavelengths. After TC was determined, the percentage change with respect to values found at harvest was calculated. TC is the percentage change after 24 h with respect to values found at harvest.

Reduced ascorbic acid content was determined following Roura et al. (2001). Briefly, 20 g lettuce portions were extracted with 100 mL metaphosphoric acid solution (60 g·kg⁻¹) for 3 min using a commercial blender.
Quality of Butterhead Lettuce

(Multiquick, MR 5550 CA Braun, Espanola S.A., Barcelona, Spain) with a 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 No. 42 filter paper. Temperature during ascorbic acid extraction was maintained at 0°C. Three aliquots (5 mL each) of filtrates were titrated with 2,6-dichloroindophenol. Ascorbic acid content was calculated as milligrams per 100 grams of fresh mass. After AA was determined, the percentage change with respect to values found at harvest was calculated. AA is the percentage change after 24 h with respect to values found at harvest.

MBC were evaluated with the methodology of Ponce et al. (2002). Ten grams of lettuce portions was macerated in a buffer solution (PO\(_4\)K\(_3\), pH 7.2). Enumeration of mesophilic bacteria was with plate count agar incubated at 32–35°C for 48–72 h. After MBC was determined, the value (\(N\)) was related to that found at harvest (\(N_0\)) and transformed using the log function; MBC is \(\log(N/N_0)\).

Overall visual quality was evaluated by a sensory panel following Agüero et al. (2011b). Quality parameters including color (shade and uniformity), brightness, crispness, wilting, bacterial decay, and physiological disorders (mainly midrib and edge browning of lettuce heads) were analyzed and scored with a 9-point scale: 9 = excellent, 7 = good, 5 = fair (accept limit), 3 = poor, and 1 = extremely poor. The average of the indices was used as an estimation of OVQ. Evaluations were carried out immediately after removal from storage. A panel including nine trained judges, aged 30–55 years, all members of the Food Engineering Group and with sensory evaluation experience in leafy vegetables, was responsible for sensory evaluations. Coded (three-digit) samples were randomly presented to judges, who made independent evaluations. Mean values obtained for each plant were used to calculate the mean value in crate layers.

Statistical Analysis

Means were separated with least squares mean (LSMEAN) together with standard deviations (Kuehl, 2001). Data were analyzed using SAS (ver. 9.0; SAS Institute). The General Linear Model procedure was used to carry out the analysis of variance (ANOVA).

A statistical model was used to evaluate the effects of environmental conditions to which heads were exposed and head placement within crates. Variation sources used as factors of the two-way ANOVA were COND (optimal or suboptimal), LAY (layer within the crate: lower, middle, or upper), and the COND × LAY interaction. A second statistical model was used to evaluate packaging effect (crate or none) and environmental conditions (optimal or suboptimal). For this purpose, a two-way ANOVA was applied with the factors PACK, COND, and PACK × COND. Data used for this analysis corresponded to mean
values of lettuce heads from the same crate and to the mean of uncrated heads. For each model, differences among results obtained for different factor levels were evaluated with the multiple comparisons Tukey-Kramer test. For both models, PROC UNIVARIATE was applied to validate ANOVA assumptions (Kuehl, 2001).

RESULTS AND DISCUSSION

Weight Loss

The first ANOVA (Table 1) yielded a significant interaction among LAY and COND. Regardless of placement within the crate and storage condition, crated lettuce heads had higher WL and WL_d values when exposed to the highest temperature and lowest relative humidity (b condition; Table 2). This could be attributed to the WVPD of each storage condition, the driving force for water evaporation. This parameter was calculated as a function of temperature and relative humidity (Guevara-Arauza et al., 2006) and was 73 times higher for the suboptimal condition (971.1 ± 74.6 Pa) than for the optimal situation (13.2 ± 7.7 Pa).

When crates were exposed to optimal conditions, heads in the lower layer had the highest WL_d. Weight of heads above them, as well as the pressure exerted during packing, could be responsible for the high WL_d. Use of wooden crates to transport lettuce from the field causes mechanical damage to heads. The amount of damaged tissue depends on head size and care in handling during packing. If lettuce heads are large, they will be tightly packed, with 21 heads per crate, likely resulting in increased mechanical damage.

Table 1: Analysis of variance for weight loss (WL%) and weight loss after waste disposal (WL_d%) occurring after exposure of lettuce heads packed in different layers in wooden crates to 0–2°C, 97%–99% RH or 20–22°C, 60%–62% RH for 24 h.

| Source of variation | df | Sum of squares | Mean squares | F       | p       |
|---------------------|----|----------------|--------------|---------|---------|
| WL                 |    |                |              |         |         |
| Cond               | 1  | 1057.8         | 1057.8       | 4,991,411 | <0.0001 |
| Layer              | 2  | 100.8          | 50.4         | 237,944  | <0.0001 |
| Cond × Layer       | 2  | 87.1           | 43.5         | 205,458  | <0.0001 |
| Error              | 246| 0.05           | 0.0002       |         |         |
| Total              | 251| 1245.8         |              |         |         |
| WL_d               |    |                |              |         |         |
| Cond               | 1  | 40,286         | 40286        | 1.9E8   | <0.0001 |
| Layer              | 2  | 576            | 288          | 1,359,012 | <0.0001 |
| Cond × Layer       | 2  | 180.2          | 90.1         | 425,249  | <0.0001 |
| Error              | 246| 0.05           | 0.0002       |         |         |
| Total              | 251| 41,042         |              |         |         |
Mondino et al. (2007), quantifying postharvest losses of lettuce in the Rosario, Argentina, region, found that 100% of producers used wooden crates with a packing content with upper layers exceeding the physical limit of the wooden crate (Figure 1). This generates increases in WL due to discarded leaves as crates are emptied.

Heads from the middle layer of crates, which were exposed to suboptimal conditions, had less WL than those from upper and lower layers. This could be related to the relative humidity generated in the wooden crate (Figure 2B). Although the WVPD was high outside the crate, head proximity within the crate generated increased relative humidity of the air around heads. The wooden crate and the upper layer of heads likely constituted a moisture barrier. Respiration and transpiration from heads generated humidity that remained in the internal parts of the crate. Lettuce heads placed in the middle layer had higher weights. Heads from the upper layer had a WL 62% higher than those from the middle one. After discarding waste, WLd values from different layers were more similar, with values in the upper layer being 13% higher than in the middle layer.

The second ANOVA (Table 3) yielded a significant interaction among PACK and COND. Despite being exposed to an environment with very low WVPD, the heads packed in crates and exposed to optimal storage conditions had WLs, whereas plants individually exposed to this condition did not (Table 4). Damage suffered by heads packed in crates led to higher WL and waste compared to heads individually exposed to these conditions. Temperature and relative humidity in the environment around packed lettuce were different from those in the environmental chamber. Changes occurred in environmental variables in different layers inside crates during 24 h after harvest (Figure 2). Lettuce heads from the upper layer reached the chamber temperature fastest; heads from the middle layer had the longer delay in reaching this temperature.

### Table 2: Interaction of storage condition and layer on weight loss (WL%) and weight loss after waste disposal (WLd%) in lettuce heads in layers in wooden crates exposed for 24 h to 0–2°C, 97%–99% RH (optimal) or 20–22°C, 60%–62% RH (suboptimal).

| Storage condition | Layer   | WL%  | WLd%  |
|-------------------|---------|------|-------|
| Optimal           | Upper   | 2.43 | 9.20  |
|                   | Middle  | 2.11ns | 7.72ns |
|                   | Lower   | 1.47* | 12.23* |
| Suboptimal        | Upper   | 7.09 | 36.35 |
|                   | Middle  | 4.37* | 32.28ns |
|                   | Lower   | 6.56* | 35.45ns |

ns, *Nonsignificant or significant at $P \leq 0.01$, least squares means analysis.
These delays could be responsible for higher WL detected in heads exposed to optimal conditions when crated than when not crated. High temperature increases tissue respiration and transpiration. Although relative humidity was high in the chamber, high temperature implies high WVPD, which favors water and WL. In contrast, WL and WL$_d$ of uncrated heads exposed to suboptimal conditions were higher than those for heads from crates exposed to the same condition, showing the protective effect of packaging (Table 4). Average weight losses (WL$_d$) of 34.69% for crates were mainly due to mechanical damage and incipient dehydration in upper lettuce heads, whereas the 38.76% WL$_d$ in uncrated heads was principally due to wilting and dehydration.

Literature on the WL of lettuce heads during storage is lacking. In most works, different combinations of temperature and relative humidity and different lettuce varieties were employed (Chandra et al., 2008; Martínez and
Table 3: Analysis of variance for weight loss (WL%) and weight loss after waste disposal (WLd%) produced after exposure of crated or uncrated lettuce heads to 0–2°C, 97%–99% RH (optimal) or 20–22°C, 60%–62% RH (suboptimal) for 24 h.

| Source of variation | df | Sum of squares | Mean squares | F       | p       |
|---------------------|----|----------------|--------------|---------|---------|
| WL                  |    |                |              |         |         |
| Cond                | 1  | 1361.7         | 1361.7       | 191,178 | <0.0001 |
| Pack                | 1  | 56.2           | 56.2         | 7897    | <0.0001 |
| Cond x Pack         | 1  | 176.7          | 176.7        | 24,815  | <0.0001 |
| Error               | 44 | 0.31           | 0.007        |         |         |
| Total               | 47 | 1595           |              |         |         |
| WLd                 |    |                |              |         |         |
| Cond                | 1  | 14,868.5       | 14868.5      | 1,380,196 | <0.0001 |
| Pack                | 1  | 69.8           | 69.8         | 6479.9  | <0.0001 |
| Cond x Pack         | 1  | 422.3          | 422.3        | 39,201.1 | <0.0001 |
| Error               | 44 | 0.47           | 0.01         |         |         |
| Total               | 47 | 15,361         |              |         |         |

Table 4: Interaction of storage condition and packaging on weight loss (WL%) and weight loss after waste disposal (WLd%) in crated and uncrated lettuce heads exposed for 24 h to 0–2°C, 97%–99% RH (optimal) or 20–22°C, 60%–62% RH (suboptimal).

| Storage condition | Packaging | WL%  | WLd%  |
|-------------------|-----------|------|-------|
| Optimal           | Crated    | 2.00 | 9.72  |
|                   | Uncrated  | 0.00*| 0.00* |
| Suboptimal        | Crated    | 6.01 | 34.69 |
|                   | Uncrated  | 12.92| 38.76ns |

ns, *Nonsignificant or significant at P < 0.01, least squares means analysis.

Artés, 1999; Martínez-Romero et al., 2008). Direct comparison with our results is difficult. Lettuce variety is a key factor to be considered because each variety has particular morphological properties. Iceberg lettuce has a high density, with small total surface exposed to the environment. Butterhead lettuce has a more open structure, with a greater exposed surface, favoring water and weight losses.

Water Status Indices

Water status indices (RWC, WC, FW, BW, and FW/TW) in crated and uncrated heads after 24 h were affected by treatment (Figure 3). RWC is the relation between the amount of water in tissue and the maximum amount of water that tissue can maintain (Agüero et al., 2011a). Head placement within the crate did affect RWC. Regardless of placement within the crate,
RWC decreased in all heads. However, RWC decreased $2.75\% \pm 0.11\%$ and $4.35\% \pm 0.40\%$ in heads under optimal and suboptimal conditions, respectively. Reductions in RWC are usually associated with storage environments with high WVPD. Decreases in RWC occurred in lettuce, kiwi (*Actinidia chinensis* Planch.), and rambutan (*Nephelium lappaceum* L., cv. Jitlee) fruit during storage under low relative humidity (Agüero et al., 2011a; Burdon and Clark, 2011; Landrigan et al., 1996). Reduction in RWC decrements can be due to reductions in leaf fresh weight and increases in turgid weight when tissue deterioration is high. When plant tissue is exposed to a saturated environment, damaged plant cells do not offer any resistance to water intake, resulting in a high turgid weight and a low RWC value (Agüero et al., 2008).

Analysis of RWC mean values for crated and uncrated heads showed that RWC increased ($4.70\% \pm 1.59\%$) in uncrated heads exposed to optimal
conditions, whereas a decrease (2.75% ± 0.11%) occurred in crated heads exposed to the same conditions. This RWC increase could be a response of lettuce tissue to high relative humidity and low temperature, with minimal or no differences in water vapor pressure between the head and the environment around it, causing cells to be saturated. Agüero et al. (2011a) found increases in RWC when butterhead lettuce was exposed to low WVPD. When suboptimal conditions were used, uncrated lettuce heads had higher RWC decreases (7.88% ± 1.97%) than crated heads (4.35% ± 0.40%). This fact was probably related to development of a more saturated atmosphere surrounding heads within crates (Figure 2B).

There were no changes in WC of crated lettuce exposed to optimal conditions for heads in layers. Lettuce heads from crates exposed to suboptimal conditions exhibited decreased WC with the largest decline in upper layer heads but no differences between middle and lower layers. There were no differences in WC of crated (Figure 3C) and uncrated heads (Figure 3D) when environmental conditions were optimal. Under suboptimal conditions, heads in the upper layer had the highest water loss. Other factors, in addition to a suboptimal environment, could be responsible for increased water loss in the upper layer. The most significant could be excessive mechanical damage generated by packing nine lettuce heads in the same layer. Water loss in vegetables depends on WVPD and is influenced by anatomical and morphological characteristics, tissue ripeness, and damaged surfaces, among others, and include temperature, relative humidity, and air movement (Ngure et al., 2009).

Crated lettuce heads (Figure 3E) and uncrated heads (Figure 3F) had decreased FW values at 24 h after harvest. No significant differences were observed in the FW behavior among layers in crates exposed to optimal conditions (mean decrease of 4.58% ± 0.66%). Heads from crates exposed to suboptimal conditions had FW decreases as a function of placement within the crate with FW declines of 12.92% ± 1.34%, 9.26% ± 0.97%, and 3.68% ± 1.57% for upper, middle, and lower layers, respectively. The FW reductions were higher in uncrated than in crated heads. Whereas a mean FW reduction of 8.62% ± 4.66% occurred in crated heads exposed to suboptimal conditions, a reduction of 51.8% ± 4.93% was detected in uncrated heads exposed to the same conditions. These differences may be attributed to protection generated by the packaging system allowing development of a saturated atmosphere between heads inside the crates (Figure 2B). Under optimal conditions, a smaller difference was detected between crated and uncrated lettuce heads, with decreases of 11.79% ± 1.84% and 4.58% ± 0.66%, respectively.

Increments in BW occurred in crated heads (Figure 3G) and uncrated heads (Figure 3H) exposed to the same conditions. Regardless of placement within crates, similar BW increases occurred at optimal conditions (mean increase of 7.36% ± 1.48%). Differences occurred among layers in crates.
exposed to suboptimal conditions (23.40% ± 0.65% for middle and lower layers without differences between them; and 12.78% ± 0.78% for the upper layer). Uncrated heads exposed to optimal and suboptimal conditions had higher BW increments than heads in crates (42.26% ± 1.70% and 20.68% ± 1.10%, respectively). In addition to the protective effect due to the packaging system, lower increases in crated heads under to suboptimal conditions could be due to the thermal profile that developed inside the crate (Figure 2A), with a gradual temperature reduction avoiding the rapid reduction that could increase retention of water (as BW).

The FW/TW decreases were detected in all lettuce heads whether crated or not, placement within the crate, and the storage condition (Figures 2I and 2J). Plants from crates under optimal conditions had a reduction in the FW/TW values of 4.61% ± 2.5%, and heads from crates exposed to suboptimal conditions had a decrease of 13.26% ± 3.3% without significant differences among layers. Uncrated heads had higher FW/TW decreases, and this effect was more important under suboptimal conditions with a loss of 38.8% ± 3.4% (Figure 3J).

Water movement took place in crated and uncrated heads and under both storage conditions. The FW decrease may be due to water loss from evaporation when heads were exposed to a high WVPD environment or as physiological responses to adverse environmental conditions or to harvest stress. Under these conditions, free water is bound within the cell, causing a change in tissue water status. Increases in BW and decreases in FW and FW/TW were detected and occurred in the tissue in a saturated atmosphere.

**Mesophilic Bacteria Counts**

The most abundant microbial population in leafy vegetables is the mesophilic microflora (Watada et al., 1996). Changes occurred in MBC in crated or uncrated heads under optimal and suboptimal conditions (Figures 3A and 3B). Heads from wooden crates exposed to optimal conditions had increments of 0.45 ± 0.15 log in the upper layer, and similar (1.10 ± 0.16 and 1.05 ± 0.20 log) values for heads in the middle and lower layers. Temperature within crates could be responsible for these differences. Heads in the upper layer had a more rapid temperature decline (6.9°C·h⁻¹) during the first 2 h; for middle and lower layers it was 3.5 and 5.6°C·h⁻¹, respectively (Figure 2A). Crated heads exposed to suboptimal conditions had MBC increments in all layers; higher ones were detected in the middle and lower layers (1.24 ± 0.20 log for both layers considered together) than in the upper layer (0.98 ± 0.14 log).

The comparison between crated and uncrated heads exposed to optimal and suboptimal conditions showed in differences in MBC. In general, MBC was higher in crated than in uncrated heads. The delay in temperature decrease in crated lettuce heads under optimal conditions (Figure 2A) favored mesophilic bacteria growth in the middle and lower layers. For crated heads
exposed to suboptimal conditions, high humidity and temperatures in crates (Figure 2B) represented a more favorable environment for mesophilic microorganisms compared to uncrated heads exposed to the same conditions. Fonseca (2006), studying the effects of environment humidity on microbial populations of lettuce at harvest, found that high relative humidity favored microorganism growth.

### Ascorbic Acid Content

Numerous factors (principally temperature, salt and sugar concentrations, pH, oxygen, enzymes, among others) affect degradation of AA (Davey et al., 2000; Lee and Kader, 2000). It has been demonstrated that AA losses occur in some horticultural products in short periods immediately after harvest (Moreira et al., 2006). These reductions could be attributed to harvest stress (Lee and Kader, 2000; Moreira et al., 2006).

Changes occurred in AA in crated and uncrated lettuce heads exposed to different environmental conditions (Figures 3C and 3D). Degradation of AA was not dependent on lettuce placement in crates; it was a function of environmental conditions to which the packed crate was exposed. In this way, heads from crates exposed to optimal conditions exhibited an average reduction of 9.93% ± 4.96%; for crated heads exposed to suboptimal conditions the decline was doubled (18.99% ± 4.05%). Higher temperature and higher WVPD, generated in suboptimal conditions, accelerated AA losses. The effect of temperature is well known (Kader, 2002): high temperature favors metabolic activity and AA losses. It is generally accepted that leafy vegetables lose vitamins, especially AA, during postharvest (Lee and Kader, 2000). However, when vegetables are subjected to unfavorable environmental conditions, it is often questioned whether temperature or water loss is responsible for these nutritional losses (Moreira et al., 2006; Nunes et al., 1998). Some argue that tissue water losses can increase AA losses due to oxidative processes (Kader, 2002; Paull, 1999).

A comparison of the results for crated heads (Figure 4C) and uncrated heads (Figure 4D) showed that those in crates had smaller AA losses. This effect was more prominent when environmental conditions were suboptimal. Uncrated heads had an AA reduction of 54.52% ± 2.80%; crated heads had a lower AA reduction (18.99% ± 0.93%). The high relative humidity generated inside crates (Figure 2B) allowed a higher AA retention than in uncrated heads. When the environment around lettuce heads had a high WVPD, nutritional losses increased, possibly due to tissue water loss.

### Chlorophyll Content

Leafy vegetables usually have reduced chlorophyll levels during storage. Chlorophyll degradation is an expression of senescence in lettuce (León et al., 2006).
Figure 4: Changes in quality indices (mesophilic bacteria count, chlorophyll content, ascorbic acid content, and OVQ) in lettuce heads placed in layers within wooden crates or uncrated heads stored for 24 h at (a) 0–2°C, 97%–99% RH or (b) 20–22°C, 60%–62% RH.

Chlorophyll changes in crated and uncrated heads exposed to optimal and suboptimal conditions occurred (Figures 3E and 3F). In crates exposed to optimal conditions, only heads from the upper layer had a slight chlorophyll content reduction (4.71% ± 2.12%); heads from middle and lower layers were not affected. Under suboptimal conditions, chlorophyll decreases occurred in heads from the middle and lower layers, which were similar (mean value of 6.85% ± 2.0%).

In general, more chlorophyll loss occurred in uncrated than in crated lettuce heads. The effect was more pronounced when conditions were suboptimal, possibly due to the protective effect inside crates. Chlorophyll loss in uncrated heads exposed to suboptimal conditions reached 50.52% ± 4.5%, whereas crated heads had chlorophyll retention of 90+% in all layers. Uncrated lettuce heads exposed to optimal conditions had retention of 87.50% ± 3.2% and retention was almost 100% in crated heads. The higher water movements in individual heads could be responsible for chlorophyll losses.

Overall Visual Quality

Lettuce sensory quality is made up of different components, including fresh appearance, color, texture, and lack of browning, among others. It is accepted
that storage time and environmental conditions introduce certain degradation in lettuce appearance, mainly characterized by texture loss, discoloration, and browning (Kader, 2002). The impact of real commercial conditions during early postharvest impacted the OVQ of lettuce heads in crate layers and uncrated heads (Figures 3G and 3H). When conditions were optimal for lettuce handling, there was an OVQ reduction of 0.7 ± 0.1 points and no differences between heads from different layers crates. Under suboptimal conditions, differences were found in OVQ from different layers, with higher OVQ decreases in heads from the upper layer (1.7 ± 0.1), followed by those from the lower layer (1.4 ± 0.1). Heads in the middle layer had the smallest OVQ loss (1.0 ± 0.2). Two factors could be responsible for these results. First, mechanical damage in heads due to packaging was higher in the lower layer due to compression from middle and upper layers and in heads from the upper layer due to lateral compression due to placement of nine plants in this layer. The other factor is relative humidity in the air space around heads. Only lettuce plants from the upper layer were exposed to an unsaturated atmosphere (Figure 2B). Lettuce heads from middle and lower layers were exposed to a more saturated environment within the crate.

When the OVQ between crated heads (Figure 4G) and uncrated heads (Figure 4H) was compared to that in crates exposed to optimal conditions had a slightly higher decrease (0.7 ± 0.1) in OVQ than uncrated heads (0.3 ± 0.1). This could be attributed to mechanical damage generated during packaging as well as temperature inside the crate delaying achievement of the optimal temperature range (Figure 2A). When environmental conditions were suboptimal, the effect was opposite, with uncrated heads having a higher OVQ reduction (2.2 ± 0.3) compared to crated heads (1.4 ± 0.2). A more saturated atmosphere inside the crate (Figure 2B), which likely prevented water loss in tissues, could be responsible for this effect. For uncrated lettuce the OVQ reduction was due to wilting caused by dehydration (high WVPD of the environment), whereas in crated heads, the OVQ decrease was associated with mechanical damage of tissues.

The system widely used in commercial butterhead lettuce (wooden crates) handling produces a different response in tissues from that in uncrated heads due to environmental conditions that developed inside the crate and to the physical damage caused by the tightly packed heads.

When environmental conditions are the best that are recommended for butterhead lettuce (0–2°C, 97%–99% RH) the temperature profile that develops inside crates slows down heat transfer. The recommended practice for butterhead lettuce, widely used in many European countries, is temperature reduction by vacuum cooling before storage under optimal conditions. Use of maintenance chambers to achieve the temperature reduction is inefficient compared to vacuum cooling. The cold temperature is achieved between 5 to 16 h depending on placement of product within crates, and this cooling delay
negatively impacts quality indices compared to uncrated heads. In addition to the temperature effect, the wooden crate system causes mechanical damage in the tissue due to pressure exerted on lettuce heads placed in wooden crates. When conditions are the best that are recommended for the handling of butterhead lettuce respiration heat, and head placement inside crates generate a delay in lowering the temperature with a direct effect in lettuce quality indices.

When the environmental conditions are suboptimal, respiration and transpiration of lettuce in crates permits development of a saturated atmosphere. Proximity of heads constitutes a physical barrier against low relative humidity. However, crated lettuce heads exposed to suboptimal conditions had greater quality losses than crated heads exposed to optimal conditions.

It is important to highlight the economic losses that take place during the first 24 h after harvest. WL results in economic loss. There is a need to improve and optimize handling of fresh produce during marketing. Upgraded technology should be implemented to maintain high produce quality achieved by producers as long as possible and ensure delivery of high-quality products to consumers.

REFERENCES

Agüero, M.V., M.V. Barg, A. Yommi, A. Camelo, and S.I. Roura. 2008. Postharvest changes in water status and chlorophyll content of lettuce (*Lactuca sativa* L.) and their relationship with overall visual quality. J. Food Sci. 73(1):47–55.

Agüero, M.V., M.R. Moreira, G. Goñi, and S.I. Roura. 2011a. Abusive isothermal conditions during first hours after harvest affect butterhead lettuce water status. J. Food Process. Preserv. 35(4):501–508.

Agüero, M.V., A.G. Ponce, A. Bevilacqua, and S.I. Roura. 2011b. Postharvest quality losses of butter lettuce as affected by leaf age and temperature. Fresh Produce 5(1):20–25.

Agüero, M.V., A.G. Ponce, M.R. Moreira, and S.I. Roura. 2011c. Lettuce quality loss under conditions that favor the wilting phenomenon. Posthar. Biol. Technol. 59:124–131.

Ahumada, O. and J.R. Villalobos. 2009. Application of planning models in the agri-food supply chain: A review. Eur. J. Oper. Res. 195:1–20.

Burdon, J. and C. Clark. 2001. Effect of postharvest water loss on ‘Hayward’ kiwifruit water status. Postharvest Biol. Technol. 22:215–225.

Chandra, D., T. Matsui, H. Suzuki, and Y. Kosugi. 2008. Changes in some physio-biochemical characteristics in lettuce during storage at low temperature. J. Biol. Sci. 8(2):398–403.

Davey, M.W., M. Van Montagu, D. Inzé, M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J. Benzie, J.J. Strain, D. Favell, and J. Fletcher. 2000. Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agr. 80:825–860.
Ferrante, A., L. Incroci, R. Maggini, G. Serra, and F. Tognoni. 2004. Colour changes of fresh-cut leafy vegetables during storage. J. Food Agr. Environ. 2(3/4):1371–1374.

Fonseca, J.M. 2006. Postharvest quality and microbial population of head lettuce as affected by moisture at harvest. J. Food Sci. 71(2):M45–M49.

Guevara-Arauza, J.C., E.M. Yahia, L. Cedeño, and L.M.M. Tijskens. 2006. Modeling the effects of temperature and relative humidity on gas exchange of prickly pear cactus (Opuntia spp.) stems. Lebensm. Wiss. Technol. 39:796–805.

He, S.Y. and Y.F. Li. 2008. Experimental study and process parameters analysis on the vacuum cooling of iceberg lettuce. Energy Conserv. Mgt. 49:2720–2726.

Hertog, M.L.A.T.M., R. Ben-Arie, E. Róth, and B.M. Nicolai. 2004. Humidity and temperature effects on invasive and non-invasive firmness measures. Postharvest Biol. Technol. 22:79–91.

Kader, A.A. 2002. Postharvest biology and technology: An overview, p. 39–47. In: A.A. Kader (ed.). Postharvest technology of horticultural crops. Univ. of California, Agriculture and Natural Resources, Davis, Calif.

Koukounaras, A., A.S. Siomos, and E. Sfakiotakis. 2006. 1-Methylcyclopropene prevents ethylene induced yellowing of rocket leaves. Postharvest Biol. Technol. 41:109–111.

Kuehl, R.O. 2001. Diseño de experimentos. [Design of experiments.] 2nd ed. Thompson Learning International, México.

Landrigan, M., S.C. Morris, D. Eamus, and W.B. McGlasson. 1996. Postharvest water relationships and tissue browning of rambutan fruit. Sci. Hort. 66:201–208.

Lee, S.K. and A.A. Kader. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. 20(3):207–220.

León, A., D. Frezza, and A. Chiesa. 2007. Evolución del color en lechuga (Lactuca sativa L.) mantecosa mínimamente procesada: Efecto del troceado y la inmersión en cloruro de calcio. V Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones [Evolution of color in minimally processed butterhead lettuce (Lactuca sativa L.): Effect of cut and immersion in calcium chloride]. Proc. V Iberoamerican Congress of Postharvest Technology and Agroexports 5:666–675.

Mahajan, P.V., F.A.R. Oliveira, and I. Macedo. 2008. Effect of temperature and humidity on the transpiration rate of the whole mushrooms. J. Food Eng. 84:281–288.

Martínez, J.A. and F. Artes. 1999. Effect of packaging treatments and vacuum-cooling on quality of winter harvested iceberg lettuce. Food Res. Intl. 32:621–627.

Martínez-Romero, D., M. Serrano, G. Bailén, F. Guillén, P.J. Zapata, J.M. Valverde, S. Castillo, M. Fuentes, and D. Valero. 2008. The use of natural fungicide as an alternative to preharvest synthetic fungicide treatments to control lettuce deterioration during postharvest storage. Postharvest Biol. Technol. 47:54–60.

McDonald, K. and D.W. Sun. 2000. Vacuum cooling technology for the food processing industry: A review. J. Food Eng. 45:55–65.

Mondino, M.C., J. Ferratto, I. Firpo, R. Rotondo, M. Ortiz Mackinson, R. Grasso, P. Calani, and A. Longo. 2007. Pérdidas poscosecha de lechuga en la región de Rosario, Argentina. [Postharvest losses of lettuce in the region of Rosario, Argentina.] Hort. Argentina 26(60):17–24.
Moreira, M.R., A.G. Ponce, C.E. del Valle, R. Ansorena, and S.I. Roura. 2006. Effects of abusive temperatures on the postharvest quality of lettuce leaves: ascorbic acid loss and microbial growth. J. Appl. Hort. 8(2):109–113.

Moreira, M.R., A.G. Ponce, C.E. del Valle, and S.I. Roura. 2005. Inhibitory parameters of essential oils to reduce a foodborne pathogen. Lebensm. Wiss. Technol. 38(5):565–570.

Ngure, J.W., J.N. Aguyoh, and L. Gaoquiong. 2009. Interactive effects of packaging and storage temperatures on the shelf-life of okra. J. Agr. Biol. Sci. 4(3):44–49.

Nguyen, T.A., P. Verboven, A. Schenk, and B.M. Nicolaï. 2007. Prediction of water loss from pears (Pyrus communis cv. Conference) during controlled atmosphere storage as affected by relative humidity. J. Food Eng. 83:149–155.

Nunes, M.C.N., J.K. Brecht, A. Morais, and S.A. Sargent. 1998. Controlling temperature and water loss to maintain ascorbic acid levels in strawberries during postharvest handling. J. Food Sci. 63:1033–1036.

Nunes, M.C.N., J.P. Emond, M. Rauth, S. Dea, and K.V. Chau. 2009. Environmental conditions encountered during typical consumer retail display affect fruit and vegetable quality and waste. Postharvest Biol. Technol. 51:232–241.

Ozturk, H.M. and H.K. Ozturk. 2009. Effect of pressure on the vacuum cooling of iceberg lettuce. Intl. J. Refrig. 32:402–410.

Paull, R.E. 1999. Effect of temperature and relative humidity on fresh commodity quality. Postharvest Biol. Technol. 15:263–277.

Ponce, A., S. Roura, C. del Valle, and R. Fritz. 2002. Characterization of native microbial population of Swiss chard (Beta vulgaris, type ciela). Lebensm. Wiss. Technol. 35:331–337.

Roura, S.I., M.R. Moreira, G. Crapiste, and C.E. del Valle. 2001. Biochemical characterization of two pepper varieties in green and red maturation stages. Italian J. Food Sci. 4(13):391–397.

Viacava, G.E., S.I. Roura, and M.V. Agüero. 2011. Improvement of water status methodology for leafy vegetables reduces time consuming, skilled labor and laboratory resources. Food Anal. Methods 4(3):307–312.

Watada, A.E., N.K. Ko, and D.A. Minott. 1996. Factors affecting quality of fresh-cut horticultural products. Postharvest Biol. Technol. 9:115–125.