Postharvest Changes in Water Status and Chlorophyll Content of Lettuce (*Lactuca Sativa* L.) and their Relationship with Overall Visual Quality

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ABSTRACT: The purpose of this study was to evaluate water status, chlorophyll content (C), and overall visual quality (OVQ) of fresh butter lettuce (*Lactuca sativa* var. Lores) as well as these indexes' evolution during storage and their relationships, if any. Whole lettuce plants were stored at optimal postharvest conditions (0 to 2 °C and 97% to 99% relative humidity). Measured parameters during each sampling day were relative water content (RWC), water content (WC), free water (FW), bound water (BW), free water to total water ratio (FW/TW), C, and OVQ. All parameters were evaluated in the external, middle, and internal zones of lettuce heads. The external zone had higher initial values of RWC, WC, and FW than the internal zone. The external zone yielded the highest FW/TW ratio (85%), indicating that external leaves had more water available to be used in degradation reactions and were more perishable, with the lowest shelf life if compared with the other lettuce zones. During storage, water status index evolution differed from zone to zone. An increase in BW and a decrease in FW were detected in all lettuce zones. RWC turned out to be a more sensitive measurement than WC. Yet RWC showed no significant correlation with any index. The OVQ parameter correlates with FW directly, or indirectly through FW/TW in all lettuce zones; therefore, FW is an objective and quantitative measurement, which impacts on the visual quality of butter lettuce. The decrease in chlorophyll content observed in the external leaves strongly correlated with the decrease in OVQ.

Keywords: butter lettuce, chlorophyll degradation, overall visual quality, shelf life, water status

Introduction

Lettuce (*Lactuca sativa* L.) is one of the most popular green salad vegetables in the world. Lettuce consumption contributes fiber, vitamin A, vitamin C, vitamin E, carotenoids, calcium, potassium, and magnesium to consumers' diet (Nicolle and others 2004). Even though its nutrient value per unit is relatively low, the per capita consumption in Argentina is high, around 20 kg/y (www.mercadocentral.com.ar), and it is currently growing (Esparza-Rivera and others 2006).

Lettuce is a highly perishable vegetable whose quality and shelf life are limited by dehydration. This process brings about a decrease in turgidity pressure in the cells as well as cellular wall degradation (Alzamora and others 2000; Abbot and Harker 2004), which affect quality attributes such as texture, turgidity, and color, producing detrimental texture changes and enzymatic browning during postharvest storage. It is important for leaf lettuce to remain well hydrated in order to maintain its sensory attributes like texture and appearance over long storage. Furthermore, the relevance of water in horticultural crops is particularly great considering that most horticultural produce is sold by weight, and water being its major component, there lies an economical interest behind keeping vegetables well hydrated (Jones and Tardieu 1998). The control of water loss from harvest to consumers is, as a consequence, critical to minimize quality loss and avoid loss to producers.

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Inadequate humidity during storage or sale is a climatic stress that reduces tissue WC (Herppich and others 1999). One of the main features of lettuce quality is its high WC, and so tissue water status measurements are essential. Moisture is lost when vegetables are stored below optimum humidity levels, generally 95% to 98% RH. The process is desorption from the surface of the vegetable, followed by moisture migration from the interior, more surface desorption, and so on, until the vegetable is thoroughly desiccated. Additionally, the study of the internal water status may allow us to know water movements and the true status of water at different storage stages in response to environmental conditions.

RWC, a common measure of plant water status in tissues, is a fraction of the amount of water held at full turgidity. Other water measurements are leaf WC, a useful indicator of plant water balance, which expresses the relative amount of water in the plant tissues, and FW, BW, and total water and the relationship between them such as FW/TW (Singh and others 2006).

Research on water availability and plant water status has generally focused on prestorage adaptive strategies of cultivars under contrasting water availability regimens (Jones and Tardieu 1998; Yamasaki and Rebello Dillenburg 1999; Diallo and others 2001; Rodríguez and others 2004; Bacelar and others 2006; Bai and others 2006; Eitel and others 2006; Romero and Botía 2006; Martínez and others 2007). However, few studies have dealt with water status under postharvest conditions and on how this influences quality parameters. Landrigan and others (1996) investigated postharvest water relationships and tissue browning of rambutan fruit. These authors found that development of browning was preceded by water loss and concomitant declines in water potential of spinterns

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and skin. Herppich and others (1999), using a "Scholander"-type pressure bomb, analyzed the effects of postharvest mechanical and climatic stress on carrot tissue water relations in order to characterize the stresses during the different steps of the whole chain. Burdon and Clark (2001) examined the effect of postharvest water loss on "Hayward" kiwifruit water status over 14 d after harvest while being subjected to dehydrating conditions. The decrease in fruit fresh weight was accompanied by decreased WC, RWC, and water potential (quantifies the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or capillary action) (Burdon and Clark 2001). Esparza-Rivera and others (2006) studied the evolution of RWC index in lettuces pretreated with ascorbic acid and stored at 5 °C. Initial RWC of "Waldmann's" leaf lettuce ranged from 94.07% to 99.79% and declined at day 14 and 21 in nontreated with ascorbic acid samples. Ascorbic acid immersed lettuce maintained its RWC for 21 d (Esparza-Rivera and others 2006).

The effect of storage conditions has been widely assessed for leafy vegetables. Physical and chemical attributes of leafy vegetables have been used as quality indicators. Among them, retention of green color, followed by chlorophyll content determination, is one of the most generally used indexes to characterize green vegetable quality.

The purposes of this study were (1) to evaluate the water status of freshly harvested greenhouse butter lettuce together with its evolution during cold storage, (2) to describe the effects of storage on quality parameters as chlorophyll content and OVQ, and (3) to describe the relationships, if any, between water status and chlorophyll content with OVQ.

Whole plants were stored under optimal postharvest conditions (0 to 2 °C and 97% to 99% RH) in order to obtain a water status base line for butter lettuce. These data will be necessary to compare leaf water status evolution during lettuce storage under adverse environmental conditions.

Material and Methods

Plant material and sample preparation

Heads of butter lettuce (*Lactuca sativa* var. Lores) were grown and harvested in Sierra de los Padres, Mar del Plata, Argentina. Greenhouse lettuce heads were cultivated by applying "mulch" technology with a black plastic film separating each plant from the soil and were harvested at optimal maturity after reaching a marketable size (approximately 24 to 30 leaves per head). Once harvested, lettuce heads were immediately precooled in refrigerated bags and transported to the laboratory within 1 h of harvesting. Plants were not subjected to any preconditioning operation, they were just identified and weighed in the laboratory. Then they 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 $g/m^2/d$), placing 2 plants per bag (28 × 55 cm, useful volume: 4 L). Bags were sealed and stored in a refrigerated chamber at 0 to 2 °C and 97% to 99% RH. Under this temperature, the respiration rate, the ethylene production, and other physiological changes were minimized. On each sampling day, 3 bags were taken from the storage chamber, 1 bag was used to assess water plant status and the other 2 to determine chlorophyll content and analyze OVQ. At zero time of storage, 6 whole plants were analyzed within 1 and 2 h after harvest.

All parameters analyzed were measured in 3 different zones of the complete lettuce head called external (outer leaves), middle (mid leaves), and internal zone (inner leaves), respectively. 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 noncompact leaves of green and dark green color, respectively. Each zone had a mean of approximately 6 to 9 leaves.

Water status indexes

Water status in lettuces was determined through the following indexes: WC, RWC, FW, BW, and the relationship between FW/TW. Water status indexes were calculated for all leaves of each zone plant.

Figure 1 depicts how each lettuce leaf was sectioned to measure the different water status indexes. At least 3 rectangles (15 cm^2) were cut from each leaf using a stainless steel cutter. The 1st rectangle, cut in the central apical area, was destined for RWC determination. As RWC depends on a large number of variables such as leaf age, environmental conditions undergone by the leaf, and leaf position (Yamasaki and Rebello Dillenburg 1999), only 1 rectangle per leaf was cut in the central apical area for its determination. The other 2 rectangles were cut on the same horizontal line, 1 cm below the 1st one, as specular images. The right rectangle was employed for FW, BW, and FW/TW determination, while the left rectangle was destined for WC. When leaf size allowed so, more rectangles were cut for WC and FW, BW, and FW/TW to obtain determinations by duplicate or triplicate.

For RWC determinations, each central apical rectangle obtained from each leaf was weighed individually to obtain fresh mass (FM). Afterward, each rectangle was 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 remained for 20 h at 4 °C in darkness. Next, rectangles were individually water drained with absorbent tissue paper and weighed to obtain their turgid mass (TM). Finally, the rectangles were dried for 24 h at 80°C in a conventional oven to determine their dry mass (DM). Values of FM, TM, and DM were used to calculate RWC, using Eq. 1 (Esparza-Rivera and others 2006):

$$\mathrm{RWC}\ (\%) = \frac{\mathrm{FM} - \mathrm{DM}}{\mathrm{TM} - \mathrm{DM}} \cdot 100 \tag{1}$$

For WC determination the left rectangles of each leaf (Figure 1) were weighed to give FM. Then, they were placed in a conventional oven at 80 °C during 24 h and, afterward, they were weighed again to obtain DM. Water content was calculated following Eq. 2:

$$\mathrm{WC}\ (\%) = \frac{\mathrm{FM} - \mathrm{DM}}{\mathrm{FM}} \cdot 100 \tag{2}$$

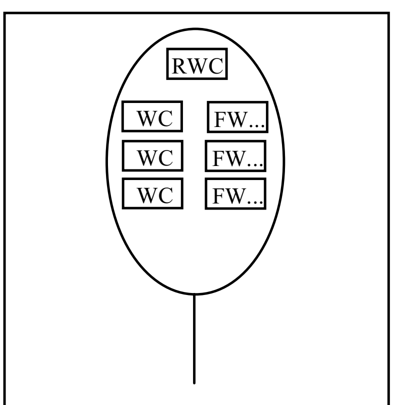

Figure 1 — Disposition of lettuce rectangles for water status indexes determinations.

Total water in leaf tissues is made up of 2 components: free solvent water and BW. These data were acquired following the methodology described by Singh and others (2006). Right rectangles (Figure 1) were weighed to obtain FM, and then frozen by placing them in 15-mL Falcon tubes for freezing in liquid nitrogen. Next the rectangles were thawed and the leaf tissue was spread out and air-dried on a table for 30 min. After this, it was weighed to obtain its air dry value (AD), oven-dried during 24 h at 80 °C, and lastly weighed (DM). The values from FM, AD, and DM were then used to measure TW, FW, and BW per unit DM using Eq. 3 to 5:

$$TW = \frac{FM - DM}{DM} \quad (3)$$

$$BW = \frac{AD - DM}{DM} \quad (4)$$

$$FW = TW - BW \quad (5)$$

The FW/TW ratio was calculated applying Eq. 5 and 3.

FW, BW, and TW were expressed as grams of water per unit of leaf dry tissue ($g \cdot g^{-1}$) .

Chlorophyll content

The chlorophyll content of each zone was determined following the methodology described by Moreira and others (2003). All leaves in each zone were homogenized with a tissue homogenizer by Braun (Kronberg, Germany), and 2 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 Corporation, Japan). Chlorophyll content was calculated applying the formula $C = 7.12A_{660} + 16.8\ A_{642.5}$, in which C is the total chlorophyll concentration (mg/L) and A_{660} and $A_{642.5}$ are the absorbance at the corresponding wavelengths. Chlorophyll content is reported as mg of chlorophyll/100 g fresh weight.

Overall visual quality

At each storage time, each individual lettuce was subjected to a sensory panel to evaluate the OVQ of each plant zone (external, middle, and internal). Evaluations were performed immediately after lettuce removal from storage conditions.

A panel comprised of 9 judges, aged 30 to 55 y, all members of the UNMDP Food Engineering Group and with sensory evaluation experience in leafy vegetables, was trained in lettuce quality evaluation. The coded (3 digit) samples were presented one at a time in random order to the judges who sat at a round a table and made independent evaluations. The judges were asked to evaluate OVQ on the basis of leaf characteristics such as color (shade and uniformity), brightness, texture, presence, or absence of defects and of all the leaves of the lettuce zones. A 9-point scoring scale was employed, in which 9 stood for excellent quality and 1 for very poor quality; the limit of acceptance was 5 (a score lower than 5 indicating poor quality).

Statistical analysis

Results reported in this paper, except for data in Figure 2, are lsmean values (least square mean, means estimators by the method of least squares) together with their standard deviations (Steel and Torrie 1992; Khuel 2001).

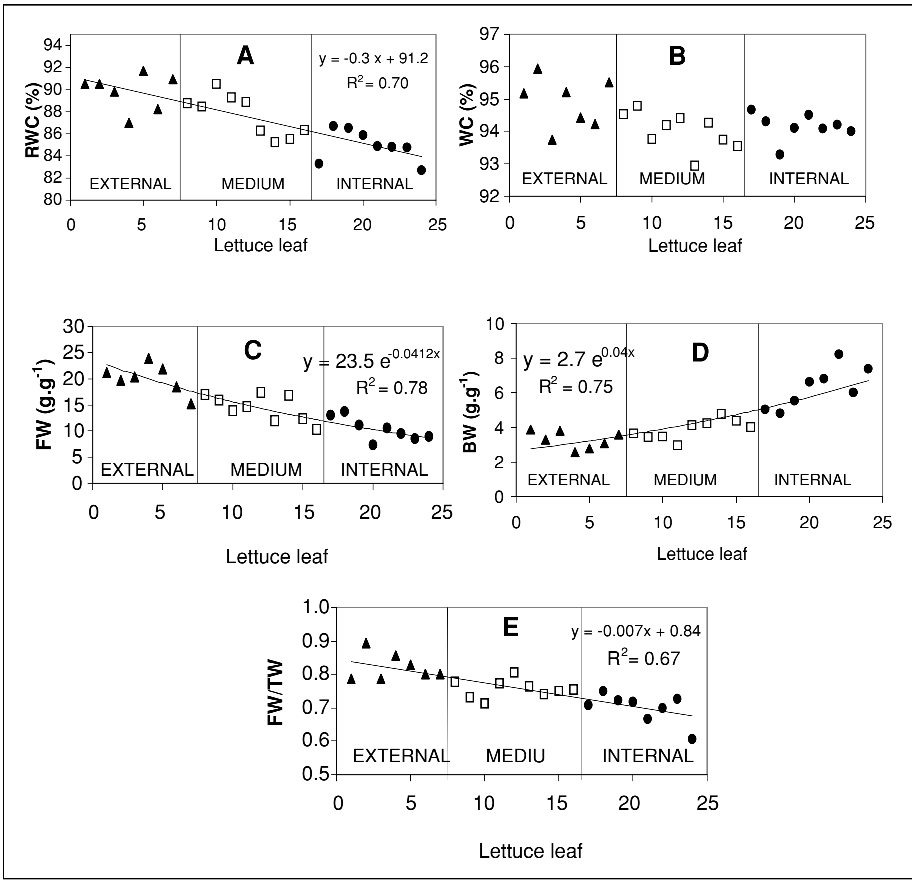

Figure 2—Water status indexes of a typical lettuce plant at day 0 of storage.

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). The factors employed as sources of variation were DAY (storage time, day of sampling), PLANT WITHIN DAY, ZONE (zone of the plant), and DAY–ZONE interaction. Differences between zones and days of storage were determined by the Tukey–Kramer multiple comparison test ($P < 0.05$). PROC UNIVARIATE was used to validate ANOVA assumptions. Correlation analyses between plant water status indexes, chlorophyll content, and OVQ were carried out through Pearson's coefficients evaluation, obtained with PROC CORR. Correlations of $P < 0.05$ were considered statistically significant.

Nonlinear regression fittings were calculated using SYSTAT 5.0 (SYSTAT Inc. 1992).

Results and Discussion

Plant water status

Table 1 lists plant water status indexes of lettuce heads at harvest.

RWC provides information about leaf WC by measuring the amount of water in the leaf tissue in relation to full saturation (Eitel and others 2006), and it indicates the maximum amount of water a tissue can hold (Yamasaki and Rebello Dillenburg 1999). Figure 2A shows the RWC profile obtained for a typical fresh lettuce head; the different plant zones were also specified. The tendency line drawn for RWC data was represented by the following regression equation: $y = -0.3x + 91.2$ ($R^2 = 0.70$, $n = 24$). No significant differences were detected in RWC values obtained for leaves from external and middle zones (87.60 ± 0.70 and 85.81 ± 0.63, respectively) (Table 1). The RWC values obtained from the internal zone (84.09 ± 0.59) proved to be significantly different ($P < 0.05$) from those from the external zone; however, they were not significantly different from those from the middle zone, thereby indicating that the values of the latter are intermediate in relation to both extreme zones (Table 1). The differences between the RWC from the internal and external zones would indicate different degrees of tissue water holding capacity, presumably due to differences in the degree of tissue development. The internal zone was composed of growing, expanding leaves. Jones and Tardieu (1998) indicated that water plays a key role in cell expansion and growth.

Esparza-Rivera and others (2006), who worked with leaves of Waldmann's dark green lettuce, found higher RWC values at harvest (higher than 95%). The differences between these values and those obtained in this work could be attributed to differences in lettuce variety, which could lead to differences in tissue holding capacity. Moreover, differences in the initial state of leaf hydration should not be discarded. Generally, normal values of RWC range from 98% in turgid and transpiring leaves to about 40% in severely desiccated and dying leaves (www.plantstress.com).

Figure 2B shows the WC profile obtained for a typical fresh lettuce head. Data did not follow any particular pattern, but WC mean values from external leaves (95.10 ± 0.19) were significantly different ($P < 0.05$) from those obtained from the middle and internal zones (94.49 ± 0.18 and 94.41 ± 0.24). Again, WC mean values from the middle and internal zones did not differ significantly from each other. The external zone yielded higher WC, which was in agreement with its higher RWC values, thus demonstrating that the outer part of lettuce plants was well hydrated at harvest.

Figure 2C to 2E show the profiles obtained for FW, BW, and FW/TW for a typical fresh lettuce head. FW decreased exponentially ($y = 23.5\ e^{-0.0412x}$, $R^2 = 0.78$, $n = 41$), while BW increased exponentially ($y = 2.7\ e^{0.04x}$, $R^2 = 0.75$, $n = 41$) from external to internal leaves in both cases. The FW/TW ratio decreased linearly following the same direction ($y = -0.007x + 0.84$, $R^2 = 0.67$, $n = 41$). Regarding the FW index, the external zone was significantly different ($P < 0.05$) from the middle and internal zones, reaching values of 23.07 ± 0.86, 15.44 ± 0.97, and 15.05 ± 1.42, respectively, while there were no significant differences between middle and internal zones. For the BW index, the external and middle zones were significantly different ($P < 0.05$) from the internal zone, with values of 4.08 ± 0.51, 4.50 ± 0.57 and 5.87 ± 0.84, respectively, while there were no significant differences between external and middle zones. For the FW/TW index, the external zone (0.85 ± 0.02) was significantly different ($P < 0.05$) from the internal zone (0.72 ± 0.03), while there were no significant differences between the external and middle zones (0.76 ± 0.02) and between the middle and internal zones. FW/TW values play a key part in the shelf life of each lettuce zone. If the plants were under unfavorable hydric environmental conditions, 85% of the TW from external zone could get lost. This fact could have a direct impact on leaf texture as well as on the sensory acceptability of the product (Burdon and Clark 2001).

Figure 3A illustrates RWC evolution in each zone during refrigerated storage. ANOVA applied to RWC data showed a significant interaction ($P < 0.05$) between factors considered in the analysis (ZONE and DAY). The RWC of internal and middle zones behaved similarly during storage (ANOVA applied to these 2 zones showed no significant interaction between ZONE and DAY). RWC values in both zones rose from 84.09 to 92.54 and from 85.81 to 90.84 up to day 8 of storage for internal and middle zones, respectively. After such increases, RWC decreased in both zones, reaching, by the end of storage, values above (internal) or similar (middle) to initial RWC values. As regards the external zone, RWC values did not show significant differences during storage.

High RWC values during storage indicated that the lettuce tissues were well hydrated (Esparza-Rivera and others 2006). These authors, who analyzed the effects of ascorbic acid on the physical and chemical properties of green leaf lettuce, reported a slight decrease in RWC values of control lettuce (without ascorbic treatment) from 99.79% to 94.04% at 21 d of cold storage (5 °C). However, they followed another experimental design: they removed 2 outer entire leaves per lettuce head at each analytical time, repackaging and storing the same lettuce head until the following analytical

Table 1 — Water status of lettuce heads at harvest.

Zone	RWC[A,B]	WC[A,B]	FW[A,C]	BW[A,C]	FW/TW[A,D]
External	87.60[a] ± 0.70	95.10[a] ± 0.19	23.07[a] ± 0.86	4.08[a] ± 0.51	0.85[a] ± 0.02
Middle	85.81[ab] ± 0.63	94.49[b] ± 0.18	15.44[b] ± 0.97	4.50[a] ± 0.57	0.76[ab] ± 0.02
Internal	84.09[b] ± 0.59	94.41[b] ± 0.24	15.05[b] ± 1.42	5.87[b] ± 0.84	0.72[b] ± 0.03
n	48	102	102	102	102

[A]Mean values with their standard deviation are reported.
[B]RWC (relative water content) and WC (water content) are reported in percent.
[C]FW (free water) and BW (bound water) are reported in g g^{-1}.
[D]Relation FW/TW (free water to total water ratio) is expressed as a fraction.
[abc] Mean values with different letter within the same column are significant different ($P < 0.05$).

S: Sensory & Food Quality

time. By so doing, the possibility of there being different RWC responses along different lettuce zones was not considered. In this study, RWC values of different zones behaved differently during storage time (Figure 3A), probably due to the different degrees of leaf development, which led to diverse physiological responses. Other authors who assessed storage conditions favoring fruits and vegetables water loss (low RH and room temperature) discovered a continuous decreases in RWC values during storage (Landrigan and others 1996; Burdon and Clark 2001). In our experimental design, lettuce was stored at optimal conditions, thereby preventing water loss (97% to 99% RH and 0 to 2 °C); and an increase in RWC values was observed in the internal and middle zones. Such behavior could answer to an adaptive response of lettuce tissues to a water saturated environment, with no difference in water vapor pressure between the vegetal and the surrounding environment.

Figure 3B illustrates WC evolution in each zone during refrigerated storage. ANOVA applied to WC data showed no significant interaction between factors considered in the analysis. Besides, the DAY factor of the statistical model was not significant; therefore no changes in WC as a function of storage days were detected. This was predictable as lettuce heads were exposed to a saturation atmosphere in the film bag. Yet, even when no measurable changes were identified in the overall WC along storage time, the RWC profiles obtained for middle and internal zones denoted changes along storage. Taking into consideration the mathematical expression of RWC (Eq. 1), the increments in RWC values could arise from 2 main reasons: an increase in the vegetable FM and/or a decrease in its TM. As no changes were detected in WC values along storage time, it was assumed that FM values remained unchanged, so the increase in RWC values may be mainly due to a decrease in the TM, i.e., a reduction in the water holding capacity of lettuce tissue. To evaluate this, the TM to DM (TM/DM) ratio was calculated. For both middle and internal zones, the TM/DM decreased significantly ($P < 0.05$) up to 16 d of storage (Figure 3F), indicating changes in the water holding capacity of lettuce tissue under water saturation atmosphere. This could denote a postharvest vegetable tissue adjustment expressed as a short time response under water saturation atmosphere. Bacelar and others (2006) noticed that the ability of olive trees to acclimate to water availability includes alterations at leaf level associated with morphological, anatomical, and physiological characteristics. Cell size changes are known to occur in different species in response to abiotic stress, such as cell size reduction under water stress (Martínez and others 2007). Along these lines, the RWC index is more sensitive than the WC index is. WC indicates water content variations of vegetable tissues arising from changes in water vapor pressure in the surrounding atmosphere. However, RWC index could express structural changes in the vegetable tissue as a response to water saturation atmosphere. Under optimal postharvest storage conditions (low temperature and high RH) no changes in WC were measured, yet RWC was more sensitive

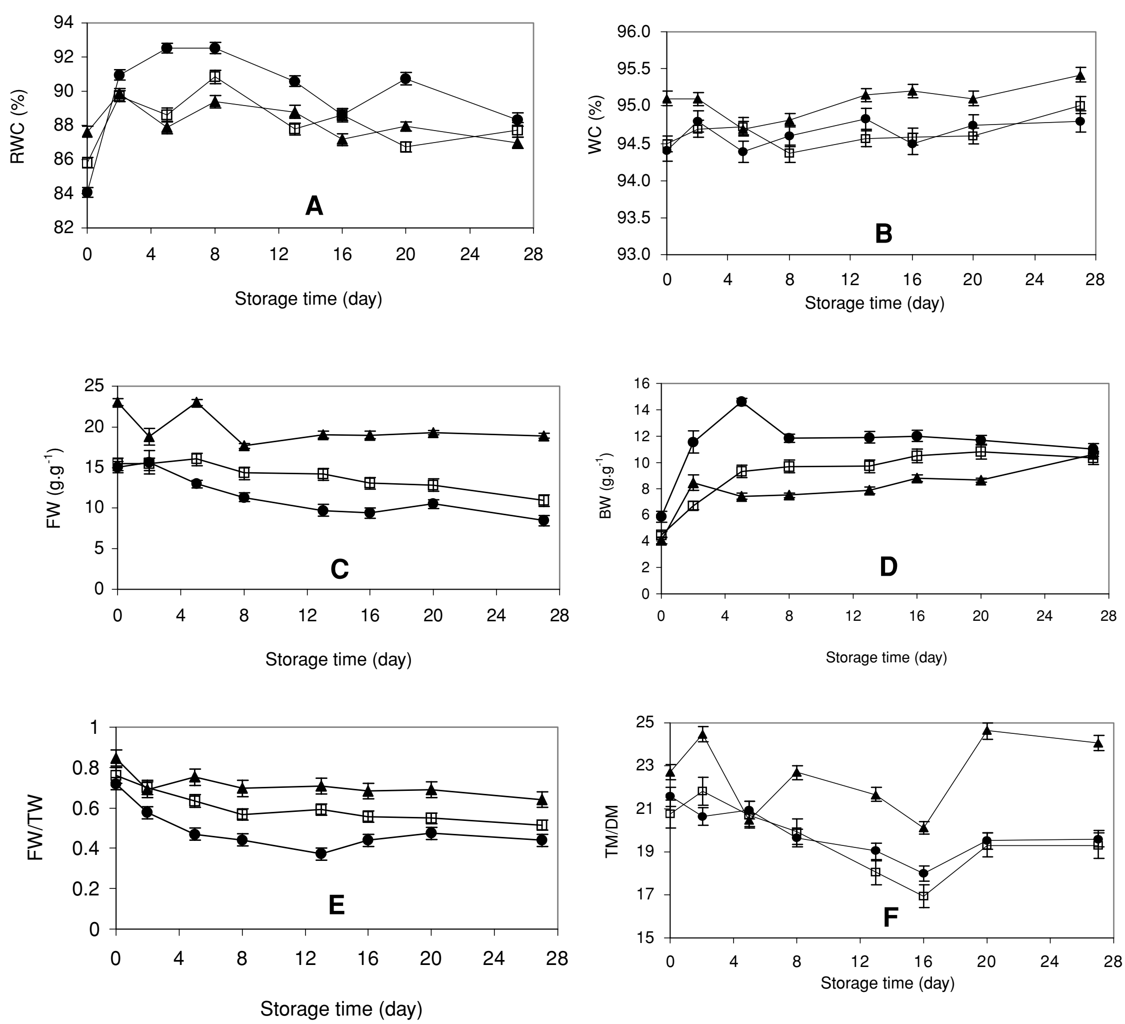

Figure 3—Water status indexes evolution in different zones of lettuce heads during refrigerated storage. External zone (▲), medium zone (□), and internal zone (•). Data presented correspond to the lsmean (least square mean, estimators of means by the method of least squares).

and allowed to detect tissue changes affected by the plant water status.

Figure 3C and 3D show FW and BW evolution in each zone during refrigerated storage. ANOVA applied to FW data showed significant interaction between factors considered in the analysis (ZONE and DAY). The FW values for the internal and middle zone behaved similarly during storage, exhibiting a gradual and significant decrease in FW content during storage. The external zone evidenced a significant ($P < 0.05$) decrease up to 8 d of storage, maintaining FW values until the end of storage. BW values for the middle and external zones behaved similarly during storage, with no significant interaction between ZONE and DAY factors. Both zones revealed a gradual increase in BW values along storage, increasing twofold at day 27. The evolution of BW data in the internal zone has a hyperbolic profile, increasing up to 5 d of storage (2.5-fold) and showing no significant changes until the end of storage time. The FW/TW corresponding to the 3 zones decreased during storage, with reductions ranging from 24% to 39% at the end of storage (Figure 3E). TW was mathematically expressed as the sum of FW and BW. This index, in agreement with WC evolution, did not suffer variations along storage (data not shown). So, changes in FW and BW during storage would indicate water movement within the plant. There seems to occur an exchange between FW and BW during storage, probably forced by changes of solute concentration in lettuce plant. A correlation between solute accumulation and an increase in the binding strength of tightly and weakly BW was reported in durum wheat leaf tissues (Rascio and others 1994). The initial increase observed in BW values for the internal zone could be related to the degree of maturity achieved by these tissue leaves (growing expanding leaves). BW in living tissue is more likely to play a major role in tolerance to abiotic stresses (El-Saidi and others 1975; Rascio and others 1998; Misik 2000). Singh and others (2006) indicated that BW acts by maintaining the structural integrity and/or cell wall extensibility of the leaves whilst an increased amount of FW could enhance solute accumulation, leading to better osmotic adjustments and tolerance to water stress. In our study, lettuce plants were exposed mainly to 3 major abiotic stresses: plant harvesting, cold storage exposition and storage time. Since, during storage, lettuce was stored at high RH, developing air-food moisture equilibrium, tissue adjustments would have been mainly forced by abiotic stress rather than water stress. In this way, the increase in BW values could answer to leaf tissue adjustment to abiotic stresses.

Chlorophyll content

Chlorophyll content in fresh lettuce was 35.65 ± 1.17, 14.96 ± 2.11, and 3.32 ± 0.99 (in mg of chlorophyll/100 g fresh weight) for external, middle, and internal zones, respectively (Figure 4). A great difference ($P < 0.0001$) in chlorophyll content was detected between lettuce zones, the content of the external zone being 10 times higher than that of the internal one due to major sun light exposition of external leaves.

The storage of almost all vegetables brings about certain degradation of chlorophyll pigments. Figure 4 depicts the evolution of chlorophyll content in each zone during refrigerated storage. ANOVA applied to chlorophyll data showed a significant interaction between ZONE and DAY factors considered in the analysis. This fact implies that the behavior of each zone was different during storage. While the external zone exhibited chlorophyll pigments degradation throughout all the storage time, the middle and internal zones showed no chlorophyll content changes during such period. Differences in chlorophyll degradation between zones could be attributed to the fact that external leaves are more exposed to environmental factors such as light and oxygen, which could hasten pigment deterioration. Also the significant decrease up to 8 d of storage in FW of external zone could favor the chlorophyll loss through water movements. Chlorophyll destruction could even arise from the increase in ethylene concentration during storage. It has been reported that the destruction of chlorophyll by ethylene could answer to the increased chlorophyllase activity (Watada and others 1990). However, in butter lettuce stored at optimal conditions, chlorophyll destruction was only evident in external zone. It is unlikely that ethylene and oxygen levels differed much between lettuce zones of the head; in this way this possible factors of chlorophyll destruction may be discarded.

During storage, vegetables containing chlorophyll undergo changes or loss of color (Abe and Watada 1991; Bolin and Huxsoll 1991; Haard 1993). During butter lettuce storage, a 1st-order kinetics for chlorophyll pigment degradation was found to better represent the experimental data for external zone leaves (Figure 5), with a kinetic constant of 0.0364/s, with $R^2 = 0.83$, $n = 24$. Chlorophyll pigment degradation has been previously modeled for different kinds of vegetables, 1st-order kinetics being the one mainly associated with its deterioration. Albanese and others (2006) found a 1st-order kinetics in chlorophyll degradation of green asparagus during refrigerated storage, and they found a similar kinetic constant value of 0.0355/s for the chlorophyll degradation. Marangoni (1996) also proposed a kinetic model for chlorophyll degradation in green tissue using the sum of chlorophyll *a* and *b*. Environmental factors such as light, temperature, humidity, oxygen and ethylene (Watada and others 1990; Yamuchi and Watada 1991) and inner plant factors such as chlorophyllase and magnesium dechelactase activity (Shioi and others 1996; Jacob-Wilk and others 1999) are responsible for the loss of chlorophyll pigments during green vegetable storage.

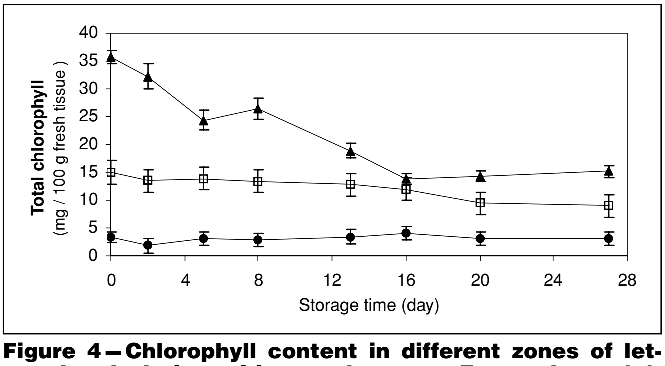

Figure 4—Chlorophyll content in different zones of lettuce heads during refrigerated storage. External zone (▲), medium zone (□), and internal zone (•). Data presented correspond to the lsmean (least square mean, estimators of means by the method of least squares).

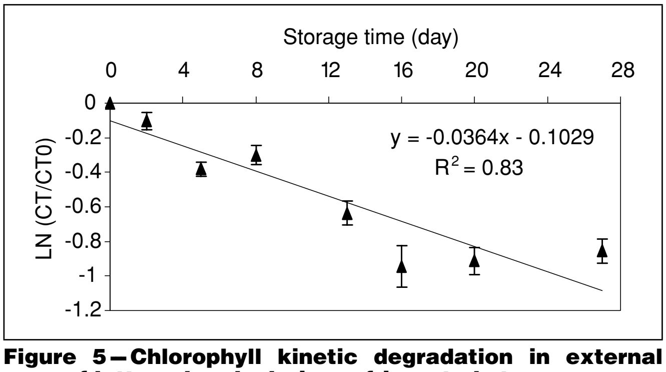

Figure 5—Chlorophyll kinetic degradation in external zone of lettuce heads during refrigerated storage.

Overall visual quality

OVQ is a sensory index closely associated with consumer acceptability. There are different quality components of lettuce OVQ, such as a fresh-looking appearance, bright green color, crispness, and mainly absence of browning. The members of the sensory panel considered that samples with OVQ scores below 5 were unacceptable. Throughout the sensory analyses, each lettuce zone was scored independently, making no comparisons among each other. Regarding fresh lettuce, each zone presented typical organoleptic characteristics mainly differentiated by leaf size, color, and texture. Even though zones differed in their sensory attributes, their initial attributes were considered with the maximum score 9.

Storage time introduces some vegetable degradation in lettuce heads appearance, mainly loss of texture, discoloration extension, and development of browning. Figure 6 shows the evolution of OVQ in each zone during refrigerated storage. ANOVA applied to OVQ data showed a significant interaction between DAY and ZONE factors considered in the analysis. This implies that each zone behaved differently during storage. While the external zone showed a decrease in OVQ from the 1st sampling day and during all the storage period, the middle and internal zones showed no reduction in OVQ until 8 d of storage. The 1st change observed by the panelists with regard to the external zone was a decrease in leaf brightness. At day 16, external leaves presented moderate discoloration and slight browning in their external edges. Visual texture was still acceptable with the mean score of 6.4. At this time, the panel described the visual quality of middle and internal leaves as good, with slight defects not objectionable and an OVQ score of 7.8 and 8.2, respectively. At day 20, external leaves presented moderate and objectionable ruptures in leaf tissues, indicating an increase in their mechanical fragility, and severe browning in the midrib base. Such defects accounted for a final score of 5.1, reaching the acceptability limit. At this time, middle leaves showed slight objectionable browning in their external leaves edges, but kept their texture, resulting in a mean score of 6.7. The internal zone maintained its good color, brightness, and texture features, reaching an OVQ score of 7.8. At the end of storage, external leaves presented an extending browning in midribs, extreme mechanical fragility with complete loss of texture, and a generalized surface discoloration, getting a final OVQ score of 4.3. The loss of green color in the external leaves would be associated with CO_2 injury, which could generalize surface discoloration. The development of browning stain in intact lettuce heads was increased by low temperatures (0 to 2.5 °C), low O_2 atmosphere, and transfer after storage to temperatures >10 °C (Brecht and others 1973a, 1973b, 1973c). Also surface discoloration in external leaves coincided with the degradation of chlorophyll content, which was greater than in the middle and internal zones (Figure 4). The middle and internal leaves showed signs of slight mechanical fragility (small ruptures were detected in the external leaf edge of some leaves). Even though both zones still showed acceptable texture, color, and brightness, leaves from the middle zone presented moderate browning in their external leaf edge, resulting in a final OVQ score below that of the internal zone, just in the limit of acceptance (5 and 7.1 for middle and internal zones, respectively).

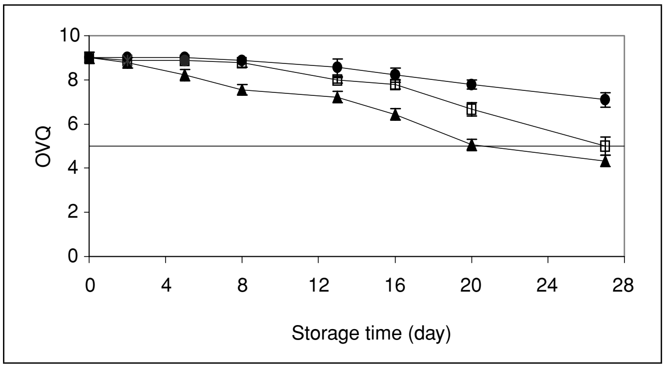

Figure 6—Overall visual quality (OVQ) in different zones of lettuce heads during refrigerated storage. External zone (▲), medium zone (□), and internal zone (•). Data presented correspond to the lsmean (least square mean, estimators of means by the method of least squares).

The internal zone is the one that best maintained its visual quality. Differences in OVQ evolution among lettuce zones could be attributed to several factors such as higher exposition of external leaves to environmental factors (light and oxygen), which could fasten deterioration; differences in FW/TW in each zone (Figure 3E); variations in phenolic metabolism among zones, which could represent differential browning potentials; different levels of phenylalanine ammonia lyase activity in each zone; and differences in CO_2 sensitivity, among others. López-Galvez and others (1996) conducted an extended discussion comparing the visual quality and shelf life of various types of lettuce and the relative importance of storage defects on OVQ.

Correlation analysis

Table 2 lists Pearson's correlation coefficients for all cross correlations among parameters analyzed: plant water status indexes, chlorophyll content, and OVQ for external, middle and internal zones, respectively.

OVQ for each lettuce zone showed differential correlations with the evaluated indexes. This was in agreement with the fact that such indexes behaved differentially in relation to the lettuce zone analyzed. In the external zone, OVQ was strongly correlated with chlorophyll content ($r = 0.876$, $P < 0.01$) and also correlated with BW ($r = -0.759$, $P < 0.05$) and FW/TW ($r = 0.705$, $P < 0.05$). As regards the middle zone, OVQ was very strongly correlated with FW ($r = 0.946$, $P < 0.001$) and also with FW/TW ($r = 0.718$, $P < 0.05$). While, in the internal zone, the changes in OVQ were correlated only with FW ($r = 0.767$, $P < 0.05$).

In the external zone the parameter with highest impact on the sensorial quality of lettuce leaves was C. The decrease in chlorophyll content observed in the external leaves strongly correlated with the decrease in OVQ. The 1st color change observed by the panelists was a decrease in leaf brightness probably induced by the 1st steps of chlorophyll degradation. At day 16, external leaves presented moderate discoloration and slight browning in their external edges. The presence of browning was considered by the panelists as a color defect. OVQ was also significantly connected with bound WC. A gradual increase in BW values during storage was observed in the external zone. As total WC remained steady along storage time, changes in BW during storage would indicate water movement in the plant. Also, FW/TW ratio decreased during storage. At day 20, external leaves presented moderate and objectionable ruptures in leaf tissue, indicating an increase in mechanical fragility as well as poor texture. It is likely that changes in water movement in the plant damaged tissue texture during storage.

OVQ of middle and internal zones were significantly correlated with free WC. In both zones the panelists took no notice of changes in green color during storage; however, at the end of storage, signs of slight mechanical fragility were detected (small ruptures in the external leaf edge of some leaves). FW movements in the plant during storage could be accountable for textural damage at the end of storage. For the middle zone, OVQ also correlated with FW/TW ratio.

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Table 2—Pearson correlation coefficients (*r*) between water status indexes (RWC: relative water content, WC: water content, FW: free water, BW: bound water, FW/TW: free water to total water ratio, and TM/DM: turgid mass to dried mass ratio) chlorophyll content (C), and overall visual quality (OVQ) in different lettuce zones.

	RWC	WC	FW	BW	FW/TW	TM/DM	C	OVQ
External zone								
RWC	1.000							
WC	−0.414	1.000						
FW	−0.379	−0.335	1.000					
BW	−0.116	0.434	−0.655	1.000				
FW/TW	−0.064	−0.387	0.827[a]	−0.964[c]	1.000			
TM/DM	0.280	0.368	−0.333	0.214	−0.250	1.000		
C	0.489	−0.400	0.432	−0.747[a]	0.697[a]	0.128	1.000	
OVQ	0.522	−0.578	0.463	−0.759[a]	0.705[a]	−0.274	0.876[b]	1.000
Middle zone								
RWC	1.000							
WC	−0.168	1.000						
FW	0.167	−0.493	1.000					
BW	0.222	0.219	−0.639	1.000				
FW/TW	−0.219	−0.288	0.789[a]	−0.965[b]	1.000			
TM/DM	0.120	0.050	0.569	−0.668	0.656	1.000		
C	−0.455	−0.123	−0.326	0.378	−0.342	−0.818[a]	1.000	
OVQ	0.298	−0.690	0.946[c]	−0.590	0.718[a]	0.403	−0.228	1.000
Internal zone								
RWC	1.000							
WC	0.247	1.000						
FW	−0.143	−0.331	1.000					
BW	0.889[b]	0.139	−0.344	1.000				
FW/TW	−0.654	−0.405	0.809[a]	−0.767[a]	1.000			
TM/DM	−0.220	−0.342	0.823[a]	−0.385	0.760[a]	1.000		
C	0.028	−0.041	−0.091	−0.234	−0.037	0.110	1.000	
OVQ	0.140	−0.468	0.767[a]	−0.068	0.411	0.503	0.098	1.000

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

Conclusions

The study of water status indexes in butter lettuce confirmed differences in the initial values of each zone as well as in their evolution during storage under optimal conditions (low temperature and high RH). The RWC method turned out to be more accurate than the traditional WC since, thanks to its determination, its is possible to detect changes in the water holding capacity of tissues along storage. Nevertheless, no significant correlation was detected, particularly between RWC and lettuce visual acceptability, nor between any of the other assessed indexes. This result limits the use of this parameter as an objective index to predict vegetable quality evolution. Even the traditional measurement of WC under optimal storage conditions did not correlate with visual quality because this index did not reveal water state in lettuce tissue.

Free and bound WC measurement in lettuce leaves in the whole plant proved to be a valuable indicator of the true status of water. Initial FW and BW of external and internal zones were clearly different. The external zone yielded the highest FW/TW (85% of its TW as FW), indicating that these external leaves had more water available to be used in enzymatic and oxidative reactions, microbiological growth, evaporation, or transpiration. As a consequence, external leaves turned out to be more perishable and had the shortest shelf life if compared with the other leaves of lettuce zones.

Under optimal storage conditions and in all lettuce zones, an increase in the amount of BW with a simultaneous decrease in the free WC was detected. This behavior could support the hypothesis of tissue adjustments forced mainly by abiotic stress (harvesting stress, low temperature stress, and time of storage). In the 3 zones, the parameter OVQ correlated with FW either directly or indirectly through FW/TW ratio. Therefore, free WC is an objective and quantitative measurement which impacts the visual quality of butter lettuce.

During whole lettuce heads trading, it is common practice to remove the external leaves as storage advances. These leaves are more perishable than middle and internal ones because of their direct exposure to environmental conditions. In this work, whole lettuce heads showed different shelf life among zones under optimal storage condition. Therefore, factors other than major environmental exposure of external leaves may be responsible for lettuce quality loss. The higher rate of OVQ loss observed in the external zone could be attributed to green color loss and to changes in true water status (FW and BW exchange in the plant).

Even when vegetables were stored under conditions preventing water loss, water movements and exchange between FW and BW took place. These water movements had a profound impact on the loss of lettuce acceptability. Also degradation in chlorophyll pigments, which occurred faster in external leaf tissues of lettuce heads, may play an important role in quality loss. Further studies should be conducted in order to prevent or reduce water exchange in lettuce tissues and control the factors that affect pigments such as light and the atmosphere.

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