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Shelf life of iceberg lettuce affected by hydro cooling and temperature of storage

C.F.M. França [1], M.N.S. Santos [2], W.S. Ribeiro [3], P.R. Cecon [4], F.L. Finger [3] (*)

[1] *Universidade Federal do Oeste da Bahia, Campus Multidisciplinar da Barra, 41100-000, Barra, BA, Brazil.*

[2] *Universidade Federal de Viçosa, Departamento de Biologia Vegetal, Viçosa, MG, 36570-900, Brazil.*

[3] *Universidade Federal de Viçosa, Departamento de Fitotecnia, Viçosa, MG, 36570-900, Brazil.*

[4] *Universidade Federal de Viçosa, Departamento de Estatística, Viçosa, MG, 36570-900, Brazil.*

(*) Corresponding author:
ffinger@ufv.br

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The authors declare no competing interests.

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Abstract: Pre cooling is applied to remove the field heat of harvested horticultural produces. The goal of this work was to determine the cooling curve and the effects of hydro cooling on quality and shelf life of iceberg lettuce 'Lucy Brown' stored at 5°C and 22°C. Through shelf life, it was determined the changes on accumulated fresh weight loss, leaf relative water content and total chlorophyll, total soluble sugars, reducing and non-reducing sugars, and starch. The field heat from iceberg lettuce heads was removed within the first 10 min when submerged into cooled water at 4°C. Hydro cooled lettuce heads accumulated water over the leaf surfaces resulting in higher rate of fresh weight loss during storage when compared to control. Lettuce stored at 5°C kept higher relative water content in the leaves throughout the shelf life. Hydro cooling treatment delayed the wilting of the external leaves in three and two days when stored at 5 and 22°C, respectively. Hydro cooling did not influence the decrease on total soluble sugars, reducing sugars, non-reducing sugars and starch throughout shelf life, but affected the leaf chlorophyll content. Independent of the temperature in which the 'Lucy Brown' iceberg lettuce will be stored, hydro cooling is recommended to prolong quality and shelf life.

1. Introduction

Senescence is a natural process common to all fresh vegetables, which is intensified after harvest, by handling and storage conditions. In addition, the rate of deterioration is quickly intensified if a vegetable or a fruit is stored under stressed conditions. Storing fresh horticultural products under extremes of high temperature or under low relative humidity, results in intense water loss, triggers senescence and finally the death of the tissues.

The major important factor affecting the postharvest shelf life for most of fresh horticultural products is the temperature during storage or display. To preserve the quality and prolong the perishables commercial quality, it is necessary to rapidly remove the field heat to an optimum temperature for subsequent storage (Brosnan and Sun, 2001). Independent of the pre cooling method to be used, the premises as an useful postharvest practice, is based on the quick reduction of product temperature. In addition, it is recommendable to reduce the temperature as soon as possible after harvest, which will increase the beneficial effects of the rapid cooling. Among the many benefits of pre cooling, in keeping the quality of a produce, is the lower respiration rate, the reduction of water loss by the product, and less contamination by pathogenic microorganisms (Brosnan and Sun, 2001).

The rapid loss of quality and limited shelf life of leafy vegetables, like lettuce, parsley and jute leaves is mainly due to their fast postharvest dehydration (Tulio Jr. *et al*., 2002; Finger *et al*., 2008; Aguero *et al*., 2011). In these products wilting of leaves occur even faster when the storage is done under high storage temperatures or without any refrigeration and inadequate packaging.

There is a variety of pre cooling techniques available including cooling rooms, hydro cooling systems, air forced cooling, ice packaging, vacuum and cryogenic cooling (Brosnan and Sun, 2001). Hydro cooling is relatively inexpensive and very effective method recommended to remove the field heat of several leafy vegetables including kale, green onions and spinach (Sargent *et al*., 2007). Álvares *et al.* (2007) determined that hydro cooled parsley leaves had less water loss, resulting in longer shelf life without the appearance of wilting symptoms compared to control. Hydro cooling also proved to be a faster method for cooling peach pulp to 1°C compared to forced air and conventional cooling room methods (Brackmann *et al*., 2009).

The expansion of large cities in developing are pushing the vegetable farms farther away from the markets, making harder for them to deliver products with good quality to urban population. This situation demands the incorporation of appropriated postharvest handling, but most of the small farmers have no capital to purchase refrigeration systems. Iceberg lettuce is the most popular leaf vegetable used in burgers, sandwiches and salads by fast food stores in most of the countries, including Brazil. Thus, there is the need to evaluate the influence of pre cooling on the shelf life of this lettuce. Therefore, the objective of this work was to determine the cooling curve and the effects of hydro cooling on quality and shelf life of iceberg lettuce heads stored under cold and room temperature conditions.

2. Materials and Methods

Heads of iceberg lettuce 'Lucy Brown' were harvested from the field at Federal University of Viçosa (642 m asl, 20°45' lat. S and 42°51' long. W) in the morning between 7 and 7:30 hours. The heads of lettuce were taken to the laboratory quickly and the heads with external leaves with brown spots, wilted, or dirty leaves were discarded. The lettuce heads weighting between 300 to 400 g were subjected to the following treatments: 1) Hydro cooling followed by storage at 5°C; 2) Control without hydro cooling and storage at 5°C; 3) Hydro cooling followed by storage at 22°C; 4) Control without hydro cooling and storage at 22°C. Hydro cooling was performed by submerging the heads in a mixture of tap water and crushed ice at proportion of 3:1 (v:v) kept at 4°C. Temperature of lettuce heads was determined at every five minutes with the help of a digital infrared thermometer. The temperature of the heads before initiating the hydro cooling treatment was between 20 to 22°C. At every 5 minutes, two heads were quickly removed from the cold water to determine the changes in the temperature, repeating the procedure up to fifty minutes. At the end of hydro cooling, the heads were removed from the cold water and allow draining for 5 min in the air before storage in the plastic boxes. Hydro cooled and control lettuces were kept in plastic boxes at 5 and 22°C for the whole experiment. The boxes (18 cm height, 25 cm wide × 48 cm length) were covered with perforated (12 holes 1.1 cm in diameter) low density polyethylene plastic sheets to protect from excessive dehydration. The relative humidity inside the boxes was always between 85 and 90%.

Loss of fresh weight of heads, leaves relative water content, chlorophyll, total soluble sugar, reducing sugar, non-reducing sugar and starch leaf contents were determined at every 12 h up to the first 48 h and then at every 24 h until the end of the lettuce shelf life.

The end of shelf life was determined when the heads were wilted, yellowed or with signs of deterioration, being unfit for commercialization. The wilting, yellowing or deterioration of 50% or more heads was used as the discard parameter.

The accumulated loss of fresh weight was obtained in relation to initial fresh mass of heads and during storage period. The leaf relative water content (RWC) was determined as described previously by Álvares *et al.* (2007) with modifications. Fifteen leaf discs with 1.1 cm in diameter were removed from the external, middle and internal position in the lettuce head, which were kept between two layers of wet sponge sheets until to obtain the leaf turgid fresh weight and then, they were oven dried at 70°C to obtain the total dry mass. The fresh weight of the disc, the turgid weight and the dry weight were used to estimate the RWC according to the formula established by Barr and Weatherley (1962).

Total chlorophyll content was determined in a combine sample of leaf discs removed from the external, middle and internal position in the head, following the method described by Inskeep and Bloom (1985) using 5,5-dimethylformamide as extractor. The absorbance of the filtrate was determined in a spectrophotometer at 647 and 664.5 nm and the results expressed in µg cm^{-2}.

Samples of five grams of leaves from the external, middle and internal position of the lettuce head were homogenized in 80% hot ethanol and centrifuged at 2000 rpm for 15 min. The pellet was then re-extracted twice with 80% ethanol, and the total soluble sugars were determined by the phenol-sulfuric acid reaction (Dubois *et al.*, 1956). From the same extract was determined the reducing sugars content by the Somogyi-Nelson method (Nelson, 1944). For total soluble sugars analysis sucrose was used as standard and glucose for the reducing sugar analysis. Afterwards, the pellet from the ethanolic extraction was dried at 65°C and then the starch was hydrolyzed in 52% perchloric acid for 30 min with shaking (McCready *et al.*, 1950). The procedure was repeated three more times. The quantification of starch was performed by the phenol-sulfuric acid reaction using sucrose as standard using the correction factor of 0.9. The total of non-reducing sugars was obtained by the difference between the total soluble sugars minus the content of reducing sugars.

The experiment was conducted in a split-plot scheme, with the treatments in the plots and shelf life in the subplots in a randomized block design with four treatments and four replicates per treatment. Each replicate was composed by one lettuce head. Individual analysis of variance was performed to evaluate the effect of the hydro cooling and temperature of storage by using the SAEG/UFV software, and the mean separation was done by Scott Knott test at 5% probability. The regression analysis was based on the regression coefficient using the t-test at 5% or 10% probability to establish the significance for the chosen regression model.

3. Results and Discussion

Initial temperature of the lettuce head showed a sharp drop within the first 10 min of hydro cooling time. The model that better explained the changes in temperature was exponential, with an estimated final temperature of 4.8°C after 10 min of hydro cooling (Fig. 1). Longer periods of cooling time did not remove additional field heat from the lettuce head. Based on the lettuce temperature record data, the total amount of heat removed from the lettuce, by the cold water under this experiment conditions, corresponded to 71% from the initial temperature (Fig. 1). The 87.5% or seven eighths cooling times recognized as the ideal theoretical reduction for the field heat presented by Brosnan and Sun (2001) was not achieved in this experiment, even after keeping the heads submerged in the cold water mixture for 50 min (Fig. 1). Using the same cooling technique of this experiment, Álvares *et al*. (2007) reported the removal of only 43% of the initial temperature in bunched parsley leaves. The reason why the hydro cooling of lettuce was much more efficient in removing the field heat than parsley remains to be the subject of further studies. Furthermore, the cooling time varied according to varieties of lettuce, as found by França *et al*. (2015) working with butter lettuce where the ideal hydro cooling time was 5 min instead

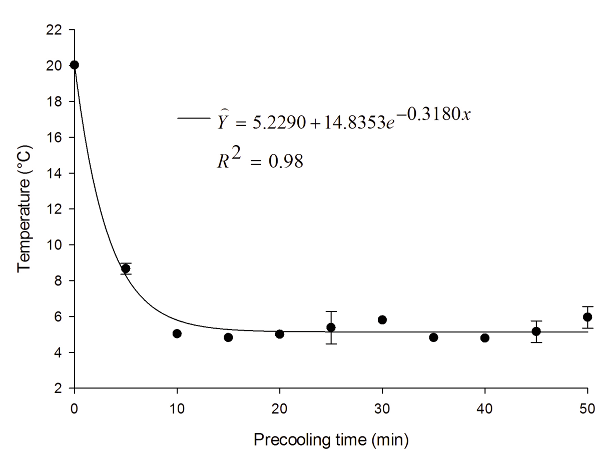

Fig. 1 - Influence of hydro cooling time period treatment on the temperature of iceberg lettuce 'Lucy Brown'.

of 10 min found for iceberg lettuce in this work (Fig. 1). The difference of hydro cooling time between the two cultivars of lettuce may relay on the thickness and compactness of the whole head. The leaves from iceberg lettuce are thicker and more compact head than butter lettuce, which can restrict the access of cold water into the more internal leaves of the head. In others leaf vegetables, including peppermint, coriander and basil, the ideal time of hydro cooling was 20, 10 and 16 min, respectively (Oliveira *et al*., 2015; Barbosa *et al*., 2016; Teixeira *et al*., 2016).

The end of shelf was established when symptoms of wilting and discoloration appeared in the external leaves (data not shown). Shelf life of hydro cooled lettuce and stored at 5°C was increased by 75% compared to not cooled lettuce, comprising a total of 168 h for the hydro cooled and 96 h for not cooled heads. For the lettuce that was hydro cooled and then stored at 22°C, the gain of shelf life was 50% or 72 h for hydro cooled and 48 h for not cooled lettuce. Álvares *et al*. (2007) also found beneficial effects to the shelf life of hydro cooled bunched parsley leaves followed by cold storage. The result of this study shows the importance of keeping the cold chain for fresh vegetables, but also shows the contribution of hydro cooling on extending the shelf life even without further cold storage. Because of the hydro cooling positive effects, the external leaves of the lettuce had a 72 h delay in showing wilt symptoms if stored at 5°C and 24 h delay for the lettuce stored at 22°C (data not shown). The increased shelf life of hydro cooled lettuce was determined by the higher leaf relative water content of hydro cooled lettuce (Table 1). The smaller effect of hydro cooling in the lettuce kept at 22°C compared to 5°C may be due to the greater gradient of water vapor between the leaf surface and the atmosphere of storage at 22°C. But, in a similar experiment with butter lettuce, the beneficial effect of hydro cooling on shelf life was greater for the lettuce stored at 22°C compared to the shelf life of hydro cooled heads followed by storage at 5°C (França *et al*., 2015).

Regardless the treatment, the rate of fresh weight loss was constant, resulting in linear accumulation up to the end of the lettuce shelf life (Fig. 2). The lowest rate of weight loss was determined in the lettuce stored at 5°C without hydro cooling (0.109% h^{-1}) and the highest for the heads hydro cooled and stored at 22°C (0.26% h^{-1}), as previously observed in a similar experiment with butter lettuce by França *et al*. (2015). The higher weight loss rate of hydro cooled lettuce was due to the water accumulated at surface and in between the leaves after being removed from the cooled water. Regardless if the lettuce was hydro cooled or not, the lower rates of weight loss found for heads stored at 5°C was determined by the smaller gradient of water vapor compared to the storage room at 22°C (Wills *et al*., 2010). Like in this experiment, when coriander leaves were stored at 5°C also had lower rates of weight loss compared to leaves stored at 20°C, regardless if the leaves were submitted to hydro cooling treatment (Oliveira *et al*., 2015). During the whole period of storage, the leaves of hydro cooled lettuce had higher relative water content when stored at 5°C (Table 1). This higher content of water found in the hydro cooled lettuce leaves was clearly observed in the appearance of lettuces, which were more turgid than those not hydro cooled, which resulted in longer shelf life due to fresher appearance. Similar results were found for hydro cooled butter lettuce heads, peppermint and coriander leaves, which also had higher relative

Table 1 - Influence of the hydro cooling and temperature of storage on the leaf relative water content during storage period of iceberg lettuce heads 'Lucy Brown'

Treatments	RWC (%)
Hydrocooled + storage at 5°C	96.4 A
Storage at 5°C	92.8 B
Hydrocooled + storage at 22°C	94.0 B
Storage at 22°C	92.4 B
CV %	3.2

Means followed by the same letter do not differ by the Scott Knott test at 5% probability.

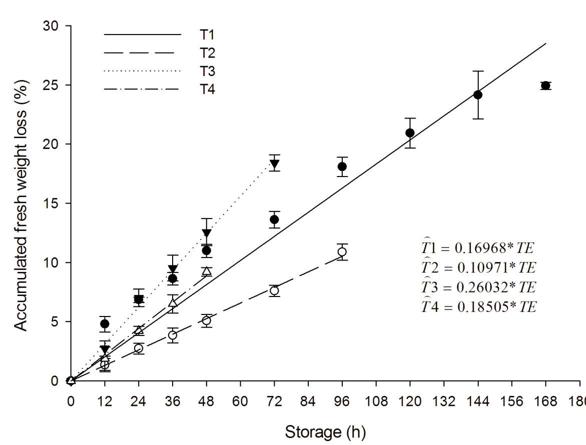

Fig. 2 - Accumulated fresh weight loss of iceberg lettuce 'Lucy Brown' submitted to the following treatments: T1) Hydro cooling for 10 min + storage at 5°C; T2) Control without hydro cooling + storage at 5°C; T3) Hydro cooling for 10 min + storage at 22°C; T4) Control without hydro cooling + storage at 22°C. TE= Time.

water content during their shelf life (França *et al.*, 2015; Oliveira *et al.*, 2015; Barbosa *et al.*, 2016). In conclusion, the hydro cooling treatment and storage 5°C resulted in higher level of water compared to not cooled heads, indicating the importance of field heat removal and followed by continuous cold chain.

Although hydro cooling reduced significantly the leaf chlorophyll content throughout storage for the lettuce stored at 5°C compared to the remaining treatments (Table 2), and the coefficient of variation was high (35.7%). This reflects the positions internal, middle and external which the leaf samples were taken. Because the relative water content present in the leaves of the hydro cooled lettuce stored at 5°C was higher, a much more favorable water status existed during storage. And at the same time, for the other treatments, the lower relative water content reflects a bigger dehydration rate of the cells, which resulted in higher chlorophyll concentration in the leaves (Table 1). But, the same effect on chlorophyll content induced by hydro cooling was not present on parsley and ora-pro-nobis leaves (Álvares *et al*., 2007; Barbosa *et al*., 2015). These differences may be related to the lower trend of parsley and ora-pro-nobis leaves in loosing water from the cell to the environment during storage.

Table 2 - Influence of the hydro cooling and temperature of storage on the total chlorophyll content during storage period of iceberg lettuce 'Lucy Brown'

Treatments	Total chlorophyll ($\mu g\ cm^{-2}$)
Hydrocooled + storage at 5°C	6.00 B
Storage at 5°C	7.74 A
Hydrocooled + storage at 22°C	7.69 A
Storage at 22°C	7.81 A
CV (%)	35.7

Means followed by the same letter do not differ by the Scott Knott test at 5% probability.

Hydro cooling had no effect on leaf carbohydrate changes during storage. However, there was significant decrease in the total soluble, reducing, non-reducing sugars and starch contents in the first 12 h of storage, either at 5 or 22°C (Table 3). In the first few hours after harvest, a much greater amount of carbohydrate is required to maintain high respiratory demand, coinciding with the highest physiological activity (Wills *et al*., 2010). In the study, during the first 48 h of storage there was a drop of 37.2, 24.5, 52.1 and 23.7% in the total soluble sugars, reducing, non reducing sugars and starch, respectively (Table 3). In a similar work, França *et al.* (2015) found similar decreases on non-reducing sugars and starch content of butter lettuce on the first 12 hours, but not on reducing sugars. The reduction of all carbohydrates found in this work, reflects the high demand of glucose and fructose to keep the respiratory activity even at low temperature of 5°C. Since, leafy vegetables do not store large amounts of carbohydrates; their storage potential for longer shelf life is much smaller than tubers and fruits, which have large amount of stored carbohydrates. Thus, further work with the use of controlled and modified atmosphere should be applied to increase iceberg lettuce shelf life.

Table 3 - General mean values of total soluble sugars (TSS), reducing sugars (RS), non-reducing sugars (NRS) and starch contents in 'Lucy Brown' icerberg lettuce stored at 5 or 22°C during the first 48 h of shelf life storage

Time (h)	TSS*	RS *	NRS *	Starch **
0	2.72 A	1.73 A	1.25 A	3.36 A
12	1.84 B	1.12 B	0.71 B	2.65 B
24	1.82 B	1.21 B	0.60 B	2.55 B
36	1.62 B	1.06 B	0.55 B	2.32 B
48	1.71 B	1.11 B	0.59 B	2.57 B
CV (%)	24.79	25.71	57.92	19.33

Means followed by the same letter do not differ by the Scott Knott test at 5% probability.

4. Conclusions

Regardless the temperature of storage, application of hydro cooling treatment removed most of the field heat with beneficial effects on quality, prolonging the shelf life of 'Lucy Brown' iceberg lettuce, by keeping higher water status in the cells and reducing discolorations in the leaves. Hydro cooling had no influence on carbohydrate metabolism of the leaves throughout storage either 5 or 22°C.

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