Effect of low temperature storage on physical and physiological characteristics of eggplant fruit (Solanum melongena L.)

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Abstract

Eggplant (Solanum melongena L.) is a perishable and chilling-sensitive tropical fruit. The chilling injury (CI) symptoms as well as some physical and physiological implications were studied in eggplants Money Maker No. 2 stored at 0 and 10 °C for 15 days. Eggplants stored at 10 °C were not damaged by temperature, whereas fruit stored at 0 °C suffered CI. Eggplant stored at 0 °C exhibited a decrease in L0 (lightness) and ΔL (oxidation potential), increase of pH and electrolyte leakage after CI symptoms are manifested. At this temperature, flesh tissue revealed ultrastructural damage. On the other hand, skin from upper fruit section showed more lightness, reddish colouration, and lower content of anthocyanins than the central fruit section at harvest and over the entire storage period at 0 °C. In fruit stored at this temperature and in upper section, changes of anthocyanin content with time were closely proportional to the Chroma evolution (lower content of anthocyanin, lower saturation of colour).

1. Introduction

Eggplant is a tropical fruit and it could present different shapes, sizes and colours, depending on the cultivar. Fruits are purple, white or striped. The purple type is commercially more important, and its colouration is caused by anthocyanins in fruit skin. Anthocyanins are pigments located in the plant cell vacuole (Timberlake, 1981) and belong to flavonoids group. Biosynthesis of different types of anthocyanins and flavonoids may continue after harvest and during air storage even at low storage temperature as found in pomegranate (Holcroft, Gil, & Kader, 1998) and blueberry (Kalt & McDonald, 1996). Sakamura, Watanabe, and Obata (1965) and Matsuzoe et al. (1999) have reported that a delphinidin derivative is the main anthocyanin present in purple eggplant.

It is well known that storage of fruits and vegetables at low temperature from harvest until consumption is an effective means for preserving quality and nutritional value. However, most fruits and vegetables originating from tropical areas, like eggplant, are chilling sensitive. Below 10 °C, eggplants suffer physiological disorders, manifested mainly by appearance of surface injuries, such as pitting and scald, seed darkening and flesh browning (Salunkhe & Desai, 1984, Chap. 4).

Eggplant quality and shelf-life are reduced by development of skin and tissue browning when stored at chilling temperatures. The information available on physical and physiological responses to chilling is dispersive. The aim of this paper was to study the evolution of chilling injury (CI) symptoms in eggplant cv Money Maker No. 2 stored at low temperatures (0 °C as chilling temperature and 10 °C as nonchilling temperature) and their effects on browning of pulp tissue, oxidation potential, electrolyte leakage (EL), ionic acidity, ultrastructural changes, surface colour and anthocyanin content.
2. Materials and methods

2.1. Plant material

Eggplant fruits (Solanum melongena L., cv Money Maker No. 2), provided by a La Plata (Argentina) farmer, were used for this study. This cultivar is from a Japanese variety, has a small size and elongate shape fruit. Harvested fruits were 11–15 cm long, weighing between 50 and 100 g each. As other cultivars, it has a dark purple calyx and very dark purple skin. In this cultivar, the calyx fruit size is important (12% of total fruit surface) and the skin colour under this zone is lighter than elsewhere in the fruit. For this reason, in the present study two sections of the fruit skin will be distinguished: central and upper.

2.2. Storage conditions

Fruits of the same shape and ripening stages were harvested. After eliminating defective fruits, eggplants were washed with water, drained, and randomly divided into groups of 6 units to be packed in perforated, low-density polyethylene (LDPE) bags and placed at 0 or 10 °C in temperature-controlled stores. For each temperature, two bags, containing 6 fruits each, were removed every sampling day from the store. All fruits were utilized to determine CI symptoms. Then 6 fruits were employed for ultrastructural analysis, colour and anthocyanin content determinations. The skin of each fruit was frozen in liquid N₂ and stored at −80 °C. Other 3 fruits were used to determine ionic acidity (pH), browning (L₀) and oxidation potential (ΔL) of the pulp; and another 3 fruits were analysed for EL. The complete experience was repeated 3 times.

2.3. Ci index in fruits

On each sampling day, both internal and external CI symptoms were visually observed. The degree of CI severity was calculated according to the following scale, similar to that proposed by Lederman, Zauberman, Weksler, Rot, and Fuchs (1997): 1 = no damage, 2 = low damage, 3 = regular damage, 4 = moderate damage, 5 = severe damage. The CI index (CIᵢ) was calculated according to the following equation:

CIᵢ = \frac{\sum \text{(Injury level} \times \text{Number of fruits on the level)}}{\text{(Total number of fruits in the treatment)}}.

Observations were made on 12 fruits for each temperature and storage time, and the results were averaged.

2.4. Determination of surface colour

A Minolta Colorimeter model CR-300 (Osaka-Japan) was used to determine the skin colour of fresh fruits. To analyse colour difference between sections (central and upper), colour parameters (a* and b*) were measured on 15 fruits harvested with different colour intensity. On the other hand, during storage, colour was characterized by the following parameters: lightness (L*), Hue angle (H¹ = tan⁻¹(b*/a*)) and Chroma (C* = (a*² + b*²)¹/₂). For these points, skin colour values were obtained using 6 fruits each time, by 3 determinations for each fruit and section.

2.5. Anthocyanin extraction and quantification

Anthocyanin were extracted from skin of each eggplant section (upper and central), separately. The skin of 6 fruits were removed, frozen in liquid N₂, powdered in a mill (model A10 Janke & Kunkel—IKA Labortechnik) and 0.5 g were extracted at least 3 times with concentrated HCl–methanol (1:100, v/v) mixture. After centrifuging at 10,000 g for 5 min, the supernatant was further diluted to 50 ml and its absorbance measured at 538 nm using a spectrophotometer (Beckman DU 650) (Esteban, Mollá, Villarroya, & López-Andréu, 1989). Anthocyanin content was expressed as OD (at 358 nm) g/ml of fresh tissue. Three extracts were prepared for each section and condition analysed, and measured by triplicate.

2.6. Browning of pulp tissue

A Minolta Colorimeter model CR-300 (Osaka-Japan) was used to determine the lightness of pulp tissue by parameter L* (0 = black and 100 = white). A 0.5 cm wide cross-section was excised from the central section whose pulp colour was rapidly measured. Results were expressed as L₀, as the mean of three fruits per storage time and temperature. It was observed that L₀ value of whitish good quality pulp was ≥86, while seed browning showed L₀ values between 81 and 82. An incipient browning of pulp and seeds showed L₀ values near 78, and severe browning of seeds and pulp implicate L₀ values ≤73.

2.7. Oxidation potential

The oxidation potential was estimated using the Larri-gaudiere, Lentinher, and Vendrell (1998) method, with little modifications. A Minolta Colorimeter model CR-300 (Osaka-Japan) was used to measure the colour parameter L* on a slice obtained as described recently. Colour was measured immediately after cutting (L₀) and after 30 min (L₃₀). The oxidation potential was expressed as ΔL = (L₀−L₃₀). All measurements were means of three fruits per storage time and temperature.

2.8. Electrolyte leakage analysis

Tissue discs (3 mm thick, 10 mm diameter) were prepared using endocarp and mesocarp tissues from the central and upper section, separately. Discs (2 g) from 3 randomly fruits were ground in 20 ml mannitol 0.6 mol/l. Conductivity of the surrounding solution was measured at 20 °C with a conductivity meter at the beginning and after 2 h. The
tissue was homogenized and centrifuged at 17,500g for 15 min at 20°C and total electrolytes measured. Results were expressed as a percentage of total electrolytes per weight of fresh tissue (EL/g). Results are the mean of duplicate measurements in each fruit section per storage condition.

2.9. Ionic acidity assay

Fruit samples were homogenized with distilled water in an Omnimixer (Dupont Instruments Sorvall®) and pH was measured with a pH meter. Homogenates were prepared in duplicate for each storage condition.

2.10. Transmission electron microscopy (TEM)

Eggplant flesh was excised from endocarp and mesocarp from central section of 6 fruits at 0, 6 and 15 days. Eggplant flesh was removed by scalpel blade in pieces of about 5 x 2 x 2 mm. The excised samples were fixed for 24 h with 6 ml/100 ml glutaraldehyde in 0.1 mol/l phosphate buffer (pH 7.4) at 4°C. Fixed samples were then washed with the same phosphate buffer for at least 1 h at room temperature and post-fixed in 2 ml/100 ml osmium tetroxide for 1 h at room temperature. The specimens were dehydrated in an ethanol gradient to absolute ethanol and embedded in Spur’s resin. Sections were cut with a diamond knife using a LKB ultramicrotome. Sections were mounted on 200 m copper slim-bar grids. The sections were stained in a plumb citrate, pH 12, for 20 min and then in a saturated solution of uranyl acetate in ethanol–water 3:100, v/v) for 45 min. Sections were rinsed twice in bidistilled water. Photographs and observations were made on a JEOL 100 CXII (Japan) transmission electron microscope at 80 keV.

2.11. Statistical analysis

A factorial design was employed defining storage temperature (3 levels) and storage time (6 levels) as factors for all studies. In some experiments, the fruit section (2 levels) was added as another factor. Data were studied by analysis of variance (ANOVA); and means were compared using LSD. All comparisons were carried out at a significant level of P = 0.05.

3. Results and discussion

3.1. CI symptoms development

No symptoms of CI were found in fruits stored at 10°C (nonchilling temperature). In turn, CI symptoms of fruit were observed during storage at 0°C (Fig. 1).

The visual aspect of fruit stored at 0°C changed after 2 days of storage, when fruit exhibited a slight skin discolouration (CI ¼ 1.4). The effect was more marked in the skin under the calyx (upper section), where colour turned from purple to light purple. After day 6, brightness losses in the skin, presence of an incipient pitting in upper section and seed browning were observed (CI ¼ 2.3). After day 13, internal pulp browning and scalds in fruit skin were evident, resulting in a CI of 4.2. Therefore, the main effect of CI symptoms was storability reduction and consumer acceptance. Fruit lost commercial quality after 9 days at 0°C.

3.2. Browning and oxidation potential changes in pulp tissue

Lightness of a recently sliced fruit (L0) was a good indication of browning evolution of fruit tissue during storage time. L0 readings of fruit stored at 10°C (Table 1) were constant over storage time, being values near 88. On the other hand, in fruit stored at 0°C, L0 (Table 1) decreased gradually up to day 15. From day 13 on, low values indicated severe browning of seed and pulp. Values of L0 and CI were related to a second-order polynomial, with high accuracy (s = 1.510; P<0.05).

The oxidation potential (∆L) was another parameter that provides information on browning of pulp tissue. At harvest ∆L was 6.21 (Table 1), but this level decreased gradually until day 6 and thereafter remained mostly constant in fruits stored either at 10 or 0°C (Table 1), though the decrease in ∆L was more pronounced at 0°C than at 10°C. A negative and exponential correlation was found between ∆L of fruit stored at 0°C and CI (s = 0.510; P<0.05).

As judged by ∆L and L0 values, fruits with cold-induced browning did not produce much further browning after being sliced. Similar results were previously reported by Larrigaudiere et al. (1998) for pears. In a previous work (Concellén, Añón, & Chaves, 2004) the enzyme polyphenol oxidase (PPO) activity was found to decrease with time after browning manifested, so the chilled fruit would not experience further browning in the present work. This decline in browning potential could also be explained by concentration decrease in fatty and organic acids or else by a decrease of phenolic synthesis (Spanos & Wroslstad, 1992).
3.3. Changes in electrolyte leakage

The EL percentage from pulp of eggplant fruit was used as an indirect measure of membrane damage, and has been used to determine the extent of CI (Murata, 1989). The initial value was similar between central and upper section and near to 3.87%/g (Table 1).

In fruits stored at 10°C (Table 1) a little variation throughout storage was observed in both upper and central sections. In contrast, at 0°C (Table 1), both upper and central sections showed a similar increase after 6 days, being the value measured approximately twice as high as the initial. After that, the upper section showed a faster increase than the central until day 13, when values were 9.5 and 5 times higher than initial, respectively. Thereafter, EL values remained practically unchanged in both sections.

Increases of EL with storage time at low temperatures were also measured in cucumbers (Kuo & Parkin, 1989). The increase showed by fruits stored at 0°C coincided with the increase in CI and browning of pulp tissue (lower L0), which possibly indicates the beginning of disruption of cell structures and membranes. Palma, Marangoni, and Stanley (1995) also found a good correlation between the increase in EL and membrane injury. On the other hand, the higher values in upper section were in agreement with high symptoms of CI in this section.

3.4. Changes in ionic acidity

At harvest, pH from pulp of eggplant fruit was 5.54. At 0 and 10°C, pH showed a modest increase at day 2 reaching a pH value of 6.06 (Table 1). Then, this value remained unaltered during the entire length of storage at 10°C. In turn, at 0°C the increase continued until day 13 and thereafter remained constant around pH 6.48.

3.5. Ultrastructural changes

Flesh tissue from eggplant was analysed by TEM at harvest and after 6 (incipient damage) and 15 days (severe damage) at 0°C. Parenchyma cells size varied considerably in the same tissue, all displaying closely appressed, turgid cells, with thin primary walls (Fig. 2a). The cells contained a large, central vacuole with intact tonoplast, and a peripheral cytoplasm confined to a narrow layer adjacent to the cell wall. The middle lamella was visible as a relatively electron-dense region between the primary walls (Fig. 2b). After 6 days at 0°C, cells damage was generally modest. Cells showed intercellular spaces (Fig. 3a) and the cytoplasm together with the plasma membrane became separated from the wall (Fig. 3b). A size increase of intercellular spaces between chilling injured cells was also reported by Brovell, Brecht, and Sherman (1998). Organelles retained their characteristic aspect. Plasma membrane was intact but tonoplast was affected in some cells.

### Table 1

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<th>Days at 10°C</th>
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<th>pH</th>
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| LSD          | 1.86 | 0.65 | 0.35 | 0.07                  | 0.07                  |

*Values are expressed as percentage of electrolyte leakage/g of flesh tissue.

Fig. 2. Transmission electron micrographs of flesh tissue from eggplant at harvest: (a) × 2700 and (b) × 20,000. CT = cytoplasm; CW = cell wall; ML = middle lamella; PW = primary wall; T = tonoplast; V = vacuole.
Chabot and Leopold (1985) have demonstrated that the plasma membrane preserved its osmotic regulatory activity during periods of chilling. After 15 days at 0°C, severe symptoms of CI were observed. Micrographs showed cellular disruption (Fig. 4a) or contraction of cytoplasm and plasmatic membrane (Fig. 4b). Cells showed irregular contour and thickness, with vesiculated appearance (Fig. 4b). Cellular material was dispersed (Figs. 4b and c) by disruption of membranes and organelles. Niki, Yoshida, and Sakai (1978) have suggested that tonoplast disruption may be responsible for irreversible chilling damage in callus tissue of *Cornus stolonifera*. They have postulated that tonoplast degradation would release toxic substances and autocatalytic enzymes that may degrade cellular components.

3.6. Changes in colour

As mentioned before, eggplant fruit *cv Money Maker No. 2* have different intensity of skin colour: dark purple in the central section and light purple in upper section. At harvest, 15 fruits were analysed and they show that upper section presents higher positive values of *a* (Fig. 5) and lower and negative values of *b* than the central section. Therefore, Hue values (*H*) for the upper section are always negative while Chroma (*C*) values are higher than of central section. Values of *L* were higher in upper than in the central section (data not shown).

During storage, visual changes in colour were well reproduced by *C*, *H* and *L* values. Central section showed less colour variations than upper, probably due to dark purple colouration present in the first section. Thus,
in central section (Table 2), values of C* were essentially constant at all temperatures. In this section, the other parameter, $H^\text{r}$, showed an increase at day 2 and decreased from day 6 on, at either 10 or 0 °C. These changes were higher at 0 °C than at 10 °C. On the other hand, in central section values of L* kept mostly unchanged throughout the storage time of fruits at 10 °C. In turn, lightness slightly decreased after 2 days at 0 °C, then remained virtually constant until day 13 and finally increased at day 15 to recover the initial value.

In the upper section, changes in colour parameters during fruit storage were more important than in the central section (Table 2). At harvest, C* values in the upper section were 14 times higher than in the central section. This ratio showed little variations along storage. C* values in upper section were generally lower than the initial during storage, regardless of temperature. C* decreased at day 2 at both temperatures and then began to increase at 0 °C up to a maximum at day 9, comparable to the initial value. The parameter $H^\text{r}$, in upper section, did not vary in practice during storage at 10 °C. At 0 °C, it kept rather constant until day 13 and then experienced a sharp increase to a positive value at day 15. Finally, parameter L* in the upper section showed slight variations along the entire storage time at 10 °C. However, at 0 °C this parameter gradually increased until the end of storage, attaining a value 30% above the initial. In general, lightness in the upper section (L*) and saturation (C*) were higher than in the central section at harvest and throughout storage at both temperatures tested.

Results indicate that only those fruits stored at 0 °C showed significant changes in colour parameters especially in the upper section. In general, surface CI symptoms were related to variation in colour parameters. For example, discolouration observed mainly in the upper skin after day 2 was related to a decrease of C*, whereas the evolution of yellow-brown colouration owing to scalds was related to a C* decrease and to increases of $H^\text{r}$ and L*. Colour changes were used to indicate evolution of CI symptoms in several fruits. Thus, in cucumber (Martínez-Romero, Serrano, & Valero, 2003), the occurrence of browned spots on the skin surface was associated with a strong reduction in the $H^\text{r}$ angle.

### 3.7. Changes in anthocyanin content

To determine anthocyanin content, wavelengths scans (240–700 nm) were carried out for samples from both sections.
sections. The same spectrum was found, so the same type of anthocyanins was present in both sections. The maximum absorbance was recorded at 538 nm, so this value was used as the wavelength for anthocyanin content determinations. Apparently, the colour difference between fruit sections was caused by the higher anthocyanin content in the central section. At harvest, anthocyanin content averaged 0.224 OD/g/ml in the central section and 0.161 OD/g/ml in the upper section, 30% below the former value.

Usually, low temperature affects pigment contents. Here, the difference in anthocyanin content between fruit sections at harvest was maintained over the storage period (Table 2). In central section (Table 2) of fruits stored at 10 °C anthocyanin contents decreased by only 13% after day 2 and remained constant from then up to the end of storage. At 0 °C, the anthocyanin content in central section decreased by 38% at day 2 and then increased up to day 9 showing a maximum 15% below the initial value.

The anthocyanin content of upper section (Table 2) decreased by 56% over the first 6 days at 10 °C and then kept practically unaltered until day 15. At 0 °C, a pronounced decrease by 73% was observed at day 2. Then, there was an increase up to day 9, to reach half the initial value, level that was maintained until end of storage. Anthocyanin levels in the upper section were always lower than in the central.

Here, although modifications of colour parameters were more pronounced in the upper section, variations in anthocyanin content were observed in both sections. To explain differences between colour and anthocyanin results, it was taken in mind that the fruit skin consists of a few layers of cells which experience progressive browning, which begins in the surface cells and continues towards the inner layers. The surface colour can only detect the browning of the surface cells, while anthocyanin content comprises the all layers of the cuticle. Therefore, the relationship between surface colour and anthocyanin content could exist only at the beginning of the storage (health cells) and also when the browning is complete in the skin (damaged cells), but not in intermediate stages. In spite of this, variations in anthocyanin content with time were related to the evolution of C* (lower anthocyanin content, lower colour).

During storage, changes of anthocyanin content were more marked at lower temperature, and at both temperatures the final contents after storage were lower than the initials. Similar results were described by López-Andréu, Molla, Fernández, and Esteban (1988) working with “Redonda Negra Lisa” variety of eggplant. Here, the decrease of anthocyanin content after day 2 could be associated with skin discolouration. This may be related to the anthocyanin function in scavenging active oxygen species (AOS), which could be produced during cold storage. Anthocyanin is one of the flavonoid compounds with stronger reducing power, and possibly plays some important role as antioxidant and regulating the redox state of the cell (Leng & Qi, 2003). Some experiments carried out in vitro showed that anthocyanin can scavenge superoxide radicals (Yamasaki, Uefuji, & Sakihama, 1996) and hydrogen peroxide (Leng & Qi., 2003). The enzyme phenylalanine ammonia lyase (PAL), necessary for flavonoids biosynthesis, was reported to undergo an activity increase in response to thermal stress (Leyva, Jarillo, Salinas, & Martinez-Zapater, 1995). Therefore, the activation of PAL enzyme re-establish levels of phenolic compounds (Peiser, López-Gálvez, Cantwell, & Saltveit, 1998). Here, it is considered that the observed increase in anthocyanin levels in both sections after day 2 could follow PAL enzyme activation. The rise of pigment levels halted after 9 days at 0 °C, when scalds appeared on the fruit skin, and then the levels declined. The chemical lability of these compounds and the possibility of acting as substrates of the enzymatic oxidation by PPO was proposed by several authors (Sakamura et al., 1965; Esteban et al., 1989).

Interestingly, the central section of eggplant was less affected by 0 °C and kept a higher content of anthocyanins. It could be proposed that its antioxidant capacity may contribute to delay or diminish damage. Wang and Stretch (2001) have also found a positive correlation between the increase of anthocyanin content during low temperature storage and the antioxidant capacity of various cranberry cultivars.

4. Conclusions

The chilling injury index (CI) of eggplant cv Money Maker No. 2 experienced a significant increase over 15 days of storage at 0 °C, and symptoms were more severe in upper section. On the contrary, no evolution of damage was observed at 10 °C. The strong increase in flesh browning at 0 °C was represented by a decrease in lightness (L0) and oxidation potential (ΔL). Moreover, at this temperature, the onset of disruption in cell structures and membranes, observed by TEM micrographs, correlated with an increase of EL. Colour parameters from upper section were more affected by storage at 0 °C than those from the central section. However, changes in anthocyanin content were observed in both sections. The anthocyanin content in central section was higher than in upper section at harvest and during storage. This was possibly a consequence of the weaker damage in central section, since anthocyanins are known to have antioxidant properties.

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