# A CASE STUDY OF TENDRAL WINTER MELONS (*CUCUMIS MELO* L.) POSTHARVEST SENESCENCE

***Bárbara Albuquerque[1,2,*], Fernando C. Lidon[1], Maria G. Barreiro[2]***

*[1] Unidade de Biotecnologia Ambiental, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Quinta da Torre, 2829-516 Caparica, Portugal*

*[2] Departamento de Fisiologia Vegetal da Estaão Agronymica Nacional, Quinta do Marquĸs, 2780 Oeiras, Portugal*

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***Summary.*** *Tendral* type cultivar melons (*Cucumis melo* L., *inodorus* group, *Winter melon* variety) were stored for a 75-d postharvest period in a ventilated chamber at 10 ºC ± 1 ºC and 75% ± 5 % RH. Qualitative degradation of the fruit was observed mainly involving proteins and ascorbic acid. It was also found that the sugar/acid ratio increased while the glucose/fructose ratio decreased, thus making the fruit sweeter. Sucrose was the predominant sugar found and glucose was the major reducing sugar. Citric acid was the major component of the organic acid fraction of the *Tendral* mesocarp. The ACC oxidase activity declined as the fruit became overripe. Membrane degradation linked to the accumulation of hydroperoxides was modulated by a progressive failure of the antioxidant enzymes system. A reduction in superoxide dismutase activity as well as in catalase activity was observed. It can be concluded that the oxidative status of *Tendral* melons during postharvest storage increased progressively.

***Keywords:*** fruit quality, oxidative stress, postharvest storage, winter melon

***Abbreviations:*** ACC – 1-aminocyclopropane-1-carboxylic acid**,** CAT – catalase, $H_2O_2$ -hydrogen peroxide, HPLC – high performance liquid chromatography, RH – relative humidity, SOD - superoxide dismutase, SSC – soluble solids content

* Corresponding author, e-mail: baba_albuquerque@hotmail.com

## INTRODUCTION

Melon (*Cucumis melo* L.) is a commercially important crop in many countries, being cultivated in all temperate regions of the world, in part due to its good adaptation to soil and climate (Villanueva et al., 2004). According to FAO, in 2002 this fruit production in Europe was higher than 3 million tons, with a 25 % increase for the last 10 years.

*Inodorus* group melons include, among other varieties, *Honeydew*, *Crenshaw*, *Casaba* and *Winter melon*. They have smooth rinds and do not have a musky odor. Previous studies with this group melons showed that its normal shelf life is about 14 days (Ryall and Lipton, 1979). When melons are stored under cool (10 ºC range) and high-humidity (80 - 90 %) conditions, they could be preserved for about 3 weeks (Hardenburg et al., 1986).

*Tendral* type cultivars (*Winter melon* variety, *Inodorus* group) are with dark skin and greenish flesh (Pardo et al., 2000) and can be stored relatively longer, allowing an extension of the commercialisation period practically until Christmas and ensuring that it attains the highest market value (Pintado et al., 2003).

Camara et al. (1995) defined quality as a set of internal and external characteristics that can be appreciated by human senses. In the particular case of melon, sensory evaluation criteria should be complemented with instrumentally determined parameters, such as pH, soluble solids content (SSC), firmness and both external and internal color, for the proper characterization of the product (Pardo, 2000).

In higher plants, senescence is characterized by the breakdown of cell wall components. Membrane disruption leads to cellular decompartmentation and loss of tissue structure (Paliyath and Droillard, 1992). Free radicals derived from oxygen are largely involved in the senescence processes, particularly in membrane deterioration (Droillard et al., 1987). They induce the peroxidation of membrane lipids, thus resulting in a loss of membrane integrity and membrane-bound enzyme activities (Bartoli et al., 1996).

This work aimed to investigate the changes in fruit quality during a 75-days postharvest period, by analysing intrinsic parameters coupled to the oxidative stress triggered during storage.

## MATERIALS AND METHODS

### Plant material, external characteristics and storage conditions

In 2003, *Tendral* melons (*Cucumis melo* L. *Inodorus* group) were grown outdoors using commercial growing conditions in Elvas (Portugal). Seeds used were maintained in the Estação Agronymica Nacional, Oeiras, Portugal. Fruits were collected

in late August. They weighed 2.31 ± 0.09 kg, and had a diameter, height and volume of 14.7 ± 0.2 cm, 20.5 ± 0.5 cm and 2.242 ± 0.156 $dm^3$, respectively. They were stored in a ventilated room (10 ºC ± 1 ºC and 70 ± 5 % RH) for 75 days.

**Physicochemical analysis**

The analyses of flesh firmness, pulp colour, total SSC and titratable acidity were carried out according to Barreiro et al. (2001). They were carried out 20, 40, 60 and 75 days after harvest. The measurements were done in three replicates of five fruits each.

*Flesh firmness:* Flesh firmness was estimated in the equatorial hipodermal mesocarp tissue, using a Bellevue penetrometer, by the resistance of the flesh to the penetration of a standard plunger (1 cm long x 8 mm diameter) and expressed as a mean force in Newtons.

*Pulp colour:* The hipodermal mesocarp tissue colour was measured using a Minolta CR-300 colorimeter to provide a specific colour value based on the amount of light transmitted through the commodity. Colour values were expressed on the CIE Colour System with the L*C*Hº axis representing lightness, chrome and hue angle, respectively.

*Total SSC:* A hand refractometer Atago ATC-1 was used for measuring total SSC in extracted juice, at 20 ºC. The refractometer measures the refractive index, which indicates how much a light beam will be slowed down when it passes through the fruit juice.

*Titrable acidity:* Titratable acidity was determined by titrating 10 ml of fruit juice with 0.1 N NaOH, to an end point of pH 8.2, as indicated by a pH meter CD 7000 WPA. The volume of NaOH needed was used to calculate the titratable acidity, using a multiplication factor of 0.64 (since citric acid is the major acid presented).

**Physiological analyses**

*Protein content:* Protein content was measured as described by Lowry et al. (1951), with minor modifications, to minimize the absorbance of interfering substances (Lidon, 1994). After addition of 5 ml of Lowry C reagent and 500 µl of Folin reagent to 500 µl of diluted melon juice (1 g of fruit mesocarp flesh homogenized with 10 ml distillated water), samples were maintained at 25 ºC for 30 min. The absorbance was determined at 540 nm, using three replicates of five fruits on days 20, 40, 60 and 75 after harvest.

*Electrolyte leakage:* Electrolyte leakage was measured using the method of Gemma et al. (1994), with few modifications, 20, 40, 60 and 75 days after harvest. Three replicates of five fruit discs mesocarp tissue (8 mm in diameter) were incubated in a beaker with 10 ml distilled water for 3 h with slight agitation at 25 ºC.

Conductivity of solution was measured using a GLP31 (Crison) dip cell and a conductivity bridge. Samples were submitted to 90 ºC for 1 h, and electrolyte leakage was measured again to determine total electrolyte leakage.

*Acyl lipids peroxidation:* Acyl lipids peroxidation was based on the thiobarbituric acid (TBA) test (Böhme and Cramer, 1971), using three replicates from five fresh fruit discs of mesocarp tissue 20 and 60 days after harvest. This test, which measures malonaldehyde as an end product of lipid peroxidation, was performed using 0.1 % of trichloracetic acid (TCA) solution. Homogenates were centrifuged at 15000 x *g* for 10 min and 0.5 ml of the supernatant obtained was added to 1.5 ml 0.5 % TBA in 20 % TCA. The mixture was incubated at 90 ºC in a shaking water bath, and the reaction was terminated by placing the reaction tubes in an ice-water bath. The samples were centrifuged at 10000 x *g* for 5 min and the absorbance of the supernatant was read at 532 nm. The absorbance was determined at both 440 and 600 nm to minimize the interference of carbon skeletons. A coefficient of 155 mM $cm^{-1}$ was used.

*Sugar quantification:* Sugar extraction was carried out on 20, 60, 75 days after harvest following the method of Hudina and Stampar (2000). Sample of 20 g of 10 fruit fresh mesocarp flesh were dissolved in 100 ml distillated water and centrifuged at 15000 x *g* for 15 min at 4 ºC. Filtration was carried out through Whatman No 4 and Millipore 0.45 µm filters. Sugars were identified and quantified by HPLC using a Waters R401 refractive index detector and a Sugar-Pack (Waters) column kept at 90 ºC. A flow rate of 0.5 ml $min^{-1}$ was applied to an aqueous mobile phase of EDTA-Ca (50 µL $L^{-1}$). An aliquot of 20 µl was injected. Each sample was analyzed in three replicates.

*Acid quantification:* Acid extraction was similar to that of sugars, but thereafter, for the isolation and characterization, a Beckman Gold 168 diode-array detector together with an Aminex HPX 87H (BioRad) column were used. A flow rate of 0.5 ml $min^{-1}$ was applied at room temperature to a mobile phase of 5 mM $H_2SO_4$. An aliquot of 20 µl was injected. Each sample was analyzed in three replicates.

*Ascorbic acid quantification:* The concentration of ascorbic acid was determined 20, 60 and 75 days after harvest as previously described (Romero-Rodrigues et al., 1992). A sample of 20 g mesocarp flesh from 10 fruits were homogenized with 30 ml of 6 % metaphosphoric acid and then centrifuged at 15000 x *g* for 25 min at 4 ºC. Filtration was carried out twice through Whatman No 4 filter paper. Volume was brought to 100 ml with 6 % metaphosporic acid and samples were again filtered through Millipore 0.45 µm filters. Ascorbic acid was quantified using HPLC with a Waters 440 detector and a Spherisorb ODS2 5 µm (Waters) column. A flow rate of 0.4 ml $min^{-1}$ was applied at room temperature to a mobile phase of $H_2SO_4$ (pH 2.3). An aliquot of 20 µl was injected. Each sample was analyzed in three replicates.

## Enzyme assays

In the assay conditions for measuring enzymes activities, the applied substrate was saturated in order to obtain maximum apparent activities.

*Enzymes implicated in the control of the oxidative stress:* These enzyme activities were determined 20 and 60 days after harvest using fresh mesocarp tissue discs. The analyses were done in triplicate with five fruits each. Superoxide dismutase activity was determined following the method of McCord and Fridovich (1969). Catalase activity was measured according to Patra et al. (1978). The analyses of ascorbate peroxidase followed the method of Nakasano and Asada (1981) whereas dehydroascorbate reductase and glutathione reductase activities were measured following the procedure of Dalton et al. (1986).

*ACC oxidase extraction and quantification:* ACC oxidase extraction was performed using a modified method of Dong et al. (1992). Samples of 100 g from 10 fruits mesocarp flesh were homogenized with 150 ml of 400 mM potassium phosphate buffer (pH 7.2) containing 10 mM sodium bisulphite, 3 mM sodium ascorbate, 5 mM dithiothreitol (DTT) and 4 mM 2-mercaptoethanol. The homogenate was squeezed through 4 layers of cheesecloth, centrifuged at 15000 x *g* for 40 min at 4ºC and the supernatant discarded. The pellet was resuspended in 20 ml 25 mM HEPES (pH 7.2) containing 1 mM DTT, 3 mM sodium ascorbate and 30% glycerol (v/v) and stirred for 15 min. Triton X-100 (0.8 %) was added and the mixture, stirred for 15 min and centrifuged at 15000 x *g* for 30 min at 4ºC. The supernatant was assayed. Sample analysis was carried out according to Vioque and Castellano (1994). A volume of 0.9 ml of a standard reaction mixture (100 mM HEPES (pH 6.7), 1 mM ACC, 0.2 mM $FeSO_4$, 10 mM sodium ascorbate, 16 % $CO_2$ in the gas phase) was added to 0.1 ml of enzyme solution. Incubation was carried out at 30 ºC for 30 min, in sealed 10 ml vials. The production of ethylene was determined by gas chromatography, using a Gas Chromatographs Pye Unicam Series 204, with a Porapak Q column and a flame ionization detector. A nitrogen flow rate of 30 ml $min^{-1}$ was used as a gas carrier. In the oven, temperature was set to 100 ºC, the injection port was kept at room temperature, the detector being maintained at 150 ºC. Three replicates of each sample were injected. Ethylene was identified and quantified by comparison with a peak area of a standard ethylene gas sample (29 μL $L^{-1}$), 20, 60 and 75 days after harvest.

## Quality parameters

Sensorial quality was determined at individual stations by a 10-member preference panel. Each station displayed melon cubes of middle mesocarp tissue taken from the equatorial region of the fruit. The panellists judged the external appearance, texture and flavour of melons. A continuous scale of 5 was used for each parameter (1- Bad;

3 – Acceptable; 5 – Very Good). Global consumer acceptance was determined by the sum of external appearance, texture and flavour (multiplied by a 3, 7 and 10 factor, respectively).

### Statistical analysis

Statistical analysis of data was performed using one-way analysis of variance (ANOVA) (P d" 0.05) applied to the studied parameters. Based on the ANOVA results, a Tukey's test was performed for mean comparison, for a 95 % confidence level. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level.

## RESULTS AND DISCUSSION

The effect of storage on flesh colour (Table 1) affected mainly the hue angle which implicated the development of the yellow colour in fruit. Even though there was no change in fruit colour (a negative correlation factor of 96.2% between C* and H$^o$ was found). In contrast, total SSC, although displaying slight fluctuations, remained stable, as previously documented by other authors (Ogle and Christopher, 1957; Dumas de Vaulx and Aubert, 1976; Evensen, 1983). Fruit juice pH increased during the 75 days of storage (Fig. 1) leading to a decrease of titrable acidity (c. a. 44 %), due to organic acids degradation and consumption. These data further supported the finding of Barreiro et al. (2001). The initial pH of the juice correlated closely during the postharvest period in 89 %, whereas that of titrable acidity reached c.a. 93.5 %. In

**Table 1.** Physicochemical characterization of *Tendral* melon fruit during the postharvest storage period. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of Tukey's test.

| Days after harvest | Colour | Firmness (N) | SSC (%) |
|---|---|---|---|
| **20** | L 68.469 ± 1.310 **(a)**<br>C 15.508 ± 0.436 **(a)**<br>H$^o$ 107.754 ± 0.477 **(a)** | 15.63 ± 1.08 **(a)** | 10.01 ± 0.61 **(a)** |
| **40** | L 68.499 ± 1.012 **(a)**<br>C 15.477 ± 0.416 **(a)**<br>H$^o$ 106.784 ± 0.621 **(ab)** | 16.99 ± 0.80 **(b)** | 9.43 ± 0.44 **(a)** |
| **60** | L 67.852 ± 1.433 **(b)**<br>C 17.451 ± 0.672 **(a)**<br>H$^o$ 103.129 ± 0.690 **(c)** | 16.48 ± 0.48 **(ab)** | 10.56 ± 0.47 **(a)** |
| **75** | L 63.001 ± 2.090 **(b)**<br>C 16.486 ± 0.743 **(a)**<br>H$^o$ 104.353 ± 0.851 **(bc)** | 19.25 ± 0.77 **(b)** | 9.63 ± 0.67 **(a)** |

addition, both parameters displayed a correlation of 90.2 % which indicates the occurrence of similar patterns for the quantification of free and conjugated acids.

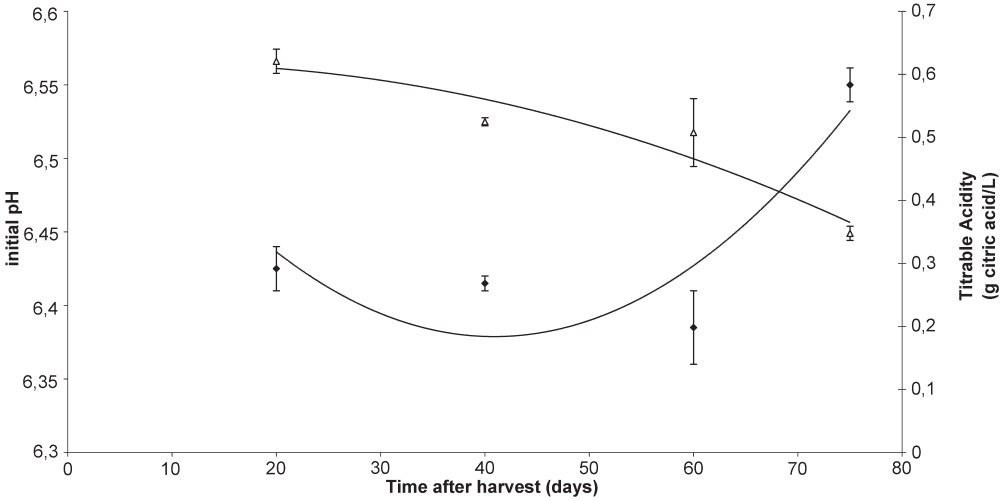


**Fig. 1**. Initial fruit juice pH (♦) ($r^2$ = 0.910) and titrable acidity (Δ) ($r^2$ = 0.997) during the postharvest storage period of *Tendral* melons. Each value is the mean of three replicates of five fruits each ± standard error.

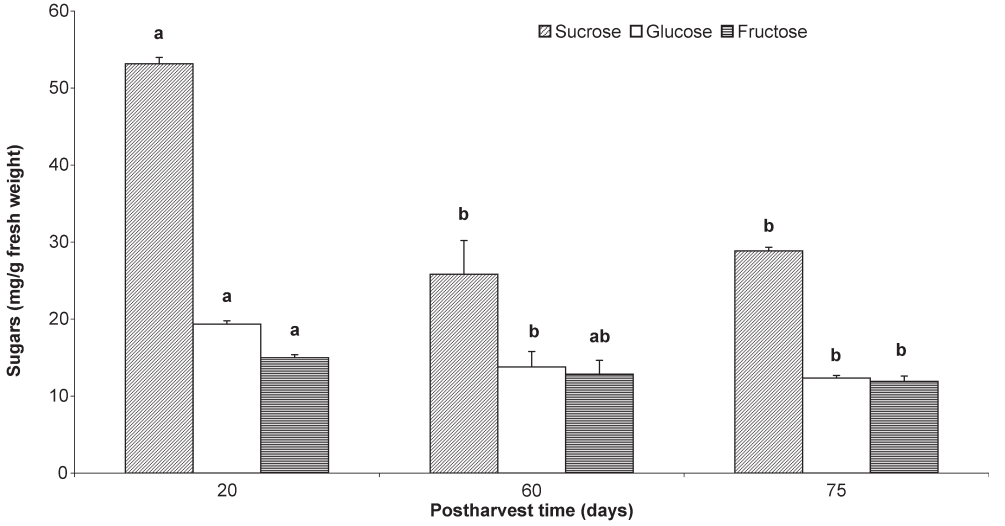


**Fig. 2**. Variation in sugar content during the 75-days storage period of *Tendral* melons. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of Tukey's test

**Table 2.** Protein and ascorbic acid contents and ACC oxidase activity (expressed as ethylene produced) during the postharvest storage period. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of Tukey's test. * - not determined, FW – fresh weight.

| Days after harvest | Protein (mg $g^{-1}$FW) | Ascorbic acid (mg $g^{-1}$ FW) | $C_2H_4$ (nmol $g^{-1}$ $h^{-1}$) |
|---|---|---|---|
| **20** | 8.48 ± 0.360 **(a)** | 0.14 ± 0.009 **(a)** | 46.723 ± 0.741 **(a)** |
| **40** | 12.77 ± 0.556 **(b)** | * | * |
| **60** | 8.40 ± 0.611 **(a)** | 0.10 ± 0.003 **(b)** | 58.519 ± 0.993 **(b)** |
| **75** | 4.91 ± 0.521 **(c)** | 0.08 ± 0.000 **(c)** | 44.211 ± 2.954 **(a)** |

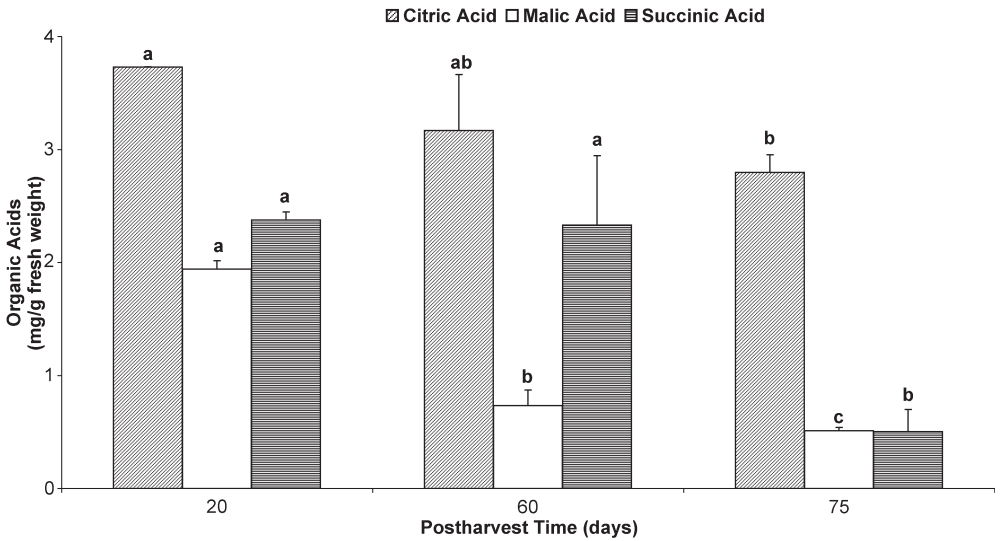


**Fig. 3.** Variation in organic acid content during the 75-days storage period of *Tendral* melons. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of Tukey's test.

Furthermore, our data confirm previous findings indicating that sucrose was the predominant sugar (Hubbard et al., 1989), with glucose being the major reducing sugar (Fig. 2). At harvest, sucrose represented about 60.7 % of total sugar content, while glucose and fructose constituted c.a. 22.1 % and 17.1 %, respectively. There was a significant reduction of sugars during the storage period (40 %), due to natural degradation. In fact, they become metabolically consumed in the respiratory chain due to phosphorylated equivalents synthesis (Lester and Bruton, 1986). In melon fruits, these processing capabilities do not seem regular, considering the strong decrease in sucrose content (44.6 %), especially during the first 40 days of storage. Only a slight decline in glucose content (20.5 %) occurred and a minimal reduction in fructose content (5.3 %) was observed. This result suggested that sucrose was

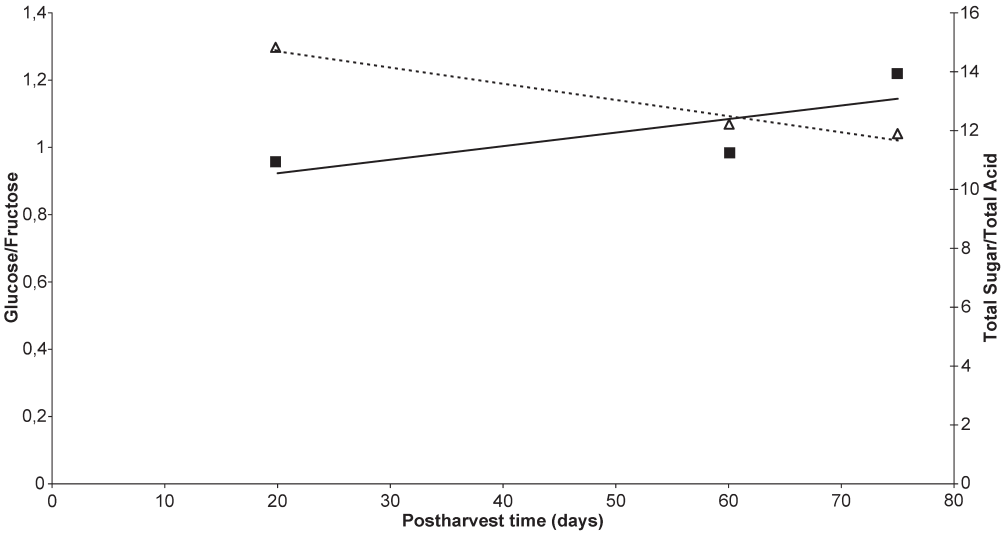


**Fig. 4.** Glucose/fructose and total sugar/total acid ratios during the storage period of *Tendral* melons (Δ - Glucose/fructose ratio ($r^2 = 0.983$); ■ - Sugar/acid ratio ($r^2 = 0.609$).

hydrolyzed to its respective monomeric isomers probably due to an increase in the invertase activity (Lingle and Dunlap, 1987; Schaffer et al., 1987; McCollum et al., 1988; Hubbard et al., 1990).

Leach et al. (1989) found that in the cultivars of *Cucumis melo*, citric acid was the major component of the organic fraction which agrees with our findings, since it contributes with about 46 % at harvest (Fig. 3). Malic and succinic acids constituted only 24.1 % and 29.5 %, respectively. By the end of storage, a 53 % decrease of total organic acid content was observed. A similar pattern has long been detected for many other fruits (Kays, 1991). However, as with sugars, this trend is different for the three acids analyzed indicating the existence of other inhibitory steps of these metabolites. In fact, the glucose/fructose ratio diminished during fruit senescence (Fig. 4). Since fructose is "sweeter" than glucose, fruits become more attractive for the consumer. This ratio correlated in 99.2 % with time. The total sugar/total acid ratio displayed an inverse correlation. It increased during the postharvest period showing a correlation of 78 %. The initial content of proteins also increased (Table 2), probably as a result of the turn over of new proteins coupled to senescence. The subsequent decline is certainly due to an enzyme activity (Abu-Goukh and Abu-Sarra, 1993). As previously reported (Evensen, 1983), a huge decrease of the ascorbic acid content (44%) also occurred during the storage period (Table 2). This parameter displays the increasing degradation ratio of the organic molecules (namely proteins and acyl lipids), since the redox equivalents of ascorbic acid are progressively affected. This decrease develops during the storage period (Adisa, 1986). Results published by

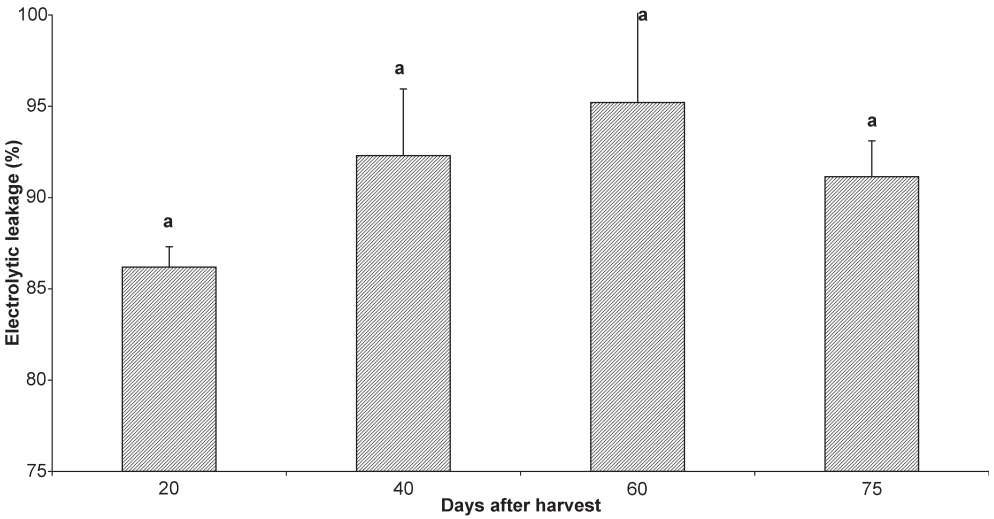


**Fig. 5**. Electrolyte leakage from *Tendral* melons during the storage period after 3 h of incubation. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of Tukey's test

Ververidis and John (1991), for *Ogen* melon, indicated that ACC oxidase activity in homogenized extracts supplemented with iron and ascorbate increases rapidly, and then it declines as fruits become overripe. Our data were analogous and eventually also correlated with lipid peroxidation, which is enhanced during storage (Table 3), reflecting the degradation of membrane polar acyl lipids (Lacan and Baccou, 1998), and justifying the evolution of ethylene, probably also implicating its production due to the synthesis of oxyradicals (as shown by the modification of the enzyme activities, SOD and CAT). These alterations of membrane properties were also supported by the measurement of electrolytic leakage from discs of muskmelon pulp. In fact, this process, which is a good indicator of membrane damage (Lacan and Baccou, 1996), slowly increased during the 75 days of storage (Fig. 5). Coupled to the already reported parameters, as seen in Table 3, the progressive failure of antioxidant enzyme systems (namely, the activities of SOD and CAT) suggested an enhanced level of reactive oxygen species. The SOD and CAT activities were significantly lower (by 30.8 % and of 24.4%, respectively). It is well known that the balance between SOD and CAT activities in cells is crucial for determining the steady-state level of superoxide radicals and $H_2O_2$ (Mittler, 2002). Thus, these data showed a progressive oxidation of *Tendral* melon tissues during postharvest resulting from the accumulation of lipid hydroperoxides in parallel with a decline in the ratio of SOD/CAT activities. In this context, the assumption supports Rogiers et al. (1998). Nevertheless, the rate of glutathione reductase activitiy was not limiting (Table 3).

**Table 3.** Activities of enzymes implicated in the control of the oxidative stress in the flesh of winter *Tendral* melon, during storage. For SOD, one unit of activity is defined as the amount of enzyme required to inhibit the reduction rate of cytochrome c by 50 % under the assay conditions. Each value is the mean of three replicates of five fruits each ± standard error. Different letters indicate significant differences in a multiple range analysis for 95 % confidence level, on the basis of a Tukey's test.

| Days after harvest | 20 days | 60 days |
|---|---|---|
| Superoxide dismutase (units $mg^{-1}$ Prot) | 58.7 ± 2.0 **(a)** | 40.6 ± 2.7 **(b)** |
| Catalase (µmol $O_2$ $mg^{-1}$ Prot $min^{-1}$) | 10.4 ± 0.6 **(a)** | 7.8 ± 0.3 **(b)** |
| Ascorbate peroxidase (µmol Asc $mg^{-1}$ Prot $min^{-1}$) | 1.1 ± 0.0 **(a)** | 0.7 ± 0.1 **(b)** |
| Dehydroascorbate reductase (µmol Asc $mg^{-1}$ Prot $min^{-1}$) | 3.2 ± 0.2 **(a)** | 2.5 ± 0.1 **(a)** |
| Glutathione reductase (µmol NADPH $mg^{-1}$ Prot $min^{-1}$) | 3.9 ± 0.2 **(a)** | 5.0 ± 0.1 **(b)** |
| Lipoperoxides (nmol MDA $g^{-1}$ FW) | 18.9 ± 0.4 **(a)** | 34.2 ± 1.1 **(b)** |

The resulting alterations coupled to the oxidative stress during the postharvest period affected *Tendral* melon quality parameters. Twenty days after harvest fruits showed a global consumer acceptance of 14.320 ± 1.031 (a) and this parameter reached 16.412 ± 0.470 by day 60 (b). The main conclusion of this work is that in spite of the alterations of the stored melons a better flavour and aroma developed, mainly due to the acid degradation and eventually implicating alkaloids and sugar accumulation.

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