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Influence of Hot Air and Modified Atmosphere Packaging on Chilling Injury Incidence and Metabolism of Soluble Sugars in Eggplant Fruit (*Solanum melongena L.*)

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***Abstract*— In this study, the influence of hot air (HA) and passive modified atmosphere packaging (MAP) on chilling injury (CI) and the metabolism of soluble carbohydrates on eggplant fruits stored at 3 °C for 9 days were investigated. Thus, the content of soluble sugar and the activities of enzymes associated to sugar metabolism, e.g. sucrose synthase (SS-synthesis and SS-cleavage), acid invertase (AI), neutral invertase (NI) and sucrose phosphate synthase (SPS) were analyzed. The CI symptoms in the control were manifested in association with an accumulation of sucrose. The fruit treated with HA and MAP showed significantly higher sucrose content with a significantly higher SS-synthesis activity and mildest CI symptoms respect the control. Thus the treatments with HA and MAP applied were effective in reduce CI incidence in the eggplant fruit. This would be associated with the increase in sucrose content and higher activity of SS-synthesis during cold storage.**

***Index Terms*— Sucrose accumulation, hot air, modified atmosphere packaging, chilling injury.**

I. Introduction

Due to their high respiration rates the vegetables are highly perishable commodities [1]. Visual quality is an important attribute that influences fruits and vegetables marketability. Low temperature is a technique usually applied to enhance storage life of fresh produce since reduces losses in storage and retains quality by slowing down the metabolic activities rate of fruits and vegetables.

However tropical and subtropical fruits are sensitive to low temperature storage and develop chilling injuries (CI) when stored at low temperature for prolonged period [2]. Therefore in these fruit the CI is a serious problem in postharvest handling [3]. Different authors reported that low temperature reduce CI in several crops such as avocados, cucumbers, tomatoes, zucchini squash, eggplants, lemons, limes, grapefruits, papayas, mangoes and sweet peppers [2].

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The *Solanum melongena* L. eggplant belongs to a family tropical, whose sensitivity to CI is generally associated to storage and processing concerns. This fruit suffers physiological disorders at temperatures below 12 °C and the symptoms are manifold, such as tissue browning, pitting, discoloration of the skin and seed browning [3]. Among the non-chemical technologies for post-harvest decay control like the irradiation and the modified atmosphere packaging, the heat treatment could be one of the most effective [4].

The heat treatment applied in fruits can delay the ripening process, induce tolerance to CI and also reduce skin surface damage during post-harvest storage, thus increasing shelf life and marketing potential [4]. For example, CI symptoms along the storage of orange [5], pomegranate [6] and 'Flavorcrest' peach [7] were markedly alleviated by heat treatments. Likewise, treating banana fruit stored at 8 °C with 38 °C hot air (HA) for 3 days was reported as lessening CI [8]. Although heat treatment may be beneficial for treated horticultural crops, its inappropriate use e.g. exposing fruits to lethal temperature or for extended time, might damage them [9].

Another way to store fruits and vegetables is the modified atmosphere packaging (MAP). They are wrapped in semi-permeable polymeric films that restrict the transfer of the respiratory gases from them [1]. With this technique, the packaged fruit is surrounded by high humidity and a composition of gases with high levels of CO_2 and low O_2. Altogether these factors appear to be beneficial in reducing CI in both climacteric and non-climacteric fruits [10]. The MAP can reduce CI in banana [11] and papaya [10].

The low temperatures induced changes in soluble carbohydrates in plants, including soluble sugars such as sucrose, glucose and fructose [12, 13]. Such sugars are significant energy sources and influence to the sensory quality of fruit [14]. On the other hand, Der Agopian et al. [15] argued that soluble sugars can modified the stress resistance in banana fruit exposed to low temperatures.

Yu et al. [16] reported that during cold storage, the soluble sugars reduce CI of fruit. It has been reported that reducing sugars improve CI resistance in different fruits such as loquat [17] and apricot [18]. Shao et al. [17] observed in loquat fruits that heat treatment induced an increased of reducing sugars when these were under chilling stress. On the other hand, the tolerance to CI was related to the content of sucrose instead of the reducing sugars as in fruits of mandarin [19]

and peach [13]. Different studies have been carried out in fruits stored under refrigeration; nevertheless the relationship between chilling tolerance and carbohydrates content is unclear [13].

The metabolism of soluble carbohydrates is controlled by enzymes when the plants are subjected to cold stress. Thus, sucrose phosphate synthase (SPS) (E.C. 2.4.1.14) and sucrose synthase (SS-synthesis) (EC 2.4.1.13) are responsible for the synthesis of sucrose from fructose and glucose [13] while the enzymes invertases (E.C. 3.2.1.26) [such as acid invertase (AI) and neutral invertase (NI)] and sucrose synthase (SS-cleavage) catalyze the irreversible hydrolysis of sucrose to glucose and fructose [13]. According to Ni et al. [20] the enzymatic activities of the synthesis of S-S and SPS are directly related to the content of sucrose.

The purpose of this work was to study the influence of MAP and HA in the sugar content of eggplant fruit (*Solanum melongena L.* cv Black nite) and the changes associated to enzyme activity during their cold storage.

II. Materials and Methods

A. Plant material and preparation

The eggplant (cv Black nite) grown in Santiago del Estero (Argentine) showing commercial maturity and volume of about 500 cm^3 were harvested and selected in terms of their shape, size, and free of visual defects. They were disinfected with chlorinate water (150 ppm, 5 min), drained and air-dried.

B. Treatments applied and storage conditions

The treatments applied were as follows:

-Modified atmosphere packaging (MAP): The fruits were MAP-packaged individually in 25 µm thick non-perforated low-density polyethylene bags (PE-LD). The O_2 transmission rates ranged between 7000-8000 cm^3/m^2.d a 25 ºC, while those of CO_2 varied about 20000-26000 cm^3/m^2.d a 25 ºC. In turn, the water vapor transmission rates of the film were from 3-5 g/m^2.d.

-Hot air (HA): The fruits were heat-treated at 35 °C for 1 h in an incubator and packaged afterwards in perforated PE-LD bags with of 190/m^2 perforations, 13 mm diameter each.

-The control fruits were packaged in perforated PE-LD bags. Each treatment involved five fruits and was stored at 3 ºC. Analyses were carried out on days 0, 3, 6 and 9.

In addition, stored fruits at 20 °C in perforated bags were used as another reference.

C. Evaluating of CI index

The CI index was evaluated on each sampling day by observing both internal and external CI symptoms. Its severity was calculated by the formula below and graded according to a 1-5 numerical scale similar to that of Concellón et al. [3] (2007), namely: 1 = no damage; 2 = low damage; 3 = regular damage; 4 = moderate damage; 5 = severe damage.

$$\text{CI Index} = \frac{\sum (nj \times i)}{N}$$

where nj is the number of fruits with the "i" mark (score 1 to 5) and N is the total amount of fruit. Scores greater than 2.5 corresponds to fruits with excessive damage that makes them unfit for consumption.

D. Measurement of soluble sugars

For the measurement of soluble sugars, 5 g of sample of the central section of each fruit were taken by triplicate and were homogenized with 20 mL of refrigerated ethanol to 95% for 2 min and agitated for 15 min. The resulting homogenate was filtered and the residue was extracted with refrigerated ethanol to 8 % (v/v). The extracts were evaporated with vacuum at 35 °C and filled to a final volume of 25 mL with distilled water. The extract obtained was used for the analysis of high performance liquid chromatography (HPLC) by passing it through a 0.45 µm membrane filter. Soluble sugars were measured using a HPLC (Agilent 1100, USA) equipped with a refractive index detector (Agilent 1100, USA) and a Kromasil®100A amino acid column (USA).

Sample (20 µL) was analyzed by HPLC using acetonitrile/water solution (80:20 v/v) as solvent with flow rate of 1 mL min^{-1} at 35 °C. The identification of individual sugars was performed by comparing the retention times and peak areas with the individual sugar standards. Concentrations were prepared between 5 and 100 µg/mL of the standard sugar solutions. The results of individual sugars were expressed as mg/g fresh weight (FW).

E. Starch content

The starch content was determined out of the insoluble residue after ethanol extraction according to the method by Lafta and Lorenzen [21]. Thus, 50 mg of the residue was taken and rehydrated using 2 mL of water and then heated for 1 hour at 90 °C. The resulting gelatinized starch sample was mixture with 1 mL of amyloglucosidase solution (20 mM NaF, 100 mM acetate buffer, pH 4.5) and incubated for 48 h at 40 °C and the glucose released was determined by HPLC as indicated above. The resulting of starch content was expressed as mg of glucose released from starch/g FW.

F. Enzyme extractions

For SPS and SS, fruit samples were extracted with a buffer solution like that used by Cao et al. [12] which contains 400 mM Tris–HCl buffer (pH 8.5), 10 mM $MgCl_2$, 5 mM EDTA, 10 mM 2-mercaptoethanol, 10 mM ascorbic acid (AA), 20% (v/v) glycerol, 1% (v/v) Triton X-100, 2 mM phenylmethylsulphonyl fluoride (PMSF), 10 µg/mL of leupeptin and 10 µg/mL of chymostatin. The samples were crushed together with the insoluble polyvinyl polypyrrolidone and centrifuged at 12000 g at 4 ºC for 15 min. These crude extracts obtained were used to measure the enzymatic activities. A crude extract in 400 mM HEPES-NaOH (pH 8.8) was used to measure the content of the enzyme invertase, instead of Tris-HCl buffer (pH 8.5).

G. Enzymes analysis assays

The enzymatic activities of SPS and SS were tested according to Miron and Schaffer [22]. Assay mixture for SPS contained 50 mM HEPES-NaOH buffer (pH 7.5), 25 mM fructose 6-phosphate, 15 mM $MgCl_2$, 25 mM glucose 6-phosphate, 25 mM UDP-glucose and crude extract. To determine the SS-synthesis activity, the crude extract together with 25 mM fructose and 25 mM UDP-glucose, in 50 mM HEPES-NaOH (pH 7.5) containing 15 mM $MgCl_2$ was incubated at 30 °C during 15 min. The enzymatic activity of SS-cleavage was measured of the reaction between 50 mM HEPES-NaOH (pH 7.0), 60 mM sucrose and 20 mM UDP and crude extract.

The NI activity was determined in a mixture of incubation containing 200 mM of sucrose, 200 mM HEPES-NaOH (pH 7.5) and the crude extract. The AI activity was determined under the same conditions described above, with exception of that the reaction mixture contained 100 mM acetic acid/sodium acetate buffer (pH 5.0). For the case of AI, the aliquot was neutralized before determining glucose content [12].

In all the cases, the reacting mixtures were incubated at 30 °C for 60 min and then added with 100 µL of 30 % (w/v) KOH. The sugars formed were separated and quantified by HPLC as for determining carbohydrates above. In turn, when determining the various enzymatic activities, an extract sample soaked in a 100 °C water bath for 10 min was used as blank.

H. Statistical analysis

The experiments were performed using a randomized design based on a bifactorial design. The statistical analyses were performed with Infostat software (version 2011, National University of Cordoba, Cordoba, Argentina). Mean separations were carried using Least significant difference tests at $P < 0.05$.

III. Results and Discussion

A. CI index

The control fruit presented CI symptoms after 3 days of storage, with a CI value of 2.3. This is due to the appearance of external pitting on the surface of the fruit. This damage became more evident after 6 days of storage, since there was a greater browning of the flesh and darkening of the seed, exceeding the limit established for commercialization (CI = 2.5) (Fig. 1).

On the ninth day of storage, the CI index of the fruit treated with HA reached 1.9, while that of those treated with MAP 1.3, i.e. the former was significantly higher ($P < 0.05$) than the latter. Although those fruits treated with MAP conserved best their original external features, both treatments (HA and MAP) showed incipient damages and kept below the limiting value of 2.5 up to day 9 of storage. The fruits conserved at 20 °C resulted unsuitable for commercialization after 6 days of storage because of the manifest signs of damage they showed due to senescence (data not shown).

These results agree with those of Yu et al. [23] who reported that treating with hot air (HA) clearly delays and reduce the CI index in peach fruit stored at 5 °C. Similar results were found by Malakou and Nanos [24] who found that the combination of heat treatment and MAP improved the quality of peach fruit. Fallik [25] reported that the protection of heat treatment against CI may be related to the accumulation of heat shock proteins.

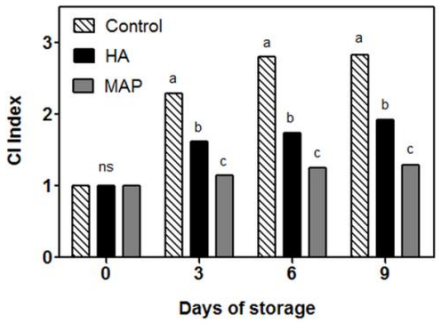

Fig. 1: Effect of HA and MAP treatments on CI index in eggplant fruit stored at 3 °C for 9 days. Different letters at each storage time represent significant differences at $P < 0.05$ according to LSD test.

B. Changes in the content of glucose, fructose, maltose, sucrose and starch

The free sugars present in the mature eggplant fruits cv. Black nite were glucose, fructose, sucrose, and maltose. Hexose (glucose and fructose) initials levels were approximately 9-10 mg/g FW, i.e. almost ten times higher than maltose level (Table 1). Additionally, it was observed that the content of glucose, fructose, and maltose remained practically constant throughout storage. There were no significant differences ($P > 0.05$) between the fruits stored at 3 °C or 20 °C (data of the latter not shown). In the fruits treated with HA and MAP, variations in the way these sugars evolved were not observable ($P > 0.05$). No were significant differences found among treatments and treatments as to the control.

Immediately after harvest, the sucrose content was of 1.3 mg/g FW which remained practically constant at 20 °C along the 9 days of experiments either among treatments or treatments as to the control (data not shown).

Table 1: Effect of HA and MAP treatments on glucose, fructose and maltose in eggplant fruit stored at 3 °C for 9 days.

Parameter/ Treatment	Storage time at 3 °C		
	Day 0	Day 3	Day 9
Glucose (mg/g FW)			
Control	9,84 a^A	1,04 a^A	9,84 a^A
HA	9,84 a^A	7,79 a^A	8,65 a^A
MAP	9,84 a^A	9,06 a^A	9,06 a^A
Fructose (mg/g FW)			
Control	9,25 a^A	9,42 a^A	9,79 a^A
HA	9,25 a^A	8,14 a^A	7,77 a^A
MAP	9,25 a^A	7,77 a^A	7,77 a^A
Maltose (mg/g FW)			
Control	0,97 a^A	0,99 a^A	0,89 a^A
HA	0,97 a^A	1,02 a^A	0,86 a^A
MAP	0,97 a^A	0,89 a^A	0,89 a^A

Means in the same row with different superscript letters or in the same column for same type sample with different upper case letters were significantly different according LSD test at P < 0.05.

As shown in Fig. 2, the sucrose content of the fruits increased during storage in all the treatments. After 9 days of storage at 3 °C, the samples treated with HA and MAP reached levels about 4.1 and 3.7 respectively in relation to the beginning while the control increased nearly 2.8 times. Out of the third day of storage at 3 ºC, the sucrose content of the treatments with HA and MAP was higher (P < 0.05) respect to control and related with the lower CI index of these treatments. However, no significant differences in sucrose content among treatments with HA and MAP throughout storage at 3 °C were found. Therefore, it could be inferred that the sucrose levels is related to CI in eggplant fruits.

Regarding the evolution of starch content, it was determined that its content decreased slowly both at 3 °C and 20 °C from approximately 6.6 mg/g FW up to 20 % less in the day 9 without differences between both temperatures (P > 0.05) (data not shown). According to these results, the observed sucrose accumulation could not be due to the degradation of this polysaccharide not to changes in glucose and fructose contents. Some authors like Couée et al. [26] and Yu et al. [23] suggests that both sucrose and hexose can contribute with the antioxidant compounds and prevent chilling stress.

In works carried out by Jiang et al. [27], Wang et al. [13] and Abidi et al. [28] reported that sucrose would have a protective effect against CI in grapes branches and peach exposed to low temperatures, respectively.

On the other hand, Couée et al. [26] and Wang et al. [13] suggested that sucrose would have effect at the cellular membrane level decreasing the inactivation of proteins and avoiding the loss of electrolytes and in this way contribute to the antioxidant effect. However, other authors reported that sugars such as glucose and fructose also induce tolerance to chilling injury in other fruits [12, 17]. For example, Shao et al. [17] reported high contents of reducing sugars such as glucose and fructose in loquat fruit, induced greater resistance to CI. However, more studies should be done on the role of reducing sugars on chilling injury.

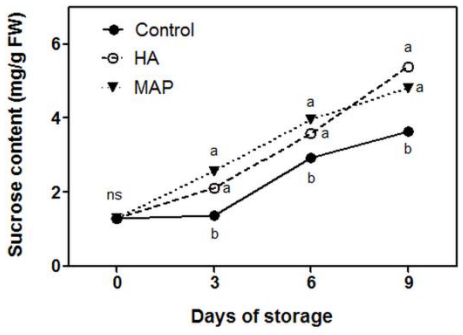

Fig. 2: Effect of HA and MAP treatments on sucrose content in eggplant fruit stored at 3 °C for 9 days. Different letters at each storage time represent significant differences at P < 0.05 according to LSD test.

C. Enzymatic activities of AI, NI and SS-cleavage

Fig. 3 shows the enzymatic activities of SS-cleavage and invertase (such as AI and NI) that are responsible of the hydrolysis of sucrose. The sucrose is hydrolyzed to glucose and fructose by the enzyme invertase. While that the enzymes SS-cleavage are reversible and cause the cleavage of sucrose to fructose and UDP-glucose [12].

In all fruits, the AI activity remained constant (P < 0.05) during cold storage at 3 °C which is similar to that of harvest time (0.5 µmol/min.g). No significant differences were found either among treatments or treatments with respect to the control (Fig. 3A). The NI activity immediately after harvest was of approximately 1.1 µmol/min.g for all the eggplants. The general trend was significantly lesser (P < 0.05) than that of the initial value during cold storage reaching levels that were approximately 4 times lower than those in fruit just harvested on day 9 of storage (Fig. 3B). However, no significant differences between treatments were found.

As to the SS-cleavage activity immediately after harvest, it was approximately 4.1 µmol/min.g. During cold storage, the general trend was a significant (P < 0.05) decrease in the SS-cleavage activity which reached levels approximately 4 times lower than in fruit just harvested at the end of storage. No significant differences among treatments were found either (Fig. 3C). In this study, this reduction in enzymatic activities of AI, NI, and SS-cleavage might be associated to lower levels or reducing sugars in the eggplant fruits during cold storage.

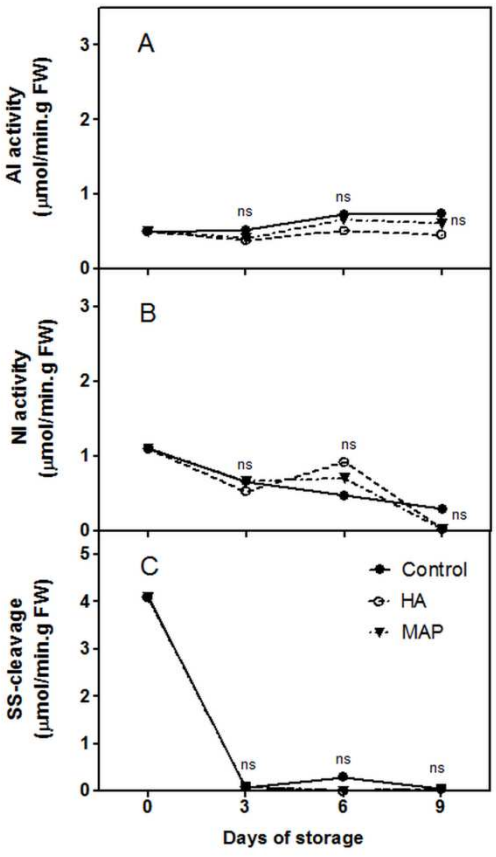

Fig. 3: Effect of HA and MAP treatments on (A) AI activity, (B) NI activity and (C) SS-cleavage activity in eggplant fruit stored at 3 °C

for 9 days. Different letters at each storage time represent significant differences at P < 0.05 according to LSD test.

D. SS-synthesis and SPS activities.

Fig. 4 shows the activities of sucrose-synthesizing enzymes such as SS-synthesis and SPS related in the catalysis of sucrose. In all the eggplants, the SS-synthesis activity was initially very low, with levels that get close to 0.001 µmol/min.g though during cold storage increased significantly (Fig. 4A). On day 9, the control reached levels that were approximately 40 times higher than that of the fruit just after harvest, while that of the fruit treated with HA and MAP were, respectively, 80 and 70 times higher than in the fruit just harvested.

The HA and MAP treatments made the SS-synthesis activity increase significantly as to the control out of the third day of the experiment. However, no significant differences were found among the samples treated with HA and MPA at the end of storage. The enzymatic activity of SS-synthesis presented a positive correlation with the sucrose content ($r = 0.99173$; $P < 0.05$). In contrast, all the treatments made the SPS activity decrease significantly ($P < 0.05$). The control decreased from initial levels of 0.082 µmol/min.g to very low values (0.008 µmol/min.g) on day 6 while the samples treated with HA and MPA showed no detectable values. Thus, the SPS activity remained unchanged in all the samples up to the end of storage (Fig. 4B).

Such an increase in the activities of the SS synthesis enzyme could lead to an increase in the level of sucrose in eggplant fruits stored at 3 °C. Besides, the lower enzymatic activities of SS-cleavage and invertase along with the higher activities of SS-synthesis suggested altogether that more sucrose was accumulated in eggplant fruits under chilling stress that effectively resulted in a prolonged storage period.

In other studies carried on peaches stored at 5 ºC, an increase of AI, NI and SS and decrease SPS, recording a reduction of sucrose levels was observed [13]. Jiang et al. [27] reported that in grape branches stored at low temperatures showed increased in the sucrose content due the increased SPS enzymatic activity was observed. For their part, Itai and Tanahashi [29] reported that AI enzymes are important in modulating the metabolism of soluble carbohydrates during refrigerated storage. Besides, Cao et al. [12] reported higher activities of SS-cleavage and invertase enzymes but lower activities of SPS and SS-synthesis that would both suggest that more sucrose was hydrolyzed and more hexose were accumulated in "Ninghaibai" loquat fruit with CI. On the other hand, Yu et al. [23] reported in peach stored at 5 ºC that treatments with HA significantly increased the enzymatic activity of SPS inducing an increased sucrose levels.

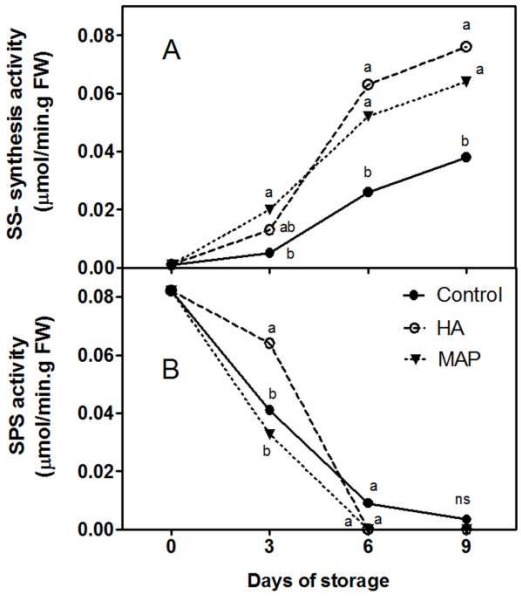

Fig. 4: Effect of HA and MAP treatments on (A) SS-synthesis activity and (B) SPS activity in eggplant fruit stored at 3 °C for 9 days. Different letters at each storage time represent significant differences at P < 0.05 according to LSD test.

IV. Conclusions

The application of MAP and HA treatments and its effect on the chilling injury of eggplant fruits stored at 3 ºC was evaluated. These treatments induced an increase of the sucrose content due to higher SS-synthesis enzymatic activities levels, getting better their chilling injury tolerance in the fruits. Therefore, HA and MAP would contribute in decrease CI in eggplants fruit (*Solanum melongena* L. cv. Black nite) maintaining their marketable quality during cold storage.

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