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Modified atmosphere packaging confers additional chilling tolerance on ethylene-inhibited cantaloupe Charentais melon fruit

Abstract Cantaloupe Charentais melon fruits are subject to chilling injury when stored at low temperatures, around 2 °C. Ethylene-suppressed cantaloupe Charentais melon, expressing a 1-aminocyclopropane-1-carboxylic acid (ACC)-oxidase gene in antisense orientation, showed strong, but not total, resistance to chilling injury, allowing an extended storage at low temperatures. Modified atmosphere packaging (MAP) is known to alleviate chilling injury symptoms in a variety of chilling-sensitive horticultural commodities. In the present work, we have compared the effects of MAP in non-retractile plastic film and storage in air on ethylene production, respiratory activity, development of chilling injury symptoms, water loss, ion leakage and accumulation of ethanol and acetaldehyde in wild-type and ethylene-suppressed melons, during storage at 2 °C and after re-warming at 22 °C. MAP reduced chilling injury and extended the postharvest life of wild-type fruit and conferred additional chilling resistance on ethylene-suppressed melons. Reduction of ethylene production and water loss are necessary to prevent chilling injury symptoms in melon.

Keywords Modified atmosphere packaging · Ethylene · Chilling injury · 1-aminocyclopropane-1-carboxylic acid oxidase antisense · *Cucumis melo* · Weight loss · Electrolyte leakage

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Introduction

Traditional cantaloupe Charentais melons (*Cucumis melo* var. *cantalupensis* Naud), such as the "Védrantais" variety, are highly aromatic and exhibit good organoleptic attributes. However, not only do they develop a very sharp climacteric phase [1], associated with a short shelf-life, but they are also sensitive to chilling injury when stored at temperatures lower than 8 °C [2].

Among all the postharvest technologies available for the limitation of chilling injury during storage of fruits and vegetables at low temperatures, modified atmosphere packaging (MAP) has the advantage of being of low-cost and easy to implement at the commercial level [3, 4, 5]. MAP utilises the properties of specific permeation to O_2 and CO_2 of polymer films to generate atmospheres that are suitable for the postharvest life of many horticultural commodities [6]. The modified atmosphere (MA) is created naturally in the sealed package, as a direct result of counterbalancing the O_2 uptake and CO_2 production of the product by the diffusion of gases across the membrane [3, 4]. In addition to the extension of shelf-life, MAP is known to reduce the incidence of some physiological disorders responsible for important economic losses of stored fruit, such as chilling injury, as has been observed in avocados [7], mangos [8] and cantaloupe melon [9]. MAP promotes the reduction of ethylene biosynthesis and sensitivity, and of water loss [3, 4]. Both effects stimulate membrane deterioration [10]. This parameter, measured as an increased ion leakage, is one of the primary chilling injury symptoms [11]. In a previous paper, we showed that ethylene suppression in cantaloupe Charentais melons expressing an antisense ACC oxidase (ACO) resulted in better chilling tolerance, although symptoms were not fully suppressed, especially when the cold storage period was extended [2]. The aim of the present work has been the evaluation of possible additional effects of MAP on the reduction of chilling injury in ethylene-suppressed melons. To this purpose, we have compared the effects of MAP, in non-retractile plastic films, and air-packaging on the postharvest behaviour of wild-type and ethylene-suppressed melons, during storage at 2 °C and after re-warming at 22 °C. By monitoring a number of physiological and biochemical parameters (ethylene production, respiratory activity, development of chilling injury symptoms, water loss, ion leakage and accumulation of ethanol and acetaldehyde), we have shown that MAP not only reduced chilling injury and extended the postharvest life of wild-type fruit, but also conferred additional chilling resistance on ethylene-suppressed melons.

Material and Methods

Plant material. Experiments were carried out using two types of F1 hybrid of cantaloupe Charentais melons, obtained by Tézier (Port les Valences, France) by crossing a transgenic line (B17) of the Védrantais genotype expressing an ACO antisense gene [12] or a Védrantais non-transformed line (A17), with another line (PL1) conferring resistance to several diseases (*Fusarium*, *Oidium*), greater firmness and a rounder shape. The resulting hybrids were either harbouring the antisense ACO gene (PL1xB17) or not (PL1xA17) and were named antisense (AS) and wild-type (WT), respectively.

Plants were grown in an insect-proof greenhouse in Murcia (Spain), under standard hydroponic conditions. Self-pollination was performed by hand and only two fruits were left per vine.

Storage conditions. AS and WT fruits were harvested 35 days after fruit set, at a time when the climacteric respiration had been initiated in the WT melons. Samples of both WT and AS fruit were taken immediately after harvest and either wrapped in non-retractile film or stored in air. Fruit were stored at 2 °C with a relative humidity (RH) of 90–95%, WT fruit for 7 and 14 days, and AS fruit for 7, 14 and 21 days. At the end of each period of cold storage, five AS and five WT fruits were taken out of the chambers, unwrapped and transferred to 22 °C for 4 days, simulating exposure on a retail shelf. Analyses of gases and other biochemical parameters were performed immediately after cold storage and during the shelf-life at 22 °C. The films used for MAP were supplied by Bolloré Technologies (France). Their technical characteristics were: thickness=15 μm, permeability to O_2=13,000 ml $m^{-2}\cdot 24\ h^{-1}\cdot bar^{-1}$, to CO_2=50,000 ml $m^{-2}\cdot 24\ h^{-1}\cdot bar^{-1}$ (23 °C and 0% RH) and to H_2O=30 g $m^{-2}\cdot 24\ h^{-1}\cdot bar^{-1}$(38 °C and 90% RH).

Analytical methods. Gases of the atmosphere inside the non-retractile bags were measured according to Pretel et al. [5]. The C_2H_4 production and the respiration rates were measured at the end of storage at 2 °C, after removal of the plastic film, and during storage at 22 °C, according to Serrano et al. [13]. Fruit weight was assessed at the beginning and at the end of the experiment in order to evaluate the percentage weight loss. Ion leakage was measured as described by Ben-Amor et al. [2]. The ethanol and acetaldehyde contents were determined according to Davis and Chace [14]. Evaluation of the severity of the chilling injury at the surface of the fruit was made by visual estimation, according to a scale ranging from 0 to 4 (0, no symptoms; 1, traces; 2, slight; 3, moderate; 4, severe). The average chilling injury index (*CI*) was obtained according to McCornack [15], using the formula:

$$CI = \frac{\sum (\text{Individualrating} \times \text{numberoffruitswiththesamerating})}{\text{Totalnumberoffruits}}$$

Results and discussion

Internal atmosphere in the package

Changes in the concentrations of O_2 and CO_2 within the bags during storage at 2 °C are presented in Fig. 1a. They show a similar pattern for bags containing WT or AS melons, with equilibria reached after 4 days, at around 1.5% for CO_2 and 15% for O_2. After 6 days, the O_2 level was slightly lower in bags containing WT fruit (14%) than in bags with AS fruit (15%).

The concentration of C_2H_4 in bags containing WT fruit showed a sharp decrease during the first 6 days of storage, from around 150 ppm, to 44 ppm and then a stabilisation at around 25 ppm (Fig. 1b). In bags containing AS fruit, the ethylene concentration always remained very low (<1ppm) and a tendency to decrease to negligible concentrations during cold storage (<0.05 ppm) was observed.

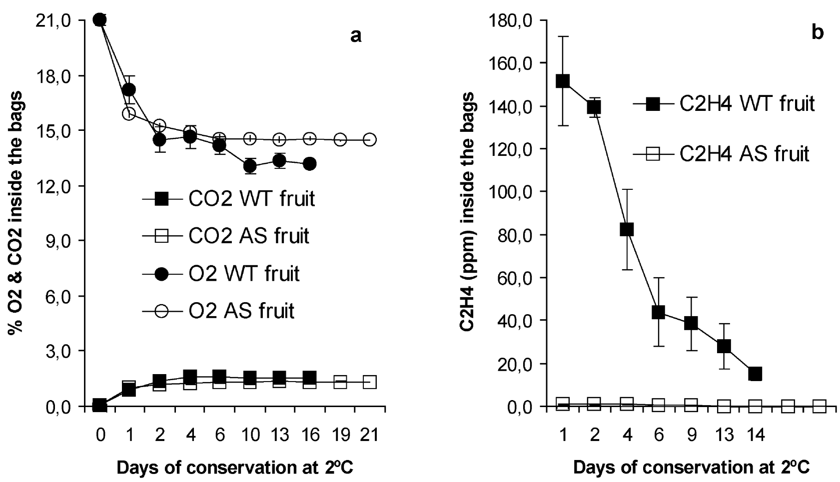

Fig. 1a, b Evolution of O_2 and CO_2 (**a**) and ethylene (**b**) concentrations at 2 °C inside the internal atmosphere of the packaging of wild-type (*WT*) and antisense (*AS*) fruits. Each value is the mean±SE corresponding to five determinations of WT and AS F1 hybrids. The surface of exchange of the packaging was 500 cm^2

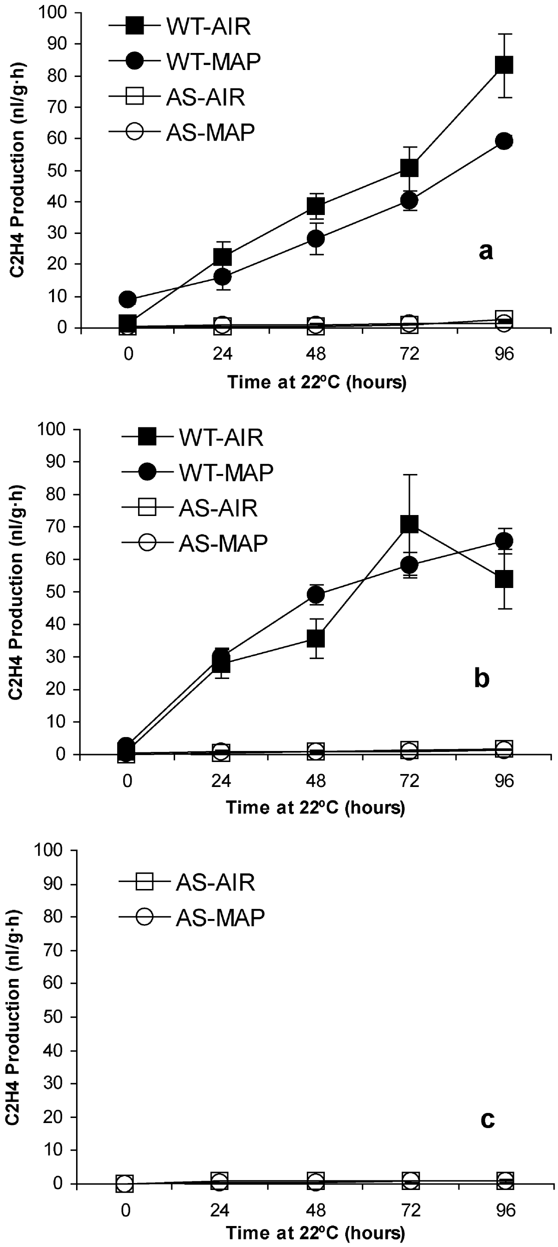

Fig. 2a–c Ethylene production, at 22 °C, of WT and AS control (*AIR*) and wrapped (*MAP*) fruits, after 7 (**a**), 14 (**b**) and 21 (**c**) days of conservation at 2 °C, and with the fruits kept in modified atmospheres already unwrapped. Each value is the mean±SE corresponding to five determinations

Ethylene production and respiratory rate

During storage at 2 °C, ethylene production and respiratory activity gradually decreased (data not shown), but after transfer to 22 °C they increased sharply (Fig. 2 and Fig. 3). There was, in general, no significant difference in ethylene production between fruit stored in air or in MAP. The only exception was for WT fruit stored for 7 days at 2 °C in MAP, which exhibited a slower increase in ethylene production rate upon re-warming compared with fruit stored in air (Fig. 2a). As expected, the ethylene production rate of AS fruit (Fig. 2a, b, c) was extremely low (<2.5 $nl{\cdot}g^{-1}{\cdot}h^{-1}$) compared to WT fruit (>82 $nl{\cdot}g^{-1}{\cdot}h^{-1}$), whether stored in air or MAP (Fig. 2a, b).

Respiratory activity increased mostly during the first 24 h of re-warming in WT fruit (Fig. 3a, c), while it increased, in general, more gradually for AS fruit (Fig. 3b, d, e). Fruit stored under MAP, especially when stored for 14 or 21 days at 2 °C (Fig. 2c, d, e), had a higher initial respiratory rate immediately upon re-warming, probably due to residual CO_2 from the modified atmosphere. But after 48 h at 22 °C there were no differences compared with fruit stored in air.

Chilling injury index

The intensity of chilling injury was evaluated, as the importance of depressed browning and pitting areas on the rind (*CI*), after 4 days at 22 °C, on fruit previously stored at 2 °C for 7, 14 or 21 days. Figure 4a shows that WT fruit exhibited severe symptoms in air, even when stored for only 7 days at 2 °C (*CI*>2). The disorder was amplified in fruit stored for 14 days at 2 °C (*CI*>3). None of these fruit were marketable. MAP storage alleviated chilling injury by more than 50%. AS fruit had very little injury (*CI*<1.25) when stored in air, even for 21 days, and were almost completely free of symptoms when stored under MAP (*CI*≤0.5). In these conditions, fruit stored under MAP were marketable even after 3 weeks of storage at low temperature.

Weight loss

As expected, weight loss was significantly reduced under MAP (Fig. 4b). The difference was particularly significant at the end of the experiment, when AS fruit stored for 21 days at 2 °C had lost almost 12% water while their counterparts stored under MAP had lost less than 6%. Interestingly, after 14 days of storage at 2 °C, AS fruit, whether in air or MAP, exhibited less water loss than WT fruit.

Ion leakage

Ion leakage is a good marker for membrane deterioration occurring during senescence and in chilling-sensitive tissues exposed to low temperature. It was always higher in WT fruit compared to AS, and was proportional to the duration of the low temperature treatment (45% and 62% for fruit held at 2 °C for 7 and 14 days, respectively, Fig. 4c). Ion leakage of AS fruit was lower in all conditions. AS fruit stored for 21 days at 2 °C exhibited approximately the same level of ion leakage as when stored for 14 days.

The use of MAP limited the damage to cell membranes. Ion leakage was 7 and 9% lower, respectively, in

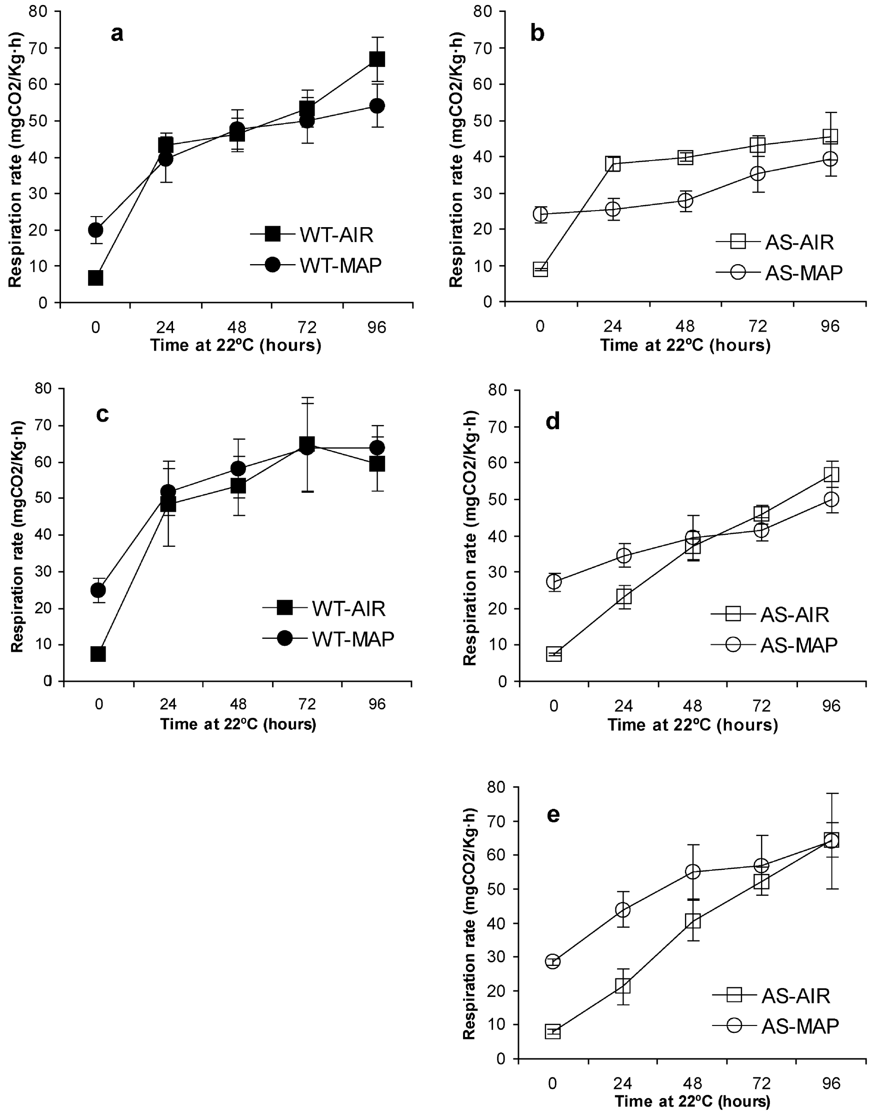

Fig. 3a–e Respiration rate, measured as CO_2 production at 22 °C, of WT (**a** and **c**) and AS (**b**, **d** and **e**) control (AIR) and wrapped (MAP) fruits, after 7 (**a** and **b**), 14 (**c** and **d**) and 21 (**e**) days of conservation at 2 °C, and with the fruits kept in modified atnmospheres already unwrapped. Each value is the mean±SE corresponding to five determinations

WT and AS fruit stored under MAP as opposed to air in the case of fruit held at 2 °C for 14 days.

Ethanol and acetaldehyde contents

The levels of ethanol in rind were much higher in WT than in AS fruit. This was particularly so for fruit that had been stored for 14 days at 2 °C (Fig. 4d). WT fruit that had been packed in the modified atmosphere exhibited higher ethanol levels (63.6 μmol/g) than fruit kept in air (27.5 μmol/g), related to higher CO_2 levels of fruit in the bag (Fig. 3). Such levels of ethanol rendered fruit inedible. In AS fruit, the level of ethanol was always very low (<2.5 μmol/g FW), even for fruit that had been stored for 3 weeks at low temperature.

The acetaldehyde content of the rind was always lower in AS than in WT fruit (Fig. 4e). WT fruit had higher levels of acetaldehyde in the absence of cold storage. Low temperature storage resulted in a loss of acetaldehyde in WT fruit whether they were packed in plastic films or not. However, there was some recovery in fruit stored for 14 days in the cold. MAP resulted in slightly lower acetaldehyde levels in WT fruit kept for 14 days in the cold and in all AS fruit, as compared to air.

Previous data had demonstrated that ethylene-suppressed melons stored in air exhibit higher tolerance to chilling injury than WT fruits [2] . The present experiments show that MAP conferred additional chilling resistance, with significant reduction of membrane deterioration. It has been reported that the development of chilling injury is associated with an accumulation of ethanol and acetaldehyde [16]. In the present case, the correlation is more complex. If, as expected, WT fruit that are sensitive to chilling accumulate more anaerobic metabolites than the less sensitive AS fruit, then MAP storage resulted in higher accumulations of ethanol and acetaldehyde although it reduced chilling symptoms. This is probably related to low O_2 and high CO_2 concentrations in the bags. In AS fruit, however, decreased chilling in-

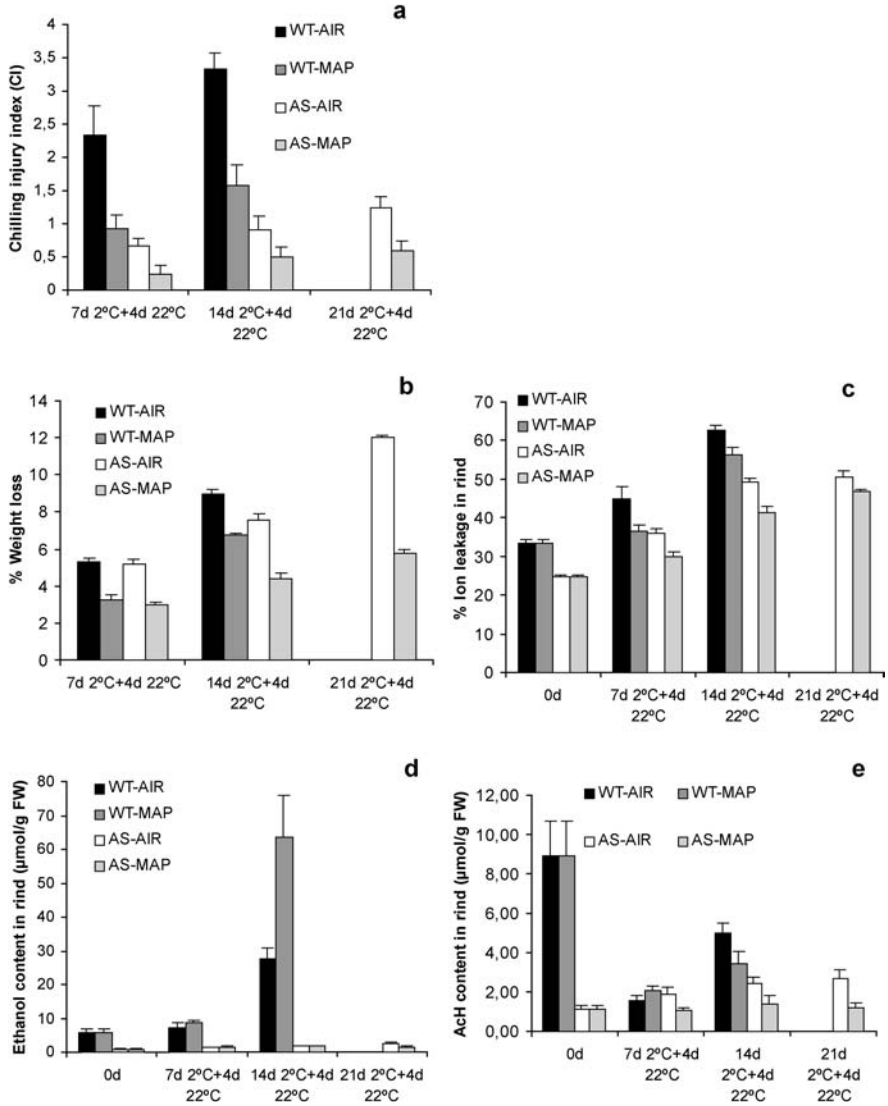

Fig. 4a–e Chilling injury index (*CI*) (**a**), percentage weight loss (**b**), percentage ion leakage (**c**) and evolution of ethanol and acetaldehyde (*AcH*) contents (**d** and **e**), of WT and AS control (AIR) and wrapped (MAP) fruits after conservation at 2 °C and 22 °C. Each value is the mean±SE corresponding to three determinations

jury of MAP-stored fruit was always associated with lower accumulations of ethanol and acetaldehyde.

It must be noted that WT fruit, before chilling, exhibited high acetaldehyde concentrations due to their advanced stage of ripening. A sharp increase in acetaldehyde has been observed previously during melon fruit ripening [17]. The WT melons used in the experiments of Ben Amor et al. [2] had lower acetaldehyde concentrations.

Alleviation of chilling injury by MA storage has been reported for several fruit species [3, 4], including melons of the *reticulatus* group [18]. The inhibitory effect of MA on chilling injury may be related to the effects of low O_2 and high CO_2 concentrations on C_2H_4 production and action [3, 4]. Inhibitors of ethylene action reduce chilling injury in avocados [7]. However, the beneficial effects of MAP can also be attributed to a reduction of water loss. Individual seal packaging, in which the internal atmosphere is not affected significantly, is known to reduce the development of low temperature tissue breakdown [6]. A correlation between weight loss and chilling injury symptoms of fruits stored in MA has been found in tomatoes [19] and melons of the *reticulatus* [18] and *inodorus* groups [20].

Our experiments lead to the hypothesis that, in AS melons, the bulk of the resistance is due to the suppression of ethylene production [2]. The residual sensitivity of AS melons could be attributed either to the very low residual ethylene level or to water loss. The present experiments support a beneficial effect of MAP on chilling injury via reduction of water loss, because a high water loss accentuated rind pitting, associated with cell collapse [20].

Although no commercial developments are expected, AS melons have interesting postharvest behaviour. They can be stored at low temperature in MAP for at least 21 days without any symptoms of chilling injury, even after re-warming. They have the disadvantage of exhibiting inhibition of volatile aroma production [21], rind colour development and softening [22]. However, full aroma production, rind colour and softening can be restored by treating AS melons with ethylene at the appropriate concentration and for the appropriate time [23]. In addition, because they can stay longer on the vine, they can reach a higher sugar content than WT fruit [22].

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References

1. Hadfield KA, Rose JKC, Bennett AB (1995) J Exp Bot 46:1923–1925
2. Ben Amor M, Flores F, Latché A, Bouzayen M, Pech JC, Romojaro F (1999) Plant Cell Environ 22:1579–1586
3. Zagory D, Kader AA (1988) Food Technol 42:70–77
4. Kader AA, Zagory D, Kerbel EL (1989) Crit Rev Food Sci Nutr 38:1–30
5. Pretel MT, Serrano M, Martínez G, Riquelme F, Romojaro F (1993) Lebensm Wiss Technol 26:8–13
6. Ben-Yehoshua S (1985) HortScience 20:32–37
7. Pesis E, Ackerman M, Ben-Arie R, Feygenberg O, Feng X, Apelbaum A, Goren R, Prusky D (2002) Postharvest Biol Technol 24:171–181
8. Pesis E, Aharoni A, Aharon Z, Ben-Arie R, Aharoni N, Fuchs Y (2000) Postharvest Biol Technol 19:93–101
9. Rodov V, Horev B, Vinokur Y, Copel A, Aharoni Y, Aharoni N (2002) HortScience 37:950–953
10. Cohen E, Shapiro B, Shalom Y, Klein JD (1994) J Am Soc Hortic Sci 119:983–986
11. Wang CY (1982) HortScience 17:173–186
12. Ayub R, Guis M, Ben-Amor M, Gillot L, Roustan JP, Latché A, Bouzayen M, Pech JC (1996) Nat Biotechnol 14:826–866
13. Serrano M, Martínez-Madrid MC, Pretel MT, Riquelme F, Romojaro F (1997) J Agric Food Chem 45:1668–1672
14. Davis PL, Chace WG (1969) HortScience 4:117–119
15. McCornack AA (1976) Proc Fla State Hortic Soc 89:200–202
16. Schirra M (1992) Postharvest Biol Technol 2:315–327
17. Horvat RJ, Senter SD (1987) J Food Sci 4:1097–1098
18. Lester GE, Bruton BD (1986) J Am Soc Hortic Sci 111:727–731
19. Hakim A (1997) Approaches to reduce chilling injury of tomato fruit. Paper VI: chilling injury in mature green tomatoes affected by polyethylene packaging during cold storage. PhD thesis, Department of Plant Production, Horticultural Section, University of Helsinki, Finland, pp 238–244
20. Edwards ME, Blennerhassett RM (1990) Austr J Exp Agric 30:693–697
21. Bauchot AD, Mottram DS, Dodson AT, John P (1998) J Agric Food Chem 46:4787–4792
22. Guis M, Botondi R, Ben Amor M, Ayub R, Bouzayen M, Pech JC, Latché A (1997) J Am Soc Hortic Sci 122:748–751
23. Flores FB, Ben-Amor M, Jones B, Pech JC, Bouzayen M, Latché A, Romojaro F (2001) Physiol Plant 113:128–133