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Assay of a potential post-harvest handling procedure for Cantaloupe Charentais melon fruit with inhibited ethylene production

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Abstract: Antisense ACC-oxidase Cantaloupe Charentais melons, unsuitable for autocatalytic ethylene production, were harvested at different ages and exposed to a standard post-harvest manipulation to reproduce the handling chain that occurs from harvesting to retail sale. The stages were as follows: storage in a chamber at 2 °C during 2 or 3 weeks, second storage at 8 °C for 4 days, treatment with 50 ppm ethylene for 2 days and, finally, maintenance at 20 °C for four more days. The date of harvest and the different periods of storage at 2 °C assayed did not affect the final de-greening of the rind, while the change in pulp colour depended on the harvest date. Storage conditions also influenced the texture of the pulp and acidity. Weight loss was dependent mainly on date of harvest, like soluble solids content, although the period of storage at 2 °C influenced too. The impact of cold on the appearance of the fruits is discussed.

Keywords: *Cucumis melo*; ethylene treatment; fruit quality; storage; post-harvest handling; genetically modified fruit

INTRODUCTION

The Cantaloupe melon (*Cucumis melo*, L. var. *cantalupensis* Naud.) is a climacteric fruit whose ripening process is regulated by the plant hormone ethylene.¹ Cantaloupe Charentais is a highly aromatic variety and presents excellent organoleptic properties, but suffers a severe climacteric crisis along with a sharp increase in the autocatalytic biosynthesis of ethylene. This provokes rapid ripening and is associated with a very short conservation period (no longer than 3–5 days at room temperature).² This characteristic greatly limits its possibilities of commercialisation to long-distance markets.

The genetic engineering has been applied in order to overcome this major disadvantage. The strategy followed was to block the ethylene production by the inhibition of the genetic expression of the enzymes responsible for its biosynthesis. In Cantaloupe melon this has been achieved by means of the antisense RNA technology.³ A genetically modified type of Cantaloupe Charentais melon was generated containing an antisense gene for the 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO, EC 1.4.3.-), the enzyme from the last

step of the biosynthesis of ethylene.⁴ The autocatalytic synthesis of ethylene in this kind of melon is lower than 0.45% of the wild-type production and it does not present a climacteric crisis.^{3,5,6} Furthermore, it is characterised by an inhibition of ethylene-dependent ripening processes and a marked resistance to chilling injury.^{3,7,8}

The aim of this study was to evaluate the final quality attained by this type of fruit after undergoing a standard procedure for post-harvest handling used for marketing purposes by agro-food companies. It consisted of the following stages: (1) conservation at 2 °C to simulate storage; (2) transference at 8 °C to reproduce transportation in a refrigerated lorry; (3) treatment with ethylene at room temperature to restore the normal ripening phenotype; and, finally, (4) maintenance at room temperature to replicate retail sale.

In this procedure two variables of interest from a practical point of view have been considered in order to evaluate their impact on the final quality of the fruit. They are the fruit age (date of harvest) and the period of conservation at 2 °C.

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To sum up, the experiment is an assay at a laboratory scale of the standard post-harvest manipulation used for fruit marketing, which fruits undergo from the time of harvest until they reach the consumer, and it is of great interest to the agricultural and food industries.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out on commercial F1 hybrids of Cantaloupe Charentais melon (*Cucumis melo* var. *cantalupensis* Naud. 'Védrantais') ACO antisense PL7×B17, obtained from the seed company Tézier S.A. (Valence, France). The genetically modified commercial hybrid PL7×B17 (AS) is a product from the interbreeding of the pure ACO antisense B17 line³ with a parental PL7 line possessing a series of beneficial attributes for the resulting hybrid, such as resistance to *Oidium* and *Fusarium* infections, a greater precocity, and a rounder and more symmetrical form, which makes it more attractive to the consumer. As a control, the non-transformed commercial hybrid PL7×A17 (WT) was used, the result of crossing the pure non-transformed line A-17 and the parental line PL7.

Cultivation was carried out in hydroponic conditions, in a greenhouse in Murcia (Spain), following the standard cultivation procedures. Pollination of the flowers was done by hand and all set fruit were marked, being the set date considered as development day 0. Only six fruit were left per plant.

Groups of fruits from the AS melon plants were periodically harvested, each consisting of 10 units, with the following set intervals: (a) 14–20 days after fruit set (DAFS), (b) 21–27 DAFS, (c) 28–40 DAFS and (d) 40–45 DAFS. They were taken immediately to the laboratory, where they were kept at 20 °C for 5 h, to allow scar formation on the peduncle cut. Each fruit group was classified according to the intervals of the set dates and was divided into two samples, each of five fruits. One of them was stored for 2 weeks in a refrigerated chamber at 2 °C and 90% RH, and the other for 3 weeks in the same conditions. Afterwards, both samples were transferred to a chamber at 8 °C and 90% RH, where they were kept for 4 days until an ethylene treatment of 50 ppm was applied for 2 days at 20 °C, according to the method described by Flores *et al.*⁹ Finally, the fruits were left at 20 °C for four more days. At the end of the experiment the quality parameters described below were determined.

The WT melon fruits were harvested at 35–40 DAFS only, when they have reached what is considered the optimal organoleptic properties for consumption (colour, firmness, sugars and acids content). Their values of quality parameters were considered as reference to compare with the ones obtained for the AS melon fruits at the end of the post-harvest handling procedure assayed. The values of the parameters of quality analysed (colour, firmness, soluble solids and titrable acidity) resulting from the

analysis of these ripe WT fruits were of use to assess if AS melons have finally achieved the demanded standards of quality.

Non-destructive analytical determinations were carried out for the different treatments that the fruit underwent, but only the results obtained at the end of the experiment, when the fruit reached the hands of the consumer, its final destination, are shown.

Colour

Rind and pulp colour was analysed by reflection, using a Minolta CR-200 chromameter (Osaka, Japan).¹⁰ The colour values were expressed in the CIE $L^*a^*b^*$ system. The a parameter was used to measure the colour of the pulp and the b parameter to measure that of the rind, because these are the best ones to reflect changes in colour of these two different tissues.¹¹ Four independent measurements were made on the rind, on different faces from the central area of each fruit, and another four were made on the pulp, in the equatorial section, separated from each other by 90°.

Firmness of the pulp

The firmness of the pulp or texture was analysed with a Penefel digital penetrometer (Copa Technologie S.A./CTIFL, France), which has a calibrated rod with a 0.5 cm² tip. This parameter was calculated as the strength (in kilograms) necessary for the rod to penetrate the flesh by 6 mm. Four measurements were taken for each fruit, separated from each other by 90°, at 1.5 cm from the exterior edge of the fruit, which was cut through the equatorial area. The results were expressed in kilograms per unit of surface (kg cm⁻²).

Weight loss

Weight loss for each melon was obtained from the weight values before and after storage. The results are expressed as a percentage.

Titrable acidity and total soluble solids content

Titrable acidity and total soluble solids content (TSS) were determined from the juice obtained, in triplicate, by homogenising 10 g of mesocarp of each fruit with 40 mL of distilled H₂O. The TSS was analysed by refractometry using an Atago P20 RL2 refractometer (Tokyo, Japan) at 20 °C and the results were expressed as °Brix. Titrable acidity was assessed by pH titration with 0.1 mol L⁻¹ NaOH to pH 8.1, the results being expressed as grams of citric acid per kg of fresh weight (g kg⁻¹).

Statistics

The data are the mean ± standard error (SE) of the determinations carried out for each sample. A variance analysis using the Student's t -test was performed to determine whether differences between means of the melon types were significant at $P \leq 0.05$.

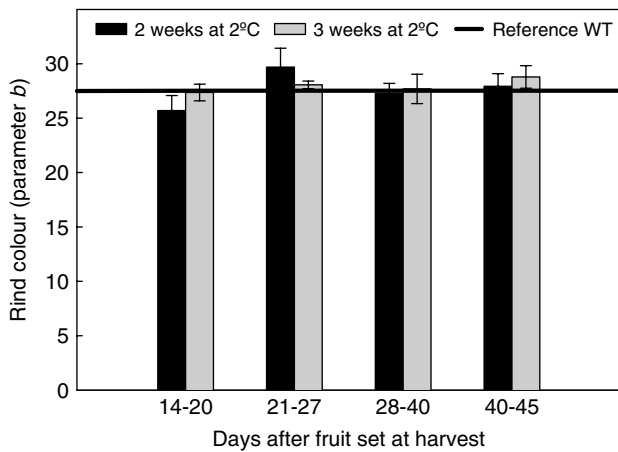


Figure 1. Colour parameter *b* in AS fruit rind for different set dates and storage periods at 2 °C of 2 or 3 weeks, in comparison with the value of the harvested 35–40 DAFS WT melon used as reference. Data are the mean ± SE of determinations made in quadruplicate for each of the five fruits per sample.

RESULTS

Colour

For the colour of the rind, at the end of the experiment, the *b* values of a normal, ripe Cantaloupe Charentais were reached, independently of its age and period of conservation at 2 °C, as it is observed comparing the AS *b* values measured with the WT reference 35–40 DAFS (Fig. 1).

An increase in parameter *b* was observed throughout the experiment, mainly when the fruit was transferred from 8 to 20 °C: this was the case for both periods of storage at 2 °C of 2 or 3 weeks (data not shown).

The most noticeable thing for the pulp colour evolution was the total absence of the development of orange-coloured pulp, which is apparent by such low values of parameter *a* measured in the youngest AS melons (14–20 DAFS), whereas the rest of the AS fruits did come close to the *a* values of the WT at the optimal ripening stage at the end of the experiment (Fig. 2).

Pulp firmness

The texture of the flesh of every AS melon fruit at the end of the post-harvest handling experiment was firmer than the WT fruit used as criterion of quality (Fig. 3). It was possible to observe a gradation in this parameter, especially in fruits stored for 2 weeks at 2 °C, except for the youngest ones (14–20 DAFS) ($P \leq 0.05$).

Weight loss

Weight loss was quantified in order to determine aptitude for conservation and, in this way, evaluate the speed at which the fruit loses water during storage, a factor which negatively affects its quality.

A scale was established in such a way that the weight loss decreased as the harvesting date for the fruit increased, and this occurred independently of whether the fruit was stored for 2 or 3 weeks at 2 °C

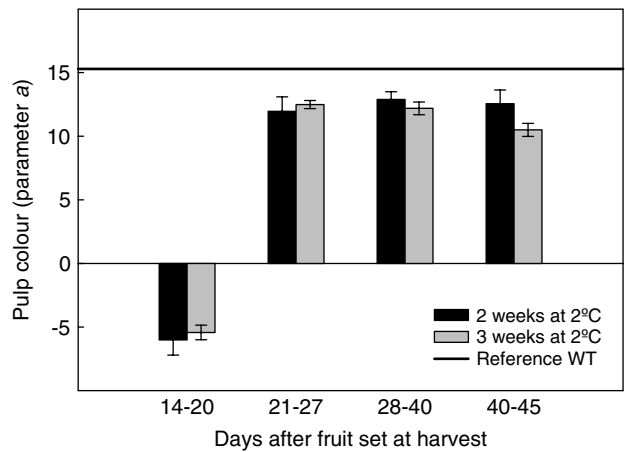


Figure 2. Colour parameter *a* in pulp of AS fruit with different set dates and storage periods at 2 °C of 2 or 3 weeks, in comparison with the value of the harvested 35–40 DAFS WT melon used as reference. Data are the mean ± SE of determinations made in quadruplicate for each of the five fruits per sample.

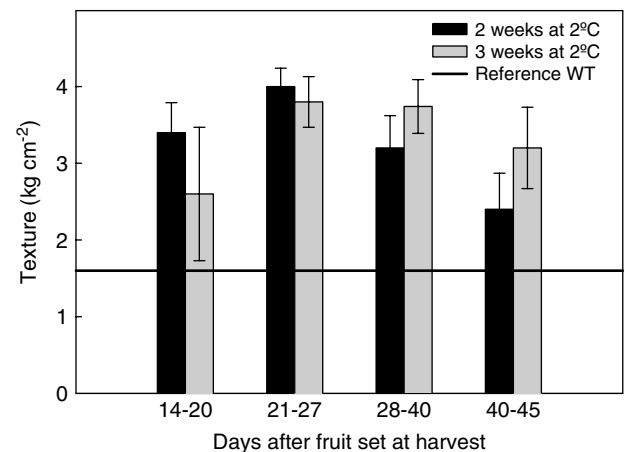


Figure 3. Pulp firmness of AS fruit with different set dates and storage periods at 2 °C of 2 or 3 weeks, in comparison with the value of the harvested 35–40 DAFS WT melon used as reference. Data are the mean ± SE of measurements made in quadruplicate for each of the five fruits per sample.

(Fig. 4). Therefore, the youngest fruits (14–20 DAFS) lost more water than those which were harvested with 21 and 27 DAFS, and they in turn lost more than the rest of fruits (Fig. 4).

Total soluble solids content and titrable acidity

Younger AS fruit showed a different behaviour compared with older fruit with regard to total soluble solids (TSS) content. Also the period of conservation at 2 °C seems to play a role in the evolution of this parameter, at least in case of the fruits with 21–27 DAFS (Fig. 5). In younger fruits the TSS content remained very much below than that of the WT value used as standard of quality. This occurred with fruits of 14–20 DAFS and 21–27 DAFS stored for 2 weeks at 2 °C, and with those of 14–20 DAFS stored for 3 weeks at 2 °C (Fig. 5). On the other hand, if they had been harvested after a larger number of days then

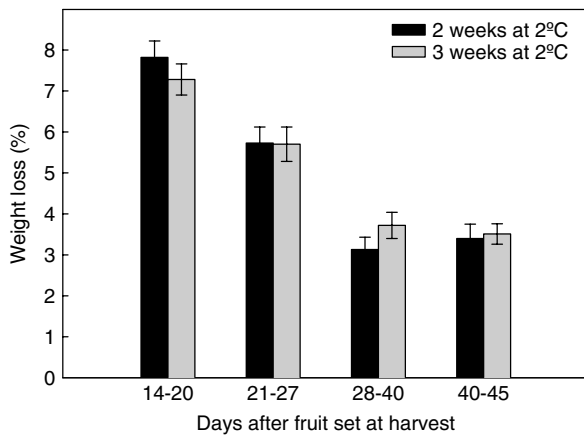


Figure 4. Percentage weight loss in AS fruit with different set dates and storage periods at 2 °C of 2 or 3 weeks. Data are the mean \pm SE of determinations made independently in each of the five fruits per sample.

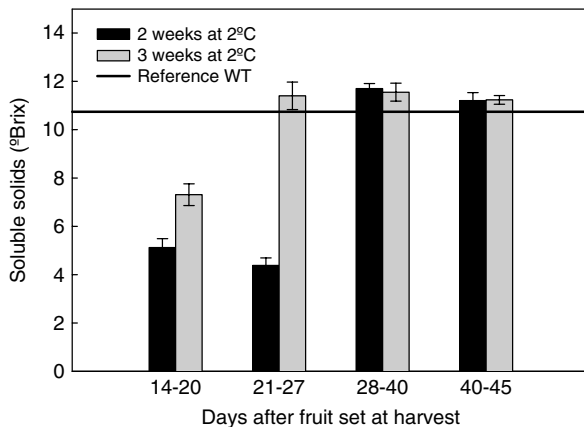


Figure 5. Total soluble solids content in AS fruit with different set dates and storage periods at 2 °C of 2 or 3 weeks, in comparison with the value of the harvested 35–40 DAFS WT melon used as reference. Data are the mean \pm SE of analyses made in triplicate in each of the five fruits per sample.

they did reach the optimal values fixed by the WT melon.

The acidity of the youngest fruit (14–20 DAFS) was the highest at the end of the post-harvest handling assay, no matter the period of storage at 2 °C (Fig. 6). But in the rest of samples a clear influence of the conservation period at 2 °C was remarked ($P \leq 0.05$). In samples of AS melons with more than 21 DAFS stored for 3 weeks at 2 °C the acidity at the end of the experiment was always higher than the standard value of quality according to the WT determination. By contrast, the values for the same fruit but stored for 2 weeks were similar to the reference marked by the ripe WT fruits (Fig. 6).

DISCUSSION

As it has been already mentioned, the experiment has been carried out using as controllable variables both, the date for harvesting the fruit, and the period of conservation at 2 °C. In normal conditions, the

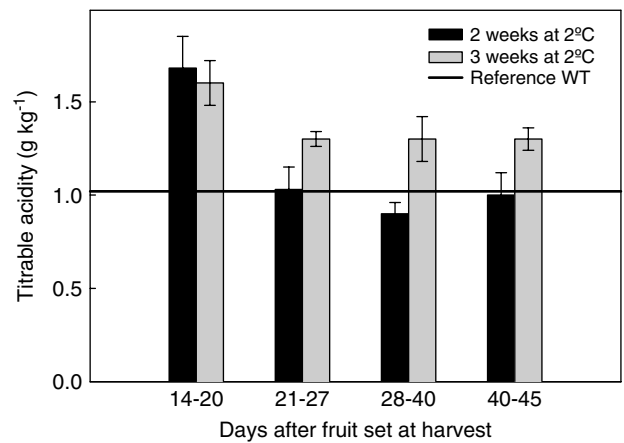


Figure 6. Titratable acidity in AS fruit with different set dates and storage periods at 2 °C of 2 or 3 weeks, in comparison with the value of the harvested 35–40 DAFS WT melon used as reference. Data are the mean \pm SE of analyses made in triplicate in each of the five fruits per sample.

other operations of the handling procedure cannot be modified because they are applied at the same time for different fruits and vegetables which are going to be transported together, and even treated with ethylene, as it is the case for bananas and melons. This is the reason why these stages are fixed previously by the distribution companies for horticultural products for the different produces to avoid a negative impact on their final quality. On the other hand the variables we introduced in the sequence only affect to the fruit object of study and in principle do not have an effect on the rest of products with which they may be transported, treated with ethylene and distributed to the markets.

The rind colour results show that it is possible to reach an optimum value after this post-harvest manipulation (Fig. 1). Previous experiments with Cantaloupe Charentais antisense ACO performed by our group have already demonstrated that it is possible to restore the normal de-greening of the rind, which is an ethylene-dependent process.^{3,9}

For the flesh colour of the melon fruit it is clear that, for developing a good orange-coloured pulp, it is necessary for the fruit to be on the plant a minimum number of days before it is harvested (Fig. 2). We have previously stated that with less than 27 DAFS on the plant the pulp has got a white greenish colour,¹⁰ due to the fact that at this stage of development the chlorophylls content is still very high, whereas the carotenoids content, responsible for the orange colour, is too low.⁵ In any case, according to what has been demonstrated by the measurements of pulp colour as well as pigments content during the development of the AS melon on the vine, in comparison with the WT melon, ethylene does not seem to intervene in the process.^{3,5,7,10}

The results of pulp softening at the end of the assay of post-harvest handling of the AS melons are somewhat disappointing, since the pulp of the fruits is too firm (Fig. 3). Anyway this result can be viewed

positively since it means that the AS fruits can stand for a longer time at room temperature without becoming too soft. This fact would allow extending the shelf life for some extra days, at least with regard to this quality parameter.

An interesting observation regarding these results of flesh firmness from a physiological point of view is the fact that AS pulp is always firmer than the WT of optimal ripening, even after an ethylene treatment of 50 ppm. This is a higher dose than the one of 2.5 ppm that stimulates the complete restoration of the pulp firmness, found in a previous study, when we investigated the restoration of several ripening processes in this type of genetically modified fruit.⁹ But there are differences between both experiments. Firstly, the fruits used in this study were hybrids while the ones used by Flores *et al.*⁹ belonged to the pure transgenic line B17. Also those pure-line fruits were not subjected to a whole post-harvest handling procedure like these hybrid ones, and the low temperatures stages may play a role in the maintenance of a higher firmness, despite the subsequent ethylene treatment.

The pattern of fruit weight loss found could be due to the contribution of several factors (Fig. 4). One of them is the lower diameter of younger fruits, whose surface/volume ratio was greater.^{1,11} Also respiration rates are greater in younger fruit.^{1,2,5} Finally, the number of lenticels not covered in the epidermis of the fruit is greater if they are younger; therefore, transpiration is assisted.^{12,13} All these factors stimulate a greater dehydration of the fruit and hence a rise in weight loss during its conservation.¹²

A weight loss of more than 5% in melon is unacceptable for commercial quality.^{12,14} This limiting value was surpassed by the youngest AS fruits (between 14 and 20 DAFS) and reached by those with 21–27 DAFS at the end of the experiment, but the rest of the melon fruits maintained a weight loss below this limiting value.

It is well known that fruit requires a minimum number of days on the vine in order to reach an optimal accumulation of sugars, which can be monitored by the determination of TSS content (Fig. 5). The evolution of this parameter, as well as sucrose (the main sugar in the fruit), throughout the development of AS and WT fruits on the plant supports this assertion.^{5,10} These results indicate that ethylene does not play any part in the evolution of this parameter; rather, developmental factors determine it. It was unexpected that storage period at 2 °C does make a difference with regard to the TSS content in sample made of fruit harvested with 21–27 DAFS. The longest period at 2 °C (3 weeks) resulted in clearly a higher TSS content, reaching the optimal value of WT fruit, all in opposition to the shortest period of storage at 2 °C (2 weeks). It is difficult to find a reason for this behaviour, since melons lack a starch reserve that could be converted to soluble sugars and therefore once separated from

the plant, melon metabolism resulted in a noticeable cessation of sugar accumulation.^{15,16}

The results of titrable acidity indicate that the stage of development at which the fruit is harvested is critical for acidity levels, but the period of storage at low temperatures also seems to be outstanding (Fig. 6). If fruit is harvested too young then the acidity content is too high and remains in this situation during the post-harvest life. The increase of the cold storage period from 2 to 3 weeks could induce metabolic alterations which are manifested as changes in acidity, these alterations taking place only in fruits that have reach a certain degree of development.

From the whole set of parameters analysed in this assay of post-harvest handling of ACO antisense melon fruit, it is possible to distinguish different behaviours. For the colour of the rind and the pulp texture no influence of the two variables introduced in the procedure (date of harvest and period of storage at 2 °C) have been observed. For another two parameters, pulp colour and weight loss, the date of harvest is the determinant for the evolution of these parameters. And for the two last ripening attributes measured, TSS content and titrable acidity, both variables do play a role in the final results.

Together with these physico-chemical changes, the visual appearance of the melons was also studied, to look for possible cold damage symptoms on the fruits during their post-harvest handling, since the fruits are stored at chilling temperatures at the beginning of the procedure.

The visual examination of whole AS melons and the pulp at the end of the post-harvest manipulation process showed that the fruit had a yellow–grey tone on the rind and that the flesh achieved a well developed orange colour, as would correspond to a ripe fruit ideal for consumption. Also, the resistance to the appearance of cold damage after conservation at 2 °C, and after subsequent periods at 8° and 20 °C, was evident, as shown by the absence of the typical alterations in the tissues because of this physiopathy.

On the other hand, when the AS fruits were collected too young, at 14–20 DAFS, they were unable to ripen normally after the different stages of the post-harvest handling procedure assayed and they did not attain the attributes appreciated by the consumer. The rind and the pulp had bright green and white colours, respectively, characteristic of an unripe melon. Furthermore, sensitivity to cold was noticeable in these melons, shown by the appearance of pitting, depressions and bruising on the rind, typical symptoms of cold damage.

The Cantaloupe Charentais melon is susceptible to cold, so a conservation period of longer than 1 week at temperatures lower than 2–7 °C induces surface pitting and brown areas on the rind, associated with a loss of its sensorial capacity.¹⁷ But we have previously observed that the ACO antisense transgenic type of this melon cultivar showed remarkable tolerance to chilling injury.² We have demonstrated that ethylene,

in combination with low temperatures, participates in the induction of cold damage in Cantaloupe Charentais melon fruit.²

But the fact that the youngest AS fruit, of 14–20 DAFS, was affected by this alteration points out that the sensitivity to this physiopathy depends on the development stage of the fruit too, i.e. in the date of harvest. That is to say, there are developmental factors which intervene in the induction of cold damage. This characteristic has already been observed in other fruits, like mangoes, peaches and peppers. If they are stored at low temperature at an immature stage they are more susceptible to chilling injury than when stored at a more advanced stage of development.^{18–20}

From the results obtained and considered as a whole, it can be concluded that the youngest AS fruits showed a significantly anomalous and different behaviour with regard to the rest. This was observed in the flesh colour, TSS content, titrable acidity and external and internal appearance, which showed that these fruits were most affected by cold damage, at the end of the post-harvest handling procedure assayed. This is why, for this type of melon fruit to reach the consumer with acceptable quality after the post-harvest period, it is essential to be harvested with no less than 21 DAFS. Also it should be taken into account the period of storage at 2 °C, which does have an effect on the evolution of two of the quality parameters analysed: TSS content and titrable acidity.

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