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Effect of temperature and cut size on the expression of *Chorismate synthase* in fresh-cut melon

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Abstract

The postharvest quality of fresh-cut melon is strongly affected by storage conditions to which it is subjected. During postharvest, fruit undergoes several stresses and its physiology is similar to the one of senescence tissues.

Chorismate synthase (CS) is a key enzyme in the shikimate pathway and catalyzes the formation of chorismate which is the precursor of numerous aromatic compounds in plants. In this work the effects of different storage temperatures and cut-sizes were studied, with the aim to individuate the molecular responses of *CmCS* gene to different postharvest conditions.

Melon (*Cucumis melo*, L. cv. Macigno) fruits were harvested at a fully ripen commercial stage, were washed in a chlorine water solution, and the mesocarp (pulp) was cut in cube-shaped portions; two sizes were chosen, 1x1 cm and 3x2 cm. Melon cubes were then stored at 20 °C or 4°C. *CmCS* was more affected by temperature showing decreased levels of expression during storage at 20 °C with respect to harvest and to cold storage. On the other hand cut-size did not determined significant changes in its expression.

Keywords: *Cucumis melo*, postharvest, storage, gene expression.

INTRODUCTION

Melon (*Cucumis melo*, L.) is a widely consumed fruit all over the world. Consumers appreciate its sensorial qualities and nutritional/nutraceutical features. In the last years, the consumption of fresh-cut fruits is increasing because of the high service content of these products. Thanks to its firmness and to its moderate perishability, fresh-cut melon can be used as an ingredient in the preparation of ready to eat fruit mixes or consumed by itself. The procedures applied in the fresh-cuts production pipeline can determine a decrement of fruit quality. Among those operations, cut is surely one of the most important. Cut induces higher transpiration, leaking, increased ethylene levels, browning, oxidative stress and senescence. The control of temperature is a crucial factor in maintaining produce quality. Lower temperatures can slow down the enzymatic reactions responsible for product degradation, but at the same time, they could stimulate tissue damage which negatively affects quality.

The shikimate pathway is in charge for the synthesis of essential aromatic amino acids and of several precursors for aromatic compounds required for UV-protection, electron transport, signaling, communication, plant defense and the wound response. Thus this pathway, which is part of the primary metabolism, represents a link between primary and secondary metabolism in higher plants (Macheroux et al., 1999). The phenylpropanoids compounds, such as flavonoids and anthocyanins, largely contribute to the antioxidant capacity and nutraceutical composition of the produce. In the fresh cut industry, the postharvest procedures can affect the gene expression and enzyme activities of the phenylpropanoids pathway and can affect the overall quality of the produce. Wounds and membrane disruption induce volatile organic compounds production, which influence the aroma of the produce. In this work it has been investigated how the expression of the gene *CmChorismate synthase (CmCS)*, responsible for the chorismate synthesis in the shikimate pathway, can be affected by different storage temperature regimes and different cut-sizes in fresh-cut melon.

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MATERIALS AND METHODS

Fruit material

Melon (*Cucumis melo*, L. cv. Macigno) fruits were harvested at a fully ripen commercial stage, when an abscission circle was observed on the fruit. Fruits were washed in a chlorine (50 $\mu\text{L L}^{-1}$ sodium hypochlorite) water solution, esocarp and endocarp were removed and discarded. The mesocarp (pulp) was cut in cube-shaped portions by using a sharp knife; two sizes were chosen, 1x1 cm and 3x2 cm. In order to evaluate the effect of the cut-size, around 170 grams of melon cubes were placed into plastic punnets and then stored at 4 °C. Samples were collected at harvest and after 4 and 7 days of cold storage. Moreover, the effect of storage temperature was evaluated on 3x2 cm melon cubes stored at 20 °C for 4 days and at 4 °C for 7 days. All analyses were performed in quadruplicate.

Total RNA-isolation and *CmCS* expression analysis

Total RNA was extracted from 100 mg of tissue using the Spectrum Plant Total RNA Kit with on-column DNase-treatment (Sigma, Italy) according to manufacture instructions. RNA concentration and integrity were assessed by NanoDrop N-1000 spectrophotometer (NanoDrop technologies). Total RNA (5 μg) was reverse transcribed using SuperscriptIII reverse transcriptase (Invitrogen, Italy) and a mix of random primers and oligo-dT (10 μM). The first strand synthesis reaction was incubated at 42 °C for 1 h. In order to avoid genomic DNA amplification, total RNA was treated with DNase I (Invitrogen, Italy). Specific primer for *CmCS* was designed based on the sequence found in the melon EST database (<http://www.icugi.org/cgi-bin/ICuGI/EST/home.cgi?organism=melon>). The SYBR green chemistry was used for gene expression analyses. Dissociation curves were performed to check the absence of primer dimers and other amplification by-products. The amplification program was: 1 cycle at 50°C for 2 min then at 95°C for 2 min; 40 cycles at 95°C for 30 s; 55°C for 1 min and 72°C for 30 s (signal acquisition stage); 72°C, 10 min and dissociation curve. Actin was used as housekeeping gene. Data are presented as relative expression, normalized on harvest time (0 days of storage) of 3x2 cm samples.

Statistical analysis

All gene expression data were subjected to a two-way ANOVA and the Bonferroni post-test was applied to evaluate significant differences between different temperatures, cut-sizes or among different days of storage.

RESULTS AND DISCUSSION

The results obtained allow identifying the molecular responses of *CmCS* gene as affected by temperature and cut-size.

1. Effect of storage temperature.

Temperature is a key factor affecting the quality of cut-size melon during storage.

In this study, different storage temperature determined significant changes in *CmCS* gene expression (Fig. 1) indicating an involvement of this gene in the stress response during melon postharvest commercial life. Samples stored at 20 °C showed a clear drop in the transcripts abundance and values were more than halved after 4 days of storage. On the other hand cold storage at 4 °C allowed maintaining constant level of expression. This could be due to the fact that low temperatures are effective in slowing down cell metabolism. As *CmCS* is the precursor of several molecules related to fruit quality, the higher levels of its expression in samples stored at 4 °C can be positively linked to a higher quality of the product.

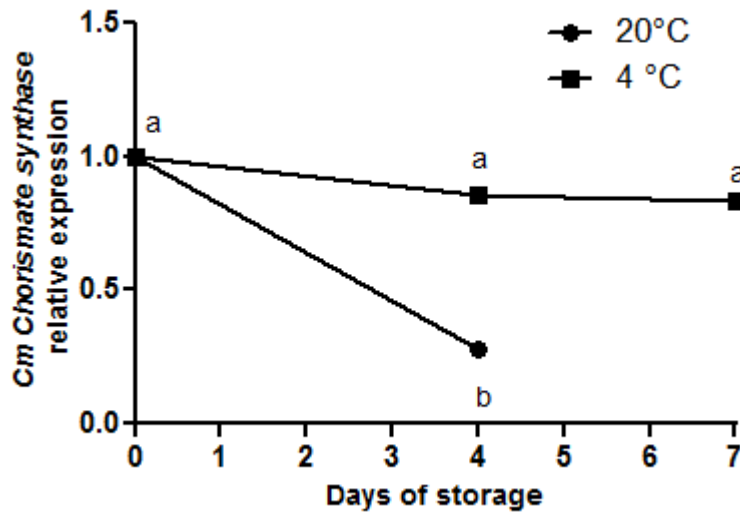


Figure 1. Relative expression of *CmChorismate synthase* during storage of fresh-cut melon at 20 °C and 4 °C. Different letters indicate significant differences ($P < 0.05$) among day of storage or temperatures.

2. Effect of cut-size.

In general, cut size did not determined significant changes in the *CmCS* expression. The difference observed between 3x2 and 1x1 cm samples were not statistically significant at any time point. However, considering the changes during storage, 1x1cm cubes showed a significant decrement in gene expression after one week at 4°C, while the levels of expression of 3x2 cm cubes, remained quite stable.

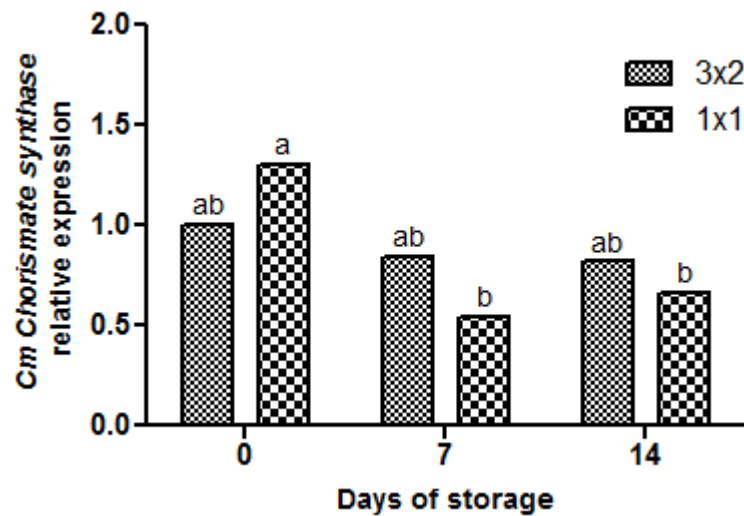


Figure 2. Relative expression of *CmChorismate synthase* during cold storage (4 °C) of fresh-cut melon. Different letters indicate significant differences ($P < 0.05$) among day of storage or cut-size.

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Book: Avanzato, D., McGranahan, G.H., Vahdati, K., Botu, M., Iannamico, L., and Van Assche, J., eds. (2014). *Following Walnut Footprints (Juglans regia L.): Cultivation and Culture, Folklore and History, Traditions and Uses*. *Scripta Horticulturae* 17 (International Society for Horticultural Science and International Nut & Dried Fruit Council). pp.442.

CONCLUSIONS

The following conclusions can be drawn from the study:

- Due to its role as intermediate point between primary and secondary metabolism *CmCS* gene, can have a role in mediating the tissue response to different postharvest abiotic stresses in melon.
- Different storage conditions can differentially affects the expression of *CmCS* gene.
- Temperature is confirmed to be a key player in the maintenance of cut-size melon quality during storage;
- Cut procedure determines significant changes in melon tissue, however

different cut-sizes did not strongly affected the expression of CmCS gene during storage at 4°C.

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