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Mild heat and calcium treatment effects on fresh-cut cantaloupe melon during storage

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Abstract

The effect of mild heat fruit pre-treatment on some properties of fresh-cut cantaloupe melon during storage was determined. Whole fruit, previously held at 4 °C, was immersed in heated water (60 °C) with and without dissolved calcium lactate (1%). Fresh-cut processing was done immediately, either after treatment or after storage at 4 °C for 24 h. Headspace gas accumulation during storage indicated reduced respiration in heat-treated fruit. Reduced lipase activity occurred in heat treated fruit during storage at 10 °C, while the fruit that was cut 24 h after treatment had a reduced peroxidase activity, unlike fruit that was processed immediately after heating. Isoelectric focussing indicated production of heat shock proteins ($PI = 5.1$ and 6.5) as a result of heat-treatment. Textural measurements showed increased hardness, chewiness and cohesiveness, but springiness decreased in heat-treated fruit. Presence of calcium in the treatment solution did not affect respiration and textural changes caused by heat treatment. Lipase activity was, however, higher in fruit heated in calcium solutions. Results indicated the potential improvement of shelf life of cut cantaloupe melon by mild heat pre-treatment of the fruit, and that the addition of calcium to the treatment water did not further improve product quality. Published by Elsevier Ltd.

Keywords: Mild heat pre-treatments; Postharvest; Minimal processing; Calcium; Heat shock proteins; Cucumis melo L.; Fruit

1. Introduction

Hot water treatment of a number of horticultural crops has been demonstrated to be effective in product shelf life improvement ([Lurie, 1998](#page-5-0)), reduction of chilling injury ([Lurie & Sabehat, 1997; Woolf et al., 1997](#page-5-0)), control of microbial and insect infestations and quarantine treatments ([Fallik et al., 2000; Fallik, 2004](#page-4-0)). It was recently reported that pre-cut heat-treatment could be used to improve the shelf life of fresh-cut cantaloupe melon ([Lamikanra, Bett-](#page-4-0)[Garber, Ingram, & Watson, 2005a](#page-4-0)). Heat-treatment reduced respiration and moisture loss during storage of the cut fruit. It also reduced total microbial count during the first storage day and prevented growth of lactic acid bacteria that occurred in untreated fruit after 8 days of storage. Sensory evaluations indicated that heat-treatment increased intensities of desirable attributes, such as fruity melon and sweet aromatic flavours, and reduced undesirable flavours, such as musty, sour, bitter, chemical and fermented.

The role of calcium in stabilising cellular membranes and delaying senescence in horticultural crops is well known [\(Poovaiah, Glenn, & Reddy, 1998](#page-5-0)). Pre- and postharvest application of calcium may delay senescence in commercial and retail storage of fruit with no detrimental effect on consumer acceptance ([Lester & Grusak, 2001,](#page-5-0) [2004](#page-5-0)). Post-cut calcium treatment of produce improves firmness retention and product shelf life [\(Garcia & Barrett,](#page-4-0) [2002](#page-4-0)), and the effectiveness of such treatment on product quality may be influenced by treatment solution temperature [\(Lamikanra & Watson, 2004a](#page-4-0)). Post-cut calcium application also inhibits lipase activity in cantaloupe melon ([Lamikanra & Watson, 2004b](#page-4-0)). Our objective was to

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determine the biochemical effects of hot water treatment on fresh-cut cantaloupe melon during storage. The effect of processing cantaloupe melon, immediately after heat-treatment, relative to holding the fruit at 4° C for 24 h before processing was assessed, as well as that of dissolved calcium in the treatment water.

2. Materials and methods

2.1. Fruit preparation

Cantaloupe melon (Cucumis melo L. var. reticulates), supplied by Del Monte Fresh Produce Co. (Coral Gables, FL), was received at 4 °C. Heat-treatment was conducted by immersing fruit in a waterbath, set at 60 $\rm ^{\circ}C,$ for a period of 60 min. For calcium treatments, calcium lactate (1%) solution was used. The treated fruit was either processed immediately or stored for 24 h at 4° C before processing. Stainless steel knives were used to cut fruit longitudinally into two halves. Seeds were removed and the fruit was then cut into 8 equal parts. The skin was removed and cubes, approximately $2-3$ cm \times 2.5 cm, were prepared in pie-like wedges cut from the 2.5-cm-wide slices. Approximately 300 g of cubes from each melon were placed into 24-ounce (about 1 l) low- profile Juice Catcher containers (SRW-24- JC; Winkler Forming Inc., Carrollton, TX), and stored at $10 \degree C$.

2.2. Determination of respiration

Immediately after cutting, cut fruit (50 g) was placed into Mason jars (0.5 l) fitted with air-tight lids equipped with rubber septums and stored at 10° C. At the designated sampling time, a needle syringe attached to a Mocon Pack Check 650 analyzer (MOCON\Modern Controls, Inc., Minneapolis, MN) was inserted into the rubber septum of the respective jar and sample gas (8 cm^3) from the headspace was analysed for CO_2 and O2 gas, respectively. Differences in respiration rate between samples during storage were assessed from the gas composition.

2.3. Enzyme extraction

Cut fruit (100 g) was homogenised with cold acetone $(300 \text{ ml}; -20 \degree C)$ in a Waring blender for 30 s. The blended mixture was further homogenised using a TekmarTM Tissumizer (Cincinnati, OH) for 30 s. The homogenate was then vacuum-filtered through Whatman No. 4 filter paper to remove the residue. The residue was successively homogenised in cold acetone and filtered until a colourless filtrate and a white mass were obtained. The mass was dried to a powder under a stream of air at room temperature. The acetone powder (0.25 g) was homogenised in Tris buffer (0.05 M; pH 7.8, 15 ml). The homogenate was centrifuged at 12,000 \times g and 4 °C for 20 min. The resulting supernatant was used for enzymatic activity assays.

2.4. Peroxidase assay

Peroxidase activity was assayed in a buffer consisting of 0.02 M Na₂HPO₄ and 0.08 M NaH₂PO₄, 20 mM guaiacol 4 mM H_2O_2 , and enzyme extract (150 µl), pH 6,in a total volume of 3 ml [\(Civello, Martinez, Chaves, & Anon,](#page-4-0) [1995\)](#page-4-0). The reaction mixture was transferred into a cuvette and incubated at 50 °C. Absorbance readings at 470 nm between 30 and 90 s. of the reaction time were used to determine the initial rates of reactions.

2.5. Lipase assay

Lipase activity was assayed using p-nitrophenyl laurate $(p-NPL)$ as substrate. Reaction mixtures (3.5 ml) contained Tris buffer (1.25 ml; 0.05 M; pH 8.2), p-NPL (1.25 ml $420 \mu M$) and the enzyme extract (1 ml). The substrate was prepared by mixing p -NPL with SDS (1:1 v/v) in 1% Triton X solution and heating in a waterbath to 65° C for 15 min [Pinsirodom and Parkin, 2001](#page-5-0). The resulting mixture was thoroughly agitated by vortexing and then allowed to reach ambient temperature before use. The reaction was started by addition of the enzyme extract and the cuvette containing the reaction mixture was immediately transferred into the spectrophotometer after vortexing. Enzyme activity was monitored at 410 nm for up to 3 min.

2.6. Texture analysis

Texture evaluations were performed on a texture analyzer (Model TA-TX2, Texture Technologies Corp., Scarsdale, NY) equipped with a 25 kg load cell and 35 mm diameter cylindrical aluminium probe. The probe compressed samples in a two-cycle test, as previously described [\(Lamikanra, Kueneman, Ukuku, & Bett-Garber, 2005 b\)](#page-4-0). Equipment settings were as follows: preset speed, 2 mm/s; test speed, 1 mm/s; distance as 30% strain; time 1 s; trigger force, 20 g. Hardness, chewiness, cohesiveness and springiness values were recorded for five fruit samples for each treatment.

3. Results and discussion

Head space $CO₂$ ([Fig. 1](#page-2-0)) concentrations indicated decreased rates of respiration in fruit treated with hot water relative to the untreated control. The rate was slightly lower in treated fruit cut without overnight storage until day 5 but, on day 8, the headspace composition was comparable to that of other heat treated fruit. The effect of post-cut application of calcium on cantaloupe melon is usually a decrease in respiration [\(Lamikanra &](#page-4-0) [Watson, 2004a; Luna-Guzman & Barrett, 2000\)](#page-4-0). Addition of calcium to pre-treatment water in this study did not reduce respiration rate relative to treated fruit without added calcium. Instead, $CO₂$ accumulation was higher in the calcium- treated relative to the corresponding treated fruit without added calcium. [Roy et al. \(1994\)](#page-5-0) found that

Days in storage

Fig. 1. Changes in headspace $CO₂$ during storage of fresh-cut cantaloupe melon. Fruit was processed unheated (fresh-cut) or after heat-treatment at 60 °C for 60 min. Treated fruit were either processed immediately after heat-treatment in water (heat) or in calcium lactate solution (heat $+$ Ca), or after 24 h storage of heated fruit in water (heat $+ 24$ h) or in calcium lactate solution (heat $+$ Ca $+$ 24 h). Error bars represent standard errors of means.

non-heated 'Golden Delicious' apples exhibited numerous deep surface cracks that formed an interconnected network on the fruit surface, unlike the epicuticular wax of heat-treated fruit that did not exhibit a similar network of deep cracks. This obstruction or elimination of deep cracks by hot water apparently limited $CaCl₂$ solutions from entering the fruit during heat-treatment. A combination of a process similar to this, which might also involve formation of calcium-induced salt bridge links at the surface of the fruit, may strengthen skin cell walls and prevent infiltration of calcium into the fruit. Additionally, the temperature gradient between the fruit initially at 4 °C and water temperature of 60 °C would favour movement of calcium ions from the fruit to water, although this could be minimised by osmotic pressure that would did the penetration of calcium ions into the fruit. Infusing calcium into uncut melons may also be limited by surface netting interfering with movement of calcium into the hyperoxidaseermal mesocarp ([Lester & Grusak, 1999,](#page-4-0) [2001](#page-4-0)).

Previous studies ([Lamikanra & Watson, 2001, 2002](#page-4-0)) indicated the ascorbate dependency of peroxidase (POD) enzymes in a number of commonly fresh-cut processed fruits whose activities appear to be related to the level of oxidative stress in cut fruit. Hot water treatment in the absence of calcium increased POD activity in fruit processed immediately after heat-treatment (Fig. 2). When the treated fruit was stored for 24 h before processing, POD activity was lower relative to unheated fruit or fruit that was processed immediately after heat-treatment. The presence of calcium in the treatment solution increased POD activity, in fruit stored for 24 h before processing, after 5 days. After 8 days of storage, POD activities were slightly higher in calcium treated fruit than in non-calcium heat- treated fruit that was held for 24 h. The mechanism by which Ca^{2+} regulates antioxidant enzymes remains to be determined [\(Hernandez et al., 2003\)](#page-4-0). Calcium chloride,

Fig. 2. Effect of storage on peroxidase activity during storage of fresh-cut cantaloupe melon. Fruit treatments and legend codes are identical to those of Fig. 1.

when applied to post-cut fruit, decreased POD activity in the extracted enzyme from cantaloupe melon [\(Lamikanra](#page-4-0) [& Watson, 2001](#page-4-0)). Reported effects of calcium application on fruit POD activities have been inconsistent, with some indicating increased enzymatic activity [\(Feng & Barker,](#page-4-0) [1993; Sams & Conway, 1993](#page-4-0)) while decreases have been reported by others [\(Antoniolli, Benedetti, Souza-Filho, &](#page-4-0) [de-Souza-Filho, 2003; Hiwale & Singh, 2003](#page-4-0)). Concentration of applied calcium may, however, be a determining factor in the effect on POD activity. [Lima et al. \(2000\)](#page-5-0) found that POD activity in ''Italia'' grape was reduced by 13.3% when 1% Ca was applied but increased by 27.2% in a 1.5% Ca application.

Hot water treatment reduced lipase activity in both fruit that was immediately processed and that was cut 24 h after heat-treatment (Fig. 3). The presence of calcium in the treatment water increased lipase activity. This is consistent

Fig. 3. Effect of storage on lipase activity during storage of fresh-cut cantaloupe melon. Fruit treatments and legend codes are identical to those of Fig. 1.

with the observed effect that was noted by [Whitaker, Klein,](#page-5-0) [Conway, and Sams \(1997\)](#page-5-0) that hot water treatment had a much greater effect than had calcium on lipid metabolism in apple fruit and that combination treatment in which fruit, heated for 4 days was later infiltrated with $CaCl₂$ (2%)- heated fruit, generally had a smaller effect than had

Fig. 4. Isoelectric focussing of acetone powder extracts from fresh-cut cantaloupe melon from heated and unheated fruit. $A = pre$ -heated fruit in water at 60° C for 1 h and processed after 24 h storage at 4 $^{\circ}$ C, and $B =$ unheated control.

the hot water treatment alone. Adjustments in membrane lipids, as a result of hot water treatment, were postulated to contribute to the beneficial effects of hot water treatment on apples. Post-cut application of calcium, however, reduces lipase activity in fresh-cut cantaloupe melon ([Lam](#page-4-0)[ikanra & Watson, 2004b](#page-4-0)).

Isoelectric focussing studies indicate the development of two protein bands ($pI = 5.1$ and 6.5) in heat-treated fruit (Fig. 4). This suggests the involvement of heat shock proteins (HSP) in the effect of hot water treatment on freshcut fruit shelf life. These proteins, which are produced in response to many stresses, including heat, can protect the cells against stress-induced damage. Exogenous calcium is generally known to support the synthesis and has also been reported to support the action of HSP [\(Vladimir, Andreev,](#page-5-0) [& Trofimova, 1998](#page-5-0)). However, calcium could differentially exert its effect on the HSP synthesis [\(Kuznetsov, Andreev,](#page-4-0) [& Trofimova, 1998](#page-4-0)). [Kuznetsov, Trofimova, and Andreev](#page-4-0) [\(1997\)](#page-4-0), using a suspension cell culture of sugarbeet, also demonstrated the possible inhibitive effect of Ca^{2+} ions on the high temperature production of some heat shock proteins. Synthesis of polyamines has often been associated with water treatment of fruits and vegetables [\(Wang, 1994;](#page-5-0) [Valero et al., 2002\)](#page-5-0). These polyamines, particularly putrescine, play important roles in heat shock responses ([Basra,](#page-4-0) [Basra, Malik, & Grover, 1997](#page-4-0)). It has been suggested that calcium and putrescine may be competing for the same

Fig. 5. Textural changes (a = Hardness, b = Cohesiveness, c = Chewiness and d = Springiness) during storage of fresh-cut cantaloupe melon. Fruit treatments and legend codes are identical to those of [Fig. 1](#page-2-0).

binding sites in the cell wall of 'Golden Delicious'' apple ([Wang, Conway, Abbott, Kramer, & Sams, 1993\)](#page-5-0). Reduction of some polyamines may also occur as a result of calcium treatment of some horticultural crops [\(Tan, Dong,](#page-5-0) [Zhang, & Li, 1998](#page-5-0)). Heat shock proteins may have contributed to the improved sensory attributes of fresh-cut cantaloupe melon from heat-treated fruit (Lamikanra et al., 2005a)

Heat-treatment of fruit most often increases firmness and reduces softening during postharvest storage (Kim, Smith, & Lee, 1993; Paul & Chen, 2000; Valero et al., 2002). Instrumental texture assessment of hardness indicated increased firmness of cut cantaloupe as a consequence of hot water pretreatment, particularly in measurements taken after 8 days of storage time ([Fig. 5](#page-3-0)a). Presence of calcium in the treatment water did not increase firmness. In a recent report, sensory evaluations of cut cantaloupe melon, from pre-heated fruit at 50 °C, did not show a significant increase in hardness as a result of heat-treatment during 5 days of storage at 10 °C (Lamikanra et al., 2005a). The difference between human sensory results and instrumental measurements may be related to differences in sensitivity levels between the two methods. The use of 60° C treatment temperature in this study might also have enhanced hardness of the heated fruit. Inhibition of solubilisation of the carbonate-soluble pectin fraction is one of the main factors contributing to the firmness retention due to heattreatment [\(Shalom, Hanzon, Pinto, & Lurie, 1996\)](#page-5-0). Loss of neutral sugar side chains during heat-treatment also leads to closer packing of the pectin strands, which hinders enzymatic cleavage, resulting in firmer fruit [\(Shalom,](#page-5-0) [Hanzon, Klein, & Lurie, 1993](#page-5-0)). Heat- treatment-induced strengthening of internal bonds was indicated by cohesiveness results [\(Fig. 5b](#page-3-0)). Chewiness, an indication of the length of time required to chew a sample to consistency, was also generally increased by heat-treatment ([Fig. 5](#page-3-0)c). Springiness or elasticity, however, decreased during storage of cut fruit from cantaloupe melon that was held for 24 h after heat-treatment before processing ([Fig. 5d](#page-3-0)). Overall, the presence of calcium in the treatment water did not profoundly alter the fruit texture relative to treated fruit without added calcium during storage.

4. Conclusion

Hot water pre-treatment of cantaloupe melon prior to processing could be used to extend the shelf life of the cut fruit. Heat-treatment reduced respiration of cut fruit during storage. Our results indicate that it is more beneficial to store treated fruit for 24 h at 4° C than to process right after treatment. Lipase and peroxidase activities are considerably lower during storage of fruit processed 24 h after treatment. Inclusion of calcium in the treatment solution inhibited the potentially beneficial effect of heat-treatment by increasing lipase activity in the fruit. Hot water pre-treatment increases firmness, chewiness and cohesiveness of cut cantaloupe melon, but may decrease springiness during storage of fruit held for 24 h at 4° C after heat-treatment before processing. Calcium, when present in the treatment solution, did not alter textural changes that occurred as a result of heat-treatment.

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