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Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality

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ABSTRACT

Fresh-cut 'Braeburn' apple slices were dipped into cold water (4 °C for 2 min) or hot water (HWT, 48 °C or 55 °C for 2 min) followed by dips into 0 or 6% w/v aqueous calcium ascorbate (CaAsc, 2 min, 0 °C) and stored in air up to 28 d at 4 °C. Microbial counts, changes in browning and sensory acceptance were determined to indicate changes in quality. Changes in antioxidant levels were measured using free radical scavenging activity (DPPH), reducing activity (FRAP), ascorbic acid content (AA) and polyphenolic content (by HPLC). CaAsc dips had a strong impact reducing the browning through increasing the flesh luminosity and hue angle. 6% CaAsc in fresh-cut apples extended the overall acceptability from less than 7 d to 14 d. Immediately after CaAsc treatment, AA content was 5 fold higher (0.25–1.25 g kg^{-1}) than those not dipped into CaAsc. However, the combination of HWT treatments and CaAsc dips led to seven fold increased levels of AA inside the apple tissue (0.25–1.85 g kg^{-1}) and consequently increased the antioxidant activity. HWT did not increase the AA content when not combined with CaAsc dips. The HWT CaAsc dip extended the overall acceptability to 21 d compared to 14 d for samples not heated but dipped into CaAsc. Shelf life was ultimately limited by sensory quality. At day 28, total plate counts were reduced from 5.3 log cfu/g (untreated slices) to 4.6 log cfu/g in the 6% CaAsc dips and further to 3.9 log cfu/g with the combination of HWT and CaAsc dip. Changes in the content of phenolic compounds with time, HWT and CaAsc dip were generally not significant except for slightly increased quercetin and phloridzin levels and decreased *p*-coumaric and procyanidins over time. The combination of HWT at 48 °C for 2 min followed by 6% CaAsc dip would be best for preserving the eating quality of apple slices.

1. Introduction

Apples are one of the most widely consumed fruits (FAOSTAT, 2011) and are a good source of phytochemicals (Boyer and Liu, 2004). Epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes (Boyer and Liu, 2004). One way to increase fresh fruit consumption is processing fruit into fresh-cut product to be sold as convenient single servings. However, fresh-cut processing results in major tissue disruption of surface cells and injury stress of underlying tissues (Toivonen, 2004). The main problem for fresh-cut apple is oxidation caused by polyphenol oxidase (PPO) that exists in particularly high amounts in apple (Whitaker, 1972). The resulting browning makes the product unsuitable to the consumer. Ranges of treatments have been applied to extend the shelf life of fresh-cut apples, mainly dipping in solutions of a wide range of anti-browning agents. Ascorbic acid, oxalic acid, oxalacetic acid, kojic acid, erythorbic acid, citric acid, and/or calcium, cysteine, 4-hexylresorcinol have all been examined at different concentrations (Son et al., 2001; Rojas-Grau et al., 2006; Tortoe et al., 2007). Among the aforementioned methods, the use of calcium ascorbate (CaAsc) has been found to be the most effective anti-browning agent and can be marketed as a minimal chemical input. Its application increases the antioxidant status and extends shelf life of apple slices (Aguayo et al., 2010). On the other hand, several methods, including heat shock, have been used to inhibit polyphenol oxidase activity since this reduces the rate of the polymerisation step of browning reactions (Kwak and Lim,

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2005; Barbagallo et al., 2012). Hot water treatment (HWT) is an effective physical treatment, free of chemical residues, and readily applicable in the fresh-cut industry during the washing process (Kim et al., 1993; Abreu et al., 2003). Previous studies have shown that HWT is sufficiently effective to maintain product quality for fresh-cut products such as lettuce (Murata et al., 2004; Moreira et al., 2006), rocket (Koukounaras et al., 2009), spinach (Gómez et al., 2008; Glowacz et al., 2013), eggplants (Barbagallo et al., 2012), and onions (Siddiq et al., 2013).

The use of non-chemical technologies that can improve product quality response by additional interactions could improve the effectiveness of CaAsc in the fresh-cut apple industry. This study sought to determine whether HWT could be recommended as a method in combination with CaAsc dips to help to maintain the sensory quality of fresh-cut apples.

2. Materials and methods

2.1. Raw material

New Zealand grown 'Mahana Red' apples (*Malus domestica* Borkh., a sport of 'Braeburn') were sourced from the refrigerated storage of a commercial supermarket. The apples had been stored for up to 6 months under controlled atmosphere (2 kPa O_2 plus 1 kPa CO_2) at 0 °C. Apples boxes were transported to the laboratory and stored at 0 °C for 12 h. The boxes were opened in a food-grade processing room (10 °C) and the fruit sorted to remove those damaged or with significant variation in background colour. Whole apple surfaces were washed by dipping in cold water (4 °C) with 5 mg L^{-1} chlorine dioxide (Oxine™, Australasia Marketing Pty Ltd. Sydney, NSW, Australia) for 10 min. The apples were then manually cored with a metal corer and cut into 8 wedge slices using a handheld knife. All the slices were dipped into cold water (4 °C) with 2 mg L^{-1} chlorine dioxide for 2 min, and the slices drained. Slices for HWT were dipped in hot water (48 °C or 55 °C) for 2 min. A stainless water-bath (Grant 40 L, with a Grant temperature controller units (±0.1 °C, 1.4 kW heater model GRAVF, U.K.) with continuous hot water recirculation and stirring was used to maintain the relevant temperature. All apple slices (heated or not) were then dipped for 2 min into 0% (control) or 6% CaAsc solution (w/w; 99.9% purity, Wolf Canyon Asia Pacific Ltd) and drained. This solution was made using water at 0 °C that had been pre-treated with 2 mg L^{-1} chlorine dioxide. Apple skins were not removed prior to treatment, as apple slices are currently marketed with skin intact. For each treatment, apple slices were randomised across packages of 15 apple slices (350 ± 20 g) per aluminium bag (25 cm × 18 cm, 80 μm thickness, Caspak, New Zealand). To maintain nearly ambient oxygen concentration in the bags, two 5-mm holes were punched through both sides of each bag. Three replicate bags per treatment were stored at 4 °C per storage duration: 7, 14, 21 and 28 d. Measures of antioxidant activity were also measured on day 0 (immediately after treatment).

2.2. Parameter evaluations

2.2.1. Colour measurement

The surface colour of the apple flesh was determined on three equidistant points in each apple slice cut surface with a Minolta chromameter (D65 light source, Minolta Camera Co., Osaka, Japan). The results were expressed as CIELAB ($L^*a^*b^*$) colour space. L^* defines the lightness and a^* and b^* define the red-greenness and blue-yellowness, respectively. The flesh colour was also measured and expressed as hue angle (h° = arctangent $[(b^*)\ (a^*)^{-1}]$) and chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$). Fifteen slices per treatment (5 slices per replicate) were measured.

2.2.2. Sensory evaluation

A panel of five people was trained to recognise and score the quality attributes of the treated apple slices. All assessments were compared to freshly cut slices. Appearance, taste and texture were scored on a nine-point scale where 1 = complete lacking or soft, to 9 = fully characteristic of fresh. A similar scale, where 1 = inedible, 3 = poor, 5 = fair (limit of marketability), 7 = good and 9 = excellent was used for the evaluation of the overall acceptability.

2.2.3. Microbial analyses

After 28 d storage, microbial growth on the slices was determined by a certified food analysis laboratory (AgriQuality, Auckland, New Zealand). Only samples dipped into 6% CaAsc and both controls (0 and 6% CaAsc) from the HWT and control were analyzed. From each of five slices, 10 g samples were blended with 90 mL of sterile peptone buffered water (Merck Darmstadt, Germany) for 1 min in a sterile stomacher bag (Model 400 Bags 6141, London, UK) using a Masticator (Colwort Stomacher 400 Lab, Seward Medical, London, UK). Appropriate dilutions were prepared. Plate Count Agar medium (PCA, Merck) was used for TPC and Rose Bengal agar medium (Merck) for the yeast and mould counts. Incubation conditions were 30 °C for 48 h for TPC, and 22 °C for 5 d for yeasts and moulds, respectively. Microbial counts were reported as log 10 colony forming units per gram of sample (log cfu g^{-1}).

2.2.4. Chemical measurements

Fruit pieces were flash frozen in liquid nitrogen and stored at −80 °C for a maximum of 2 months. To ensure uniformity, frozen samples (200 g) were either homogenised in 100 mL of distilled water in a commercial blender (Sunbeam Model PB7600, Type 504, 230–240 V, Sydney, Australia) to produce a juice extract for antioxidant activity analysis, or 150 g was ground to a fine powder in a Cryomill in liquid nitrogen for ascorbic acid content (AA) analysis.

2.2.4.1. Antioxidant activity. Two assays were used to measure the antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. All the material, method and equipment has been previously reported (Aguayo et al., 2010). All the antioxidant assays were carried out in triplicate. Calibration curves were made for each assay using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and AA as standards. The antioxidant activity (DPPH, FRAP assay) was expressed as Trolox and AA equivalent antioxidant activity per kg fresh weight of apple tissue.

2.2.4.2. Ascorbic acid evaluation. The method was adapted from Rassam and Laing (2005). A 0.2 g sample of powdered apple tissue was suspended in 1 mL of 6% metaphosphoric acid, 2 mM EDTA and 1% PVPP containing 4 mM TCEP (tris[2-carboxyethyl] phosphine hydrochloride). The slurry was vortexed for 20 s, and incubated in a heating block for 2 h at 40 °C to ensure full reduction of any dehydroascorbate. The extract was clarified by centrifugation at 4 °C for 10 min, and 20 μL of the supernatant was injected into a 7.8 × 300 mm Aminex HPX-87H HPLC column (Bio-Rad, Merck, Darmstadt, Germany). The column was run with 2.8 mM H_2SO_4 as the mobile phase, at a flow rate of 0.01 mL s^{-1}. The amount of AA was detected using a Waters 996 photodiode-array detector (Milford, MA, USA) set at 245.5 nm for absorbance using AA (Sigma, St. Louis, MO) as a standard. The peak was authenticated as AA by showing that it was completely degraded by ascorbate oxidase at pH 5.5.

2.2.4.3. Phenolic compounds evaluation. One mL of apple juice extract was combined with 0.25 mL of methanol and HCl