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Effects of calcium ascorbate treatments and storage atmosphere on antioxidant activity and quality of fresh-cut apple slices

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ABSTRACT

Fresh-cut 'Braeburn' apple slices were dipped in calcium ascorbate (CaAsc; 0, 2, 6, 12 and 20%, w/w) and stored in air or under modified atmosphere (MA) conditions for up to 28 d at 4 °C. Changes in antioxidant levels were measured using free radical scavenging activity (DPPH), reducing activity (FRAP), ascorbic acid content (AA) and polyphenolic content (by HPLC). Changes in browning, sensory quality and microbial counts were measured to indicate eating quality. CaAsc dips increased the initial levels of AA from 0.19 g kg^{-1} in the untreated control to 3.8 g kg^{-1} for the 20% CaAsc treatment. Ascorbic acid content of treated slices during storage decreased by more than 50% in CaAsc concentrations of 6, 12 and 20%. Similar patterns were observed for FRAP and DPPH activities. Untreated or 2% CaAsc treated slices stored in air or MA showed browning, microbial deterioration and poor sensory quality, thus resulting in a short shelf life (<7 d). However, apples dipped in 6 or 12% CaAsc and stored in MA packaging, or dipped in 20% CaAsc and packaged in air or MA had a shelf life of 21–28 d. Total antioxidant activity in these treatments was provided by both exogenous ascorbic acid and endogenous phenolic compounds; the latter varied in composition, but were relatively stable during storage compared with ascorbate in higher CaAsc concentration treatments. Thus, the antioxidant levels (as measured by FRAP and DPPH) were related to shelf life and it appears that an antioxidant activity remaining above 2 g kg^{-1} (DPPH or FRAP) may be a minimum level to achieve long shelf life in 'Braeburn' apple slices.

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1. Introduction

Sales of fresh-cut product continue to grow through consumers' increasing willingness to pay for prepared, ready-to-eat or ready-to-use fresh produce (Rico et al., 2007). A range of treatments have been applied to extend the shelf life of fresh-cut apples including use of natural browning inhibitors (Luo and Barbosa-Canovas, 1996; Buta et al., 1999; Rojas-Grau et al., 2006), salt and chemical treatments (Gil et al., 1998; Zuo and Lee, 2004; Varela et al., 2007), coating agents and reduced oxygen atmospheres (Anese et al., 1997; Rocculi et al., 2004; Pérez-Gago et al., 2006).

A key approach used to avoid browning in apples has been the use of reducing agents, often with the addition of calcium chloride ($CaCl_2$), in combination with modified (reduced O_2) atmospheres and low temperature storage (Sapers et al., 1990; Luo and Barbosa-Canovas, 1996). Tortoe et al. (2007) observed moderate browning on 'Golden Delicious' apple slices using ascorbic acid (AA, 0.5 M with or without sodium chloride) stored at 4 °C up to 14 d. Son et al. (2001) also reported the effect of AA on browning of fresh-cut apples. Selected combinations of treatments have been shown to be effective in the prevention of browning of apple slices for up to eight weeks at 0.2 °C (Anese et al., 1997). While flesh browning may be minimised during extended storage, other organoleptic factors such as texture and flavour may not be acceptable. An understanding of the physiology behind such changes and the development of mechanisms to prevent them is required to improve the shelf life of fresh-cut products.

The role of oxidative-related senescence in postharvest quality loss is well recognised (Hodges, 2003), but quality loss due to accelerated postharvest senescence is not consistently associated with loss of antioxidant concentration or activity (Hodges, 2003). Active oxygen species (AOS) have been associated with induced or natural senescence processes (Hodges and Forney, 2000). Changes in lipid membranes as a result of oxidative stresses can trigger lipoxygenase degradation and the generation of biological signals that result in apoptosis and necrosis (Spiteller, 2003).

Levels of AOS are regulated by their relative rates of generation and degradation which includes scavenging by enzymatic and non-enzymatic antioxidants (Hodges, 2003). *In vivo*, the intrinsic antioxidant content is compartmentalised in different organelles and may have variable impacts on senescence and programmed cell death. Calcium plays a pivotal role in cell signals related to

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AOS (Bhattacharjee, 2005). Calcium dips have been implicated in enhancing membrane stability, slowing senescence, and improving the retention of membrane integrity (Picchioni et al., 1996).

Exogenous antioxidant treatments should be able to interfere with senescence-related oxidation reactions but there is little direct evidence. Antioxidant dips for fresh-cut apples reduce the browning and presumably also reduce the levels of AOS. Ideally, there should be an efficient level of antioxidant which would decrease or even neutralise the AOS, resulting in prevention of induced senescence and thus, extension of shelf life. Apple fruit contain up to 0.25 g kg^{-1} ascorbic acid antioxidant. In our opinion, this could be supplemented by fresh-cut dips with exogenous ascorbate or other antioxidants. Apples also contain large amounts of endogenous phenolic antioxidant compounds (Kondo et al., 2002) that react with oxidisers in damaged tissues to form enzymatic browning products. The fate of the added antioxidants and the influence of the resulting levels of antioxidants on the quality of the resulting fresh-cut produce are not known.

Since calcium ascorbate (CaAsc) is becoming the commercial "industry standard" anti-browning treatment for fresh-cut apples, this study sought to investigate the influence of the concentration of CaAsc dips, subsequent storage time and modified atmosphere conditions on antioxidant status and shelf life of fresh-cut apple slices.

2. Materials and methods

2.1. Raw material

New Zealand grown 'Mariri Red' apples (*Malus domestica* Borkh., a sport of 'Braeburn') were sourced from coolstores of a commercial supermarket and transported to the laboratory and stored at 0 °C for 12 h. This apple variety was chosen due to high level of ascorbic acid (Laing et al., personal communication) and is an important commercial cultivar. The apple 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 sterilised by dipping in cold water (4 °C) with 5 mg L^{-1} chlorine dioxide (Oxine™, Australasia Marketing Pty Ltd, Sydney, NSW, Australia) for 15 min. The apples were then manually cored and cut into eight slices (using a handheld slicer). This wedge cut allowed maximum use of the apple. Slices were dipped into cold water (0 °C) with 2 mg L^{-1} chlorine dioxide for 2 min, and the slices drained. CaAsc solutions were made up using water at 0 °C pre-treated with 2 mg L^{-1} chlorine dioxide then made up to the different concentrations using CaAsc (99.9% purity, Wolf Canyon Asia Pacific Ltd) at 0 (control), 2, 6, 12 and 20% (w/w). Slices were then dipped in these CaAsc solutions for 2 min and drained. Apple skins were not removed prior to treatment, as apple slices are currently marketed with skin intact.

2.2. Packaging of apple slices and storage

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 near-ambient oxygen concentration in the air treatment bags, a 5-mm hole was punched through both sides of each bag. For the modified atmosphere (MA) treatment, most of the air inside the bags was extracted by a vacuum machine and the bags were heat-sealed (vacuum impulse seal, Model ME-4510VG, Mercier Corporation, MHsing-Chuan City, Tapei Hsien, Taiwan).

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).

2.3. Parameter evaluations

2.3.1. Gas measurements

Gas composition (O_2 and CO_2) within the bags was monitored weekly until the end of storage using a GC (using an infra-red CO_2 transducer Servomex 1505, Servomex Ltd., Sussex, UK) and oxygen sensor (CiTicel oxygen cell, Model C/2, City Technology Ltd., London). For analysis, 0.5 mL gas samples were taken from each bag using a plastic syringe through a silicone septum. At the same time, the C_2H_4 levels were determined by injection of 1 mL gas samples into a GC (Philips PU4500; Pye Unicam, Cambridge. U.K.) equipped with an active alumina column and a flame ionisation detector. Three replicates were made for each treatment and evaluation period. Laboratory conditions for temperature were 25 °C and 1 atm (≈101 kPa).

2.3.2. 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 (Model CR-300 Minolta; Ramsey, NY). 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 $[(b^*)(a^*)^{-1}]$), chroma ($C^* = [(a^*)^2 + (b^*)^2]^{1/2}$) and whiteness index ($WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$) according to Bolin and Huxsoll (1991). The more representative colour parameters (h° and WI) are reported in the results. Fifteen slices per treatment (five slices per replicate) were measured.

2.3.3. 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 slices freshly cut from whole air-stored apples of the same variety and purchase data. 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. Only appearance and overall acceptability data are presented.

2.3.4. Microbial analyses

Microbial growth on the slices was determined after 28 d storage by a certified laboratory (AgriQuality, Auckland, New Zealand). 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 yeast counts. Incubation conditions were 30 °C for 48 h for TPC, and 22 °C for 5 d for yeasts, respectively. Microbial counts were reported as $\log_{10}$ colony forming units per gram of sample (log cfu g^{-1}).

2.3.5. Chemical measurements

Fruit pieces were flash frozen in liquid nitrogen and stored at −80 °C for a maximum of two 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, Auckland, New Zealand) to produce a juice extract (for the antioxidant activity analysis), or 150 g was ground to a fine powder in a Cryomill in liquid nitrogen (for ascorbic acid content (AA) analysis).

Antioxidant activity. Two assays were used to measure the antioxidant activity; 2,2-diphenyl-1-picrylhydrazyl (DPPH) and

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