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Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples

Mahmoud Koushesh Saba [a, *], Ommol Banin Sogvar [b]

[a] Department of Horticultural Science, University of Kurdistan, Sanandaj, P.O.Box: 416, Iran
[b] Department of Horticultural Science, University of Kurdistan, Sanandaj, Iran

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ABSTRACT

Fresh cut apples have a short shelf-life due to their high rate of deterioration, water loss and cut-surface browning that can rapidly reduce fruit quality. In this study the effects of carboxymethyl cellulose (CMC) coatings in combination with $CaCl_2$ and ascorbic acid (AA) has been studied. Apple slices were dipped for 5 min in distilled water (uncoated), CMC (1% w/v) + $CaCl_2$ (0.5%), or CMC (1%) + $CaCl_2$ (0.5%) + AA (2%), and then stored at 4 °C, 90–95% (RH). The treatments suppressed browning, retained flesh firmness, and reduced soluble solid content, titratable acidity and pH changes of the slices. Browning incidence with CMC in combination with AA was lower than that of CMC solely. Vitamin C and antioxidant capacity in treatments was higher than that of uncoated. Coating treatments generally reduced total phenolic and flavonoid concentrations and polyphenol oxidase and peroxidase activity changes of slices during storage. These findings suggest that the combination use of CMC + AA may be useful for maintaining quality and reducing surface browning of fresh cut apples.

1. Introduction

Recently, there has been an increasing market demand for minimally processed fruits and vegetables. Fresh cut apples in particular are desired as a convenient snack for catering services to salad-bars, schools, and company cafeterias (Saftner, Abbott, Bhagwat, & Vinyard, 2005). However minimally processed products became more perishable because processing results in quality deterioration associated with water loss, softening, microbial contamination, increased respiration, and cut-surface browning (Rolle & Chism, 1987). Enzymatic browning is one of the most important reactions occur in many fresh-cut fruits and vegetables. The reaction in which phenolic compounds are oxidized is related to polyphenol oxidase (PPO) activity, the amount of phenolics, and the presence of oxygen (Koukounaras, Diamantidis, & Sfakiotakis, 2008). However, antioxidants are involved in browning and maintaining the commercial value of fresh cut products (Rojas-Grau, Sobrino-Lopez, Tapia, & Martin-Belloso, 2006).

Many compounds may be used to reduce browning in foods (McGhie, Hunt, & Barnett, 2005). Ascorbic acid (AA) is known as one of the most widely used compounds, since it is very effective in reducing browning, generally recognized as safe, inexpensive, and consumer friendly (Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martin-Belloso, 2013a). AA treatments reduce cut surface browning and increase apple slice shelf life, but only for a short period of time, since use of AA soften the tissue and promotes microbial growth (Gil, Gorny, & Kader, 1998) and subsequent fruit deterioration. Moreover, once the AA has been completely oxidized to dehydro-ascorbic acid, the reaction lead to convert back quinones to phenolic compounds, quinones again can be accumulated and undergo browning (Sarpers, 1993).

Edible coatings have been used to reduce the deleterious effect brought about by minimal processing. The semipermeable barrier provided by edible coatings is aimed to extend shelf life by reducing moisture and solute migration, gas exchange, respiration, and oxidative reaction rates, as well as suppress physiological disorders on fresh-cut fruits (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007; Rojas-Grau, Tapia, & Martin-Belloso, 2008). Carboxymethyl cellulose (CMC) is a linear, long-chain, water-soluble, anionic polysaccharide that can be used as fruit coating (Gol, Patel, & Rao, 2013). Purified CMC is a white to cream-colored, tasteless, odorless, free-flowing powder (Hattori, Abe, Yoshida, & Cuculo, 2004; Keller, 1986). Treatment with CMC can extend strawberry (Gol et al., 2013) and cucumber (Oluwaseun, Kayode,

* Corresponding author.
E-mail address: m.saba@uok.ac.ir (M. Koushesh Saba).

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Bolajoko, & Bunmi, 2013) shelf life and maintain quality of fruit. Edible coatings can also serve as carriers of food additives, e.g. anti-browning and antimicrobials agents, colorants, flavors, nutrients, and spices (Pranoto, Salokhe, & Rakshit, 2005).

Until recently, there have been few researches that investigated the effect of the combination of CMC and anti-browning agents for minimally processed products to inhibit enzymatic browning. The objective of this study was to develop procedures for the use of CMC coatings in combination with anti-browning agent and to extend the shelf-life of minimally processed apple slices.

2. Materials and methods

2.1. Fruit slicing and treatments

Apples (*Malus domestica* Borkh L.) of approximately same size and maturity were harvested from a commercial orchard and stored at 1 °C until processing. These fruits were rinsed gently with 200 μL L^{-1} sodium hypochlorite solution, dried naturally and cut into eight equal slices using a sharp stainless steel knife and seed cavity was removed.

Coating solutions were prepared by dissolving CMC powder in distilled water (1% w/v) and heated at 85 °C for 30 min while stirring until the solution became clear and 2.5 mL 100^{-1} mL glycerol was added as plasticizer. Fruit slices were divided in three groups and were dipped in the following solutions for 4 min, T1: distilled water as uncoated control, T2: CMC +0.5% $CaCl_2$ and T3: CMC +0.5% $CaCl_2$ +2% AA. Temperature of all solutions was 24 °C. Following, all fruit were air-dried at room temperature and then placed in polystyrene boxes, each containing 10 apple slices, and stored at 4 °C with 95% relative humidity. Three boxes were sampled at 0, 1, 3, 6, 9 and 12 days for each treatment.

2.2. Measurement of browning index

Evaluation of slices surface browning was subjectively based on a numeral scoring index (Lin et al., 2013) using a scale of 0–4, where 0 = none, 1 = slight (up to 5% surface affected), 2 = moderate (5–20% surface affected), 3 = moderately severe (20–50% surface affected) and 4 = extreme (>50% surface affected) for individual slices. Severity index of flesh browning determined as follows: ((% fruit with slight browning × 1) + (% fruit with moderate browning × 2) + (% fruit with moderately severe browning × 3) + (% fruit with extreme browning × 4))/5.

2.3. Fruit quality measurements

Fruit slice firmness was evaluated using a texture analyzer (Santam, STM-1, Iran), fitted with an 8 mm probe with constant speed of 20 mm min^{-1}. Values were expressed as Newton (N). A wedge-shaped slice of flesh taken from each fruit slice was pooled and juiced. Soluble solids content (SSC) was measured using a temperature-compensated refractometer (Brix 0–32%, Atago, Japan). Titratable acidity (TA) was measured by titrating 10 mL of juice with 0.1 N NaOH to an end point of pH 8.2 and expressed as % malic acid. The pH of fruit juice was measured using a pH meter (Metrohm 827, Switzerland).

2.4. Vitamin C assay

Vitamin C content was determined by titration with 2,6-dichlorophenolindophenol (DCPIP) (AOAC, 2000), using different AA concentrations for the standard curve, and expressed in mg of vitamin C per 100 g fresh weight (FW).

2.5. Total phenol (TP) and total flavonoid (TF) concentration

TP concentrations were measured by homogenizing 1 g of frozen tissue from each replicate with 3 mL ice cold 1% HCl–methanol solution and then centrifuged at 4 °C for 15 min at 12,000× g. The supernatant was collected and used for phenol determination. TP concentration in the extracts were determined according to the Folin–Ciocalteu procedure (Orthofer & Lamuela-Raventos, 1999), using gallic acid for the standard curve. Results are expressed as mg of gallic acid equivalent (GAE) per 100 g of FW.

TF concentrations were measured according to Bouayed, Hoffmann, and Bohn (2011). One mL aliquot of catechin standard solution (0–100 mg/L) or samples were added to 10 mL volumetric flasks containing 4 mL water. Initially 0.3 mL of 5% $NaNO_2$ was added to the flask, following 0.3 mL of 10% $AlCl_3$ was added after 5 min, and then 2 mL of 1 M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. The absorbance was measured at 510 nm, using a spectrophotometer (Unico UV-2100, USA). TF was expressed as mg catechin equivalents per 100 g FW.

2.6. Total antioxidant activity (TAA)

TAA was determined by the 2,2-Diphenyl-1-picryl-hidrazil (DPPH) radical-scavenging method according to Sanchez Moreno, Larrauri, and Saura-Calixto (1999). The absorbance was measured at 517 nm, using a spectrophotometer. Total antioxidant activity was expressed as the percentage inhibition of the DPPH radical and was determined using the following equation:

$$TAA(\%) = \frac{Abs_{sample} - Abs_{control}}{Abs_{sample}} \times 100$$

2.7. Enzyme activities

The activity of peroxidase (POX) and superoxide dismutase (SOD) was measured using 1 g of sample homogenized in 2 mL of freshly prepared 100 mM phosphate buffer (pH 7). The homogenate was centrifuged and the supernatant was used as a source of crude enzyme. All steps to obtain enzyme preparations were carried out at 4 °C.

POX activity was determined by the rate of guaiacol oxidation in the presence of hydrogen peroxide at 470 nm for 1 min, as described previously (Ghanati, Morita, & Yokota, 2002). The reaction mixture contained 50 mM phosphate buffer (pH 7), 28 mM guaiacol, and enzyme extract in a 3 mL assay volume, and the reaction was initiated by adding hydrogen peroxide at a final concentration of 5 mM. The enzyme activity was expressed as units of enzyme per milligram of protein. SOD activity was determined by measuring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm after exposure to a light for 30 min (Giannopolitis & Ries, 1977), as described previously (Koushesh Saba, Arzani, & Barzegar, 2012). A total of 1 unit of SOD activity is defined as the amount of enzyme that inhibits the NBT photoreduction by 50% under the assay conditions.

PPO enzyme was extracted by blending 1 g of frozen sample in freshly prepared 100 mM phosphate buffer (pH 6.8) containing polyvinylpyrrolidone (PVP), followed by centrifugation at 16000g for 20 min, as described previously. Aliquots of the supernatant were added to solution containing catechol at a final concentration of 0.05 M. The increase in absorbance was monitored at 410 nm for 2 min at 25 °C. PPO activity was expressed as units per milligram of protein of the homogenate (Koushesh Saba et al., 2012).