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Effect of whey protein isolate films incorporated with montmorillonite and citric acid on the preservation of fresh-cut apples

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ABSTRACT

The objective of this paper was to evaluate the effect of bioactive whey protein isolate/montmorillonite films containing citric acid on the inhibition of enzymatic browning and physicochemical properties in minimally processed apples. Whey protein isolate films incorporated with montmorillonite (3 g/100 g) and citric acid (5 and 10 g/100 g) were applied to the apples slices. All samples were packaged in polypropylene trays (14.6 cm × 11.4 cm × 6.5 cm) and stored at 5 ± 2 °C and 85 ± 3% RH for eight days. Every two days, the apples samples were evaluated for color, acidity, pH, soluble solids, water activity and polyphenol oxidase and peroxidase enzyme activity. The enzymatic browning of the apples slices was reduced for all films during storage. However, the films containing citric acid maintained the color characteristics, reducing the loss of quality associated the maintenance of acidity, soluble solids, water activity, reduction of polyphenol oxidase and peroxidase activity, thus prolonging the shelf life of the apples.

1. Introduction

Despite the growth of minimally processed products, the shelf life of fruits and vegetables is limited by degradation reactions, mainly by enzymatic browning when cut or peeled, and growth of microorganisms (Barbosa, de Araújo, Matos, Carnelossi, & de Castro, 2013; Botelho et al., 2010; Limbo & Piergiovanni, 2007). In this context, apple is a fruit, rich in vitamins, minerals, fibers and phenolic compounds that aid in the maintenance of health and its processing in pieces appear as a demand for the convenience of the consumers (Biedrzycka & Amarowicz, 2008; Liu et al., 2016). However, fresh-cut apples are susceptible to be damaged because of their enzymatic browning. Polyphenol oxidases (PPO) are enzymes responsible for enzymatic browning in apples. This enzyme catalyzes the oxidation of phenolic compounds, with by non-enzymatic formation of melanin and produce brown pigments on the surface of fruit and vegetables. Darkness leads to the development of unpleasant flavors and quality losses (Haminiuk, Oliveira, Baggio, & Masson, 2005; Nunes, Boas, & Xisto, 2011). Peroxidase (POD) is another enzyme that participates in the enzymatic browning process, destructuring the cell membranes and promoting chain reactions that lead to the formation of free radicals, thus altering the sensorial characteristics of the product (Botelho et al., 2010; Vilas-Boas & Kader, 2006).

To avoid the enzymatic browning, chemical methods such as antioxidant addition (ascorbic acid, citric acid, calcium chloride and ethylene diamine tetracetic acid), pH decrease, and polyphenol oxidases hydrolysis with proteases are used (Raybaudi-Massilia, Mosqueda-Melgar, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, 2007; Rojas-Graü, Sobrino-López, Soledad Tapia, & Martín-Belloso, 2006). Citric acid it is an organic acid, naturally found in plants, and acts as a chelator in synergy with ascorbic acid, erythorbic acid and its neutral salts. It has an inhibitory effect on PPO by lowering the pH, complexing with the copper of the active enzyme center (del Aguila et al., 2008). In this context, besides chemical methods, researches have been focused on the development of bioactive films to reduce the decay of minimally processed fruits and prolong their shelf-life.

One way of minimizing degradation reactions due to minimal processing is the use of bioactive films, which can interact positively with food and the environment, conferring desirable sensory and nutritional characteristics and thus enhancing the shelf life of the product (Endo

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et al., 2008). Whey protein films can be used as active packaging for the reduction of waste from the dairy industry and replacement of conventional plastic films. They are excellent for carrying food additives, such as antioxidants, antimicrobials, dyes, flavors, fortifying nutrients and spices (Azevedo et al., 2015; Salmieri & Lacroix, 2006). In addition, the controlled release of the active ingredient (low diffusion rate) from the packaging material into the food helps to maintain the concentration of the active ingredient at desirable levels during the storage time (Kristo, Koutsoumanis, & Biliaderis, 2008). However, films developed from whey protein isolate usually have suitable mechanical and optical properties, and being excellent barriers to oxygen, lipids and aromas, but show poor water vapor barrier properties because of their hydrophilic nature (Azevedo et al., 2015; Ramos et al., 2012; Teixeira et al., 2014). Thus, like other proteins based on bioactive films, the low moisture barrier can be improved with the incorporation of hydrophobic substances, like as nanoparticles (Azevedo et al., 2015; Kim & Ustunol, 2001; Ramos et al., 2012).

Nowadays, that food safety is a topic of world concern and relevance. Government agencies are concerned about the safety effects of the use of nanocomposites as food packaging materials, and how these nanomaterials can migrate toward the packaged foodstuffs besides the potential hazard consumer health after nanomaterials migrated (Huang et al., 2011; Huang, Li, & Zhou, 2015). The reason, is that materials at nano-size can be substantially different from individual molecules or their conventional forms, and their toxicity is not yet completely understood. Some nanomaterials are not approved in the European Union (EU) due to limited toxicity data available (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2014; Huang et al., 2015). In addition to the physicochemical and biological nature, toxicity can be dependent on the particle size, morphology, and surface behaviours of the nanomaterials (Magnuson, Jonaitis, & Card, 2011). In this context, nanoclays are one of the first polymer incorporated in nanocomposites as a novel material for food packaging (Huang et al., 2015; Silvestre, Duraccio, & Cimmino, 2011). The most common clay nanoparticle employed in these nanocomposites is montmorillonite (MMT). This clay is naturally abundant, low cost, relatively simple processability, high stability and toxin-free mineral used in foods and healthcare products (Chen & Evans, 2005; Gutiérrez, Ponce, & Alvarez, 2017; Sorrentino, Gorrasi, & Vittoria, 2007). The sodium montmorillonite clay which their dimensions typically range from 1 to 1000 nm and they are homogeneously dispersed in the polymer matrix (Jo, Park, & Kim, 2008). This nanoclay increase the resistance of biopolymers against water, improve mechanical properties and decreased permeability (Heydari, Alemzadeh, & Vossoughi, 2013; Majdzadeh-Ardakani & Nazari, 2010; Tunç, Duman, & Polat, 2016). Thus, the application of the nanoclays has been a promising option in order to improve the performance properties and reinforcing of biodegradable films.

Research has been conducted about the effects of bioactive films on physicochemical quality of minimally processed but little has been reported. These researches related about antimicrobial films, anti-browning agents on quality of minimally processed (Barbosa et al., 2013; Del Nobile, Conte, Cannarsi, & Sinigaglia, 2008; Fai et al., 2016; Ierna, Rizzarelli, Malvuccio, & Rapisarda, 2017; Olivas, Mattinson, & Barbosa-Cánovas, 2007; Perez-Gago, Serra, Alonso, Mateos, & Del Rio, 2003; Sothornvit & Rodsamran, 2008). In this context, based on the fact that citric acid acts as a chelating agent to control the polyphenol oxidase and peroxidase enzyme activity, it is believed that the addition of this compound in a bioactive film can prevent enzymatic browning and help to preserve minimally processed apples. Thus, the objective of this paper was to evaluate the effect of whey protein isolate nanocomposites incorporated with montmorillonite (MMT-Na^+) and citric acid on the enzymatic browning and quality maintenance of freshly processed apples stored under refrigerated conditions. In addition, it is important to highlight that this manuscript is an extension on previous work reported by Azevedo et al. (2015).

2. Material and methods

2.1. Material

Whey protein isolate (9400) was obtained from Hilmar Ingredients (Hilmar, CA, USA). Glycerol and sodium hypochlorite were purchased from Sigma Aldrich (Brazil), granulated anhydrous citric acid was obtained from Cargill (Uberlândia, MG, Brazil) and natural montmorillonite clay (Cloisite Na^+), d_{001} = 11.7 Å and moisture 4–9% was supplied by Southern Clay Products, Inc. (Gonzales, TX, USA). Apples of the Gala cultivar were harvested in the morning and purchased in the retail market of Lavras (MG).

2.2. Experimental design

The active films used in this study were developed using a full factorial design with the following factors: 0 and 3 g/100 g for MMT, and 0, 5 and 10 g/100 g for citric acid. The experiment was conducted using a completely randomized design with 3 replications.

To evaluate the effect of the films on the maintenance of the physicochemical characteristics of minimally processed apples during the refrigerated storage period, the experiment was conducted using a full factorial experimental design (2 × 3 × 5). The six films developed (MMT0CA0, MMT0CA5, MMT0CA10, MMT3CA0, MMT3CA5 and MMT3CA10) were evaluated at five storage times (0, 2, 4, 6 and 8 days). The experiments were performed in triplicate, and a total of 90 trials were evaluated. The experimental portion consisted of a tray containing approximately 100 g of sliced apple.

2.3. Preparation of whey protein isolate films

All the films were developed according to Azevedo et al. (2015). Whey protein isolate (WPI) films were developed, WPI and glycerol being fixed at 6 w/v and 40 g/100 g of WPI, respectively. The control film was prepared by dissolving 6 g (w/v) of WPI in 44 mL of distilled water with 2.4 g of glycerol (GLY) (based on the WPI weight) in 56 mL of distilled water, both separately subjected to continuous agitation on a magnetic stirrer for 30 min at room temperature. After stirring, the solutions were poured together and kept under continued agitation for 10 min at room temperature. The pH was then adjusted to 8 with 2 mol/L NaOH and the final solution submitted to an ultrasonic homogenizer (Sonifier Cell Disruptor Branson - Model 450D, Manchester, UK) for 10 min at an output of 80 W/25 °C. The solutions were then heated at 90 °C for 30 min in a water bath, cooled to room temperature and poured on glass plates. The others films added MMT and citric acid were developed as was the control film, the MMT (0, 3 g/100 g of WPI) and citric acid (0, 5, 10 g/100 g of WPI) being dispersed in glycerol and codified as MMT0CA5; MMT0CA10; MMT3CA0; MMT3CA5 and MMT3CA10. The thickness control was accomplished by the volume applied to the support, corresponding to 110 mL. Then, the films with dimensions of 18 × 30 cm were dried at room temperature for 48 h to ensure slow evaporation of the solvent and film formation.

2.4. Film conditioning and thickness

All films were stored at a controlled temperature of 23 ± 2 °C and 50 ± 5% relative humidity for 48 h before analysis, according to the D618-00 method (ASTM, 2000). The average film thickness was measured by reading at ten randomly chosen distinct points on each test body, using a Mitutoyo digital micrometer (accuracy 0.01 mm Mitutoyo, Suzano, SP, Brazil).

2.5. Characterization of films

The humidity of the films was determined after oven drying at 105 °C under forced air circulation for 24 h. Samples (0.2 g) of the film