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Fresh-cut fruit and vegetable coatings by transglutaminase-crosslinked whey protein/pectin edible films

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ABSTRACT

Fresh cut apples, potatoes and carrots were coated by a blended whey protein/pectin film, prepared in the presence of transglutaminase, and several properties of the coated and uncoated fruit and vegetable samples were analyzed during their storage. The linear increase in apple weight loss observed during storage was significantly reduced after 10 days when the samples were coated by whey or soy protein isolate films (about 20 and 40%, respectively) but mostly (about 80%) by whey protein film grafted with pectin and transglutaminase. In addition, the latter film was able to totally prevent the weight loss of potato and carrot samples at least until the 6th day of sample storage. Coating by the crosslinked blended film prevented microbial growth in all samples analyzed, also preserving their phenolic content and carotenoid in carrots. Finally, a marked reduction of both hardness and chewiness, detected after ten days of storage in all the uncoated samples by a texture profile analysis, was shown to be effectively counteracted by coating, whereas no significant differences in the acceptability scores of the coated samples after storage, were recorded by a sensory panel with respect to all samples tested before storage.

1. Introduction

In recent years the increasing interest towards ready-to-use fruit and vegetables stimulated new efforts of research in this field. These fresh-cut products are, in fact, at the top of the list of foods meeting the preference of consumers, apples as well as potatoes and carrots being the most popular, even though their market remains quite limited as a consequence of fast deterioration during storage and distribution (Barry-Ryan, Pacussi, & O'Beirne, 2000; Mastromatteo, Conte, & Del Nobile, 2012; Saltveit, 2003). Therefore, the search for methods that retard the negative effects on the quality of the products compromising their safety -such as presence of microorganisms, off-flavour development, browning and texture breakdown-is of great interest for all the stakeholders involved in the production and distribution of fresh-cut fruits and vegetables.

To extend food shelf-life a number of technologies based on temperature and headspace atmosphere are available, even though modified atmosphere packaging and low temperature storage are usually not sufficient in the case of fresh products because of their well known susceptibility to microbial spoilage following food cutting and slicing. Furthermore, water loss represents an important cause of postharvest deterioration, contributing to product wilting and loss of its textural quality, such as softening and loss of crispness (Kader, 1992; Medina, Tudela, Marín, Allende, & Gil, 2012).

Both microbial proliferation and water loss of fresh products can be counteracted by the use of food coatings (Cisneros-Zevallos, Saltveit, & Krochta, 1997). In fact, edible films applied onto the food's surface may extend its shelf-life by decreasing moisture transfer and solute migration, gas exchange and oxidation processes as well as by reducing or even suppressing possible physiological disorders (Baldwin, Nisperos, Chen, & Hagenmaier, 1996; Park, 1999; Rojas-Grau, Soliva-Fortuny, & Martın-Belloso, 2008). Thus, edible coatings -often containing antimicrobial agents and/or other food additives, including anti-browning agents, colorants, flavors, nutrients and spices-are gaining relevance as potential tools to reduce the deleterious effects of fruit and vegetable processing (Cagri, Ustunol, & Ryser, 2004; Conte, Scrocco, Brescia, & Del Nobile, 2009; Del Nobile et al., 2009; Kader, 1992; Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2011; Pranoto, Salokhe, & Rakshit, 2005). The effectiveness of the coating is

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strongly influenced by the characteristics of the material, thus an appropriate edible one deserves be selected according to the physical and chemical properties of the biopolymer components and taking into account film permeability and mechanical features (Lin & Zhao, 2007; Rossi Marquez, Di Pierro, Esposito, Mariniello, & Porta, 2014).

As recently as the mid-1980s there were little more than ten companies offering such products, while by 1996 this number grew to more than one thousand and, today, the use of edible films has expanded rapidly with annual sales exceeding US $ 100 million (Pavlath & Orts, 2009). In addition, the use of edible films is likely to expand dramatically in the future, especially in fruits/vegetable sector, since health-conscious consumers look for more foods requiring minimal preparation, such as cut fruit and premixed salads. Thus, many food researchers have turned their attention to develop invisible, edible, colorless, odorless and tasteless coatings for such ready-to-eat foods. However, specific studies on fresh-cut fruits and vegetables are still rather limited and their industrial application are still considered at early stage, most studies having been carried out so far only at a laboratory scale (Galgano, Condelli, Favati, Di Bianco, Perretti & Caruso, 2015).

Since we previously demonstrated that edible whey protein/pectin films obtained in the presence of the enzyme transglutaminase (EC 2.3.2.13) possess good water vapor and gas barrier properties (Porta, Mariniello, Di Pierro, Sorrentino, & Giosafatto, 2011; Di Pierro, Rossi Marquez, Mariniello, Sorrentino, & Porta, 2013), their effectiveness as coating in reducing weight loss and microbial growth during ten days of postharvest cold storage of cut apples, potatoes and carrots was investigated in comparison with both uncoated, whey protein- and soy protein-isolate coated food products (Shon & Choi, 2011). Moreover, two texture profile parameters, i.e. hardness and chewiness, of the coated and uncoated fruit and vegetable samples were analyzed after ten days of storage, and the total phenolic content in apple and potato samples, as well as carotenoid content in carrots, were also determined. Finally, a preliminary sensory evaluation of whey protein/pectin/transglutaminase-coated apple slices was also carried out.

2. Materials and methods

2.1. Materials and chemicals

Commercial whey protein isolate, containing 920 g/kg protein, was obtained from Bioline (Sidney, Australia & New Zealand). ACTIVE WM (product no. AJ301402, lot no. 00.02.03) containing *Streptoverticillium* Ca^{2+}-independent transglutaminase was obtained from Prodotti Gianni (Milano, Italy). Sorbitol, Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, pectin from *Citrus* fruits (9.4% degree of methylation), soy protein isolate containing 520 g/kg protein were obtained from Sigma-Aldrich Chemical Co. (Milano, Italy). All other chemicals and solvents were of analytical grade and purchased from common sources. Fresh apples cv. *Royal gala*, carrots cv. *Nantes* and potatoes cv. *White* were purchased from a local market (Napoli, Italy).

2.2. Film forming solution preparation

Whey protein/pectin (4/1 kg/kg) film-forming solution containing transglutaminase was prepared as previously described (Rossi Marquez, Di Pierro, Esposito, Mariniello, & Porta, 2014) by solubilizing 1.2 g of whey protein isolate, 0.6 g of sorbitol, and 0.3 g of pectin in 51 mL of water. The pH of the solution was adjusted to 5.1, and then transglutaminase (8 U/g of whey protein isolate) was added by stirring overnight at room temperature. Control whey protein film forming solution was also prepared as reported above but in the absence of pectin and transglutaminase. Soy protein (50 g/L) film forming solution, containing glycerol (25 g/L) and $CaCl_2$ (1.25 g/L), was prepared as previously described (Shon & Choi, 2011). Finally, to investigate film antioxidant activity, all film forming solutions were cast by pipetting 30 mL of each film forming solution into Petri dishes (150 mm × 15 mm) and drying at 45 °C and 30% RH.

2.3. Preparation of samples

Peeled fruits and vegetables were cut into 2 × 2 × 2.5 cm pieces by a shaped knife and then washed. Control and coated samples were assigned randomly. All samples were immersed first in a chlorinated solution (0.1 g/L) for 5 min, then in a 20 g/L $CaCl_2$ solution for 10 min and, finally, left to dry for 5 min. Coated samples were dipped into film forming solution for 5 min and then let drained for 10 min before storage, at 4–6 °C for either 2, 6 or 10 days, into thermally sealed low density polyethylene bags (free volume 6 pieces/bag) until use (1 bag each treatment), similarly to the control samples.

2.4. Weight loss

The weight loss of the uncoated and coated samples was calculated in triplicate at 0, 2, 6 and 10 days of storage as follows:

$$Weightloss(\%) = \frac{(initialweight - finalweight)}{Initialweight} \times 100$$

2.5. Microbiological analysis

Each coated or uncoated sample (10 g) was homogenized aseptically with 90 mL of Ringer's solution as described by Harrigan and McCane (1976). Serial dilutions were made using Ringer's solution, poured onto total count agar (TCA) and incubated at 37 °C for 24 h. Colonies were counted and expressed as CFU/g of sample. All tests were carried out in duplicate.

2.6. Total phenolic content

Uncoated and coated apple, potato and carrot phenolic content was determined at 0 and 10 days of storage using the Folin-Ciocalteu method described by Rocha and Morais (2002). 10 g of each sample were homogenized in 80 mL of water and then centrifuged at 200 g and 4 °C for 10 min. 0.5 mL of supernatant were mixed with 2.5 mL of Folin-Ciocalteu's reagent (100 g/L) and, then, 2 mL of sodium bicarbonate solution (75 g/L) were added. Samples were incubated for 1 h at 30 °C and then for an additional 1 h at 4 °C and, finally, absorbance was measured at 760 nm. Gallic acid was used to prepare a standard curve to determine the amount of phenols (2–12 μg). Total phenols were expressed as g of gallic acid equivalents (GAE)/kg of each sample (fresh weight).

2.7. Carotenoid extraction

Uncoated and coated carrot samples were analyzed at 0 and 10 days of storage using a previously proposed method (Jagannath, Napjappa, Das Gupta, & Bawa, 2006). Each sample (5 g) was homogenized in hexane solution containing acetone (400 mL/L) and magnesium carbonate (1 g/L) was added during the extraction. The mixture was allowed to decant slowly, then was washed twice first with 25 mL of acetone and then with 25 mL of hexane and, finally, the extracts were combined. The acetone was removed by repeated