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Development of new active packaging films containing bioactive nanocomposites

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ABSTRACT

The aim of this study was to develop and evaluate the effectiveness of active packaging films produced with a natural extract obtained from a residual stream generated during the PVPP cleaning process in the brewing industry after a process of elimination of excess of haze active polyphenols present in beer. The thermal stability of the active phenolic compounds was first established at 100 °C and 200 °C and then incorporated into ethylene vinyl acetate (EVA) and low-density polyethylene (LDPE) films by extrusion. Migration, antimicrobial activity and lipid oxidation tests showed that EVA film was the most suitable for incorporating the natural extract. Finally, EVA film was spiked with 3% and 6% (w/w) of the natural extract or functionalized nanoclays (0.6%, 1.2% and 1.8%). Functionalized nanoclays were prepared by combining untreated montmorillonite and 20% of natural extract. The films spiked with the highest concentrations of extract or functionalized nanoclays provided the best results by retarding both the oxidation of beef samples by around 60% and *S. aureus* growth. The active films developed in the present study show promise for use in the food industry.

Industrial relevance: The new active packaging films developed in this study with a natural extract obtained from a brewery waste and functionalized nanoclays (prepared with natural extract) showed the capacity to enhance the oxidative stability of beef during refrigeration with respect to control films. The use of functionalized nanoclays improves the effectiveness of the active packaging and minimizes the amount of natural extract required. The use of these active packaging films containing bioactive compounds with both antioxidant and antimicrobial properties could extend the shelf life of minimally processed meat products and should therefore be of great interest in the food industry.

1. Introduction

Spoilage of fresh beef is mainly characterized by microbial activity and by the oxidation of lipids and pigments (Sun & Holley, 2012). Lipid oxidation, which is one of the main causes of deterioration of meat quality during refrigerated storage, reduces the stability and acceptability of food and is often a decisive factor in determining the shelf-life of food (Gray, Gomaa, & Buckley, 1996). Reducing the oxidation process is an important challenge to food producers and, indeed, everyone involved in the entire food chain *from the farm to the fork*.

The shelf-life of fresh meat can be extended by using antioxidants, antimicrobials and appropriate packaging materials (Zhou, Xu, & Liu, 2010). One of the aims of food packaging is to protect the product from harmful environmental factors by acting as an inert barrier (Vermeiren, Devlieghere, van Beest, de Kruijf, & Debevere, 1999). Active packaging is currently one of the most dynamic technologies used to preserve the quality, safety and sensory properties of food. The active packaging interacts positively with product and environment to improve/preserve the quality of food longer than conventional packaging (Kerry, O'Grady, & Hogan, 2006). Some types of active packaging allow the controlled release of bioactive substances (antimicrobials or antioxidants that have previously been added to the package), thus avoiding the direct addition of the active agents to the food product (Lee, 2010). Most studies in this field have concerned active packaging developed with antimicrobial agents, which can be directly incorporated into packaging films and have been extensively studied for use with meat products (Coma, 2008; Zhou et al., 2010).

Growing concern about the potential health hazards caused by synthetic additives has led to renewed interest in the use of naturally occurring antioxidants/antimicrobials (Shahidi & Zhong, 2010). Waste products from fruit and vegetable processing provide a practical and economic source of potent antioxidants/antimicrobials that could replace synthetic preservatives (Balasundram, Sundram, & Samman, 2006; Moure et al., 2001).

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Many research studies in recent years have focused on developing active packaging containing natural antioxidants. Thus, some authors have incorporated antioxidants such as BHT, BHA, alpha-tocopherol and natural extracts into packaging films and assessed how these migrate and retard the oxidative process of foods during storage (Barbosa-Pereira et al., 2013; Moore et al., 2000). Moore et al. (2000) concluded that the incorporation of antioxidant substances (such as BHT, BHA, alpha-tocopherol and rosemary extract) into packaging may be more effective than the direct use of additives on the meat surface. Active packaging systems have been developed by using natural extracts such as rosemary, oregano and green tea with both antimicrobial and antioxidant properties to increase the stability of different meat products and thus to extend their shelf-life (Calatayud et al., 2013; Camo, Lorés, Djenane, Beltrán, & Roncalés, 2011; Nerín et al., 2006; Siripatrawan & Noipha, 2012).

In the present study, a natural extract obtained from a brewery waste stream (PVPP-WS extract) was used to develop active packaging film. This natural extract has a high content of phenolic compounds such as flavonols (catechin, gallocatechin and epigallocatechin) and hydroxycinnamic and hydroxybenzoic acids (gallic acid, caffeic acid, p-coumaric acid and ferulic acid). These compounds confer the extract with a high level of free radical scavenging activity as they act as free radical acceptors and chain breakers (Barbosa-Pereira, Angulo, Paseiro-Losada, & Cruz, 2013; Barbosa-Pereira, Pocheville, Angulo, Paseiro-Losada, & Cruz, 2013). We have also confirmed the antimicrobial activity of the PVPP-WS extract against both Gram-positive and Gram-negative bacteria (Barbosa-Pereira et al., 2013). The antimicrobial activity of different natural extracts and essential oils that contain phenolic compounds has been demonstrated in several studies. (Burt, 2004; Cushnie & Lamb, 2005; Holley & Patel, 2005; Moreno, Scheyer, Romano, & Vojnov, 2006; Puupponen-Pimiä et al., 2001; Rauha et al., 2000; Tajkarimi, Ibrahim, & Cliver, 2010).

Recent studies have focused on the use of nanocomposites in developing new types of active packaging (Bradley, Castle, & Chaudhry, 2011; Busolo & Lagaron, 2012; Silvestre, Duraccio, & Cimmino, 2011). Abdollahi, Rezaei, and Farzi (2012) developed an active bionanocomposite film with a synergic effect between nanoclays and natural extracts with antioxidant and antimicrobial activities (Abdollahi et al., 2012). In the present study, functionalized nanoclays were prepared using the natural extract obtained from a brewery waste stream and incorporated into a polymer film to develop an active packaging film.

The aim of this study was to develop active and nanocomposite packaging films with both antioxidant and antimicrobial properties and evaluate the effect of these films on the stability of packaged beef during refrigeration. For this purpose, the suitability of the natural extract for incorporation into a polymeric matrix by extrusion was assessed by testing thermal stability. Two polymeric matrices (LDPE and EVA) were compared and the optimal concentration of the natural extract and the overall migration, antimicrobial and antioxidant activities of the active films were tested.

2. Materials and methods

2.1. Chemicals

Butylated hydroxytoluene (BHT) (99.0%); 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) (98%); sodium azide (99.0%); 2-thiobarbituric acid (TBA) (≥98%) and trichloroacetic acid (TCA) (puriss. p.a. 99.5%) were purchased from Sigma-Aldrich (Steinheim, Germany). 1,1,3,3-Tetraethoxypropane (TEP) (purum ≥95% (GC)), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Denis reagent (purum) and gallic acid were supplied by Fluka Chemie AG (Buchs, Switzerland). Anhydrous sodium carbonate (99.9%), orthophosphoric acid (85% GR for analysis), methanol (GC ≥ 99.9%), absolute isooctane and absolute ethanol were provided by Merck (Darmstadt, Germany). Ultrapure water was prepared in a Milli-Q filter system (Millipore, Bedford, MA, USA).

2.2. The natural extract

The extract containing natural antioxidants was obtained from a residual stream generated during a brewery cleaning process. During storage of beer, colloidal haze can develop as a result of the formation of complexes between polypeptides and polyphenols. The negative impact of polyphenols on haze stability is minimized by using polyvinylpolypyrrolidone resin (PVPP) in a clarification step that is essential to improve beer stability and extend its shelf life. As a result of this process, a PVPP sludge loaded with polyphenolic compounds is obtained and then washed with a NaOH solution (2% w/w) at room temperature. After the NaOH-PVPP was filtered, a clean PVPP resin and a PVPP washing solution (PVPP-WS) containing phenolic compounds were obtained.

The waste PVPP-WS (1000 L) was acidified to pH 1.5 with HCl (37%), and polyphenolic compounds were extracted with ethyl acetate (1:2 ratio) by stirring for 30 min at room temperature. Organic and aqueous phases were separated by decantation and the organic phase was collected and evaporated to dryness at 40 °C under vacuum. Residual water was removed from the extract by lyophilization to obtain a dry crude extract. The PVPP-WS extract was obtained on an industrial scale and characterized in previous studies (Barbosa-Pereira, Angulo, Paseiro-Losada, & Cruz, 2013; Barbosa-Pereira, Pocheville, Angulo, Paseiro-Losada, & Cruz, 2013d).

2.3. Evaluation of the suitability of the natural extract for incorporation into food packaging films

2.3.1. Thermal stability

Experiments were carried out under an oxidizing atmosphere (air atmosphere) in an oven at two different temperatures, 100 and 200 °C, for 120 min, to determine the thermal stability of the natural extract containing antioxidant compounds. Samples of the natural extract (approx. 0.20 g) were collected at different times (5, 10, 15, 30, 60 and 120 min) and cooled at room temperature in a desiccator before gravimetric determination of the non-volatile fraction. The thermooxidative stability of the natural extract samples was assessed by measuring the phenolic content and antioxidant activity.

2.3.2. Total phenolic content—Folin–Denis

The total phenolic content of the natural extract samples was determined spectrophotometrically, by the Folin–Denis method, according to Waterman and Mole (1994). An aliquot (100 μl) of sample solution was mixed with 8.4 ml of ultrapure water in a test tube, and 0.5 ml of Folin–Denis reagent was added. After 1 min, 1 ml of Na_2CO_3 solution (10%) was added and the solution was allowed to stand for 30 min at room temperature. Absorbance of the resulting blue complex was measured at 760 nm in a dual-beam spectrophotometer (Uvikon XL, Bio-Tek Instruments, Milan, Italy). All determinations were made in triplicate. The total phenolic content of the samples was determined by comparison against the standard curve of commercial gallic acid, and expressed as gallic acid equivalents (GAE).

2.3.3. Radical scavenging activity—DPPH

The antioxidant activity of the natural extract was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method described by von Gadow, Joubert, and Hansmann (1997), with slight modifications. Sample solutions of the natural extract samples collected at selected times after exposure to temperature, according to the conditions indicated in Section 2.3.1, were prepared in methanol. Two synthetic compounds with antioxidant properties and commonly used in the food industry, BHA and BHT, were used as controls. An aliquot of each antioxidant (50 μl) was added to 2 ml of DPPH radical methanolic