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Physical properties of chitosan films incorporated with natural antioxidants

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ABSTRACT

In the past decades, as alternative to the petroleum based products, biodegradable polymers, such as chitosan, have undergone extensive investigation in order to minimize waste disposal problem. Yet, biopolymers face some constraints, namely regarding their poor mechanical and barrier properties. Thus, the purpose of this work was to test the incorporation of several natural antioxidants, both oil and water based, in the chitosan matrix and to evaluate the effect on the physical properties of the resulting biopolymers. Five essential oils (EO) (ginger, rosemary, sage, tea tree and thyme EO) and six different hydro-alcoholic extracts (HAE) (ginger, rosemary, sage, black tea, green tea and kenaf leaves HAE) were used. Thickness of films produced did not change statistically with incorporation of the natural antioxidants tested (values were found in the range 52–71 μm, including pristine chitosan with 67 μm). The color of EO films was similar to control samples, but HAE turned the material to a more saturated color and less bright. EO's and (especially) HAE's (which reduced by 15–80% the transmittance compared to pristine chitosan) improved the light barrier of films, conferring to chitosan an extra protection against oxidative processes. In general, incorporation of HAE in the chitosan increased the moisture content (from 13% in the chitosan to 25% on average of the HAE films) and solubility (from 17% in the chitosan to 22% on average of the HAE films) while swelling degree decreased (from 191% in the chitosan to 139% on average of the HAE films), due to the interaction of water, chitosan and polyphenols present on extracts used. The same trend, although with less significance, was also observed in the EO's films. HAE's and (especially) EO's (which increased the tensile strength of chitosan from 20 MPa to 26 MPa, on average) active compounds changed the mechanical properties of chitosan films. To conclude, chitosan films incorporated with natural antioxidants have shown, generally, modified physical properties. Among the tested extracts, black tea and green tea HAE's and sage, thyme and rosemary EO's can be highlighted as the most promising due to the resulting mechanical properties of the prepared films. Yet, in order to identify if these novel materials can be used by the food packaging industry, further research is demanded to evaluate the behaviour of these biopolymers when in contact with food matrices.

1. Introduction

Global packaging market has demonstrated constant growth in the past decades, reaching in 2014 the value of $812 billion dollars (2.8% higher than 2013) (all4pack, 2016). According to Smithers Pira forecast this amount will stand around $997 billion dollars in 2020, with a growing rate of 3.5% per year (Smithers Pira, 2013). In the five main categories of the global packaging market (metal, glass, paper and board, rigid and flexible plastic and wood), plastic material stood out, corresponding to almost half of materials in value (22.8% rigid plastic and 24.85% flexible plastic) (all4pack, 2016).

Data from the European market shows that 80.9% of 2015 flexible packaging market has, as main client, the food sector (all4pack, 2016). Despite of the current stage of technology, about 1.3 billion ton per year of the food produced for human consumption are still lost or wasted globally (FAO, 2011), partly because of lack of adequate packaging. Therefore, some strategies of packaging optimisation have been proposed, such as varying pack sizes and developing active and intelligent packaging that maintain food quality and also increase its shelf life (Realini and Marcos, 2014; WPO, 2016). Moreover, the increasing concern on environmental issues has pushed the industry to try to substitute the non-biodegradable petrochemical-based plastic to biode-

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gradable ones (Souza and Fernando, 2016). The latter has advantages over the former since are derived from renewable sources, but comparing their functional properties, the biopolymers (such as chitosan) have relatively poor mechanical and barrier properties, which limits its use by the food packaging industry (Rhim and Ng, 2007; Tang et al., 2012).

Strategies to improve the properties of biopolymers, go through the reinforcement of the matrices. Incorporation of antioxidant compounds in the biomaterial is an approach to meliorate the antioxidant properties of the polymers (Tian et al., 2013). Antioxidants are synthetic or natural substances used to retard deterioration, rancidity and discoloration resulting from the oxidation (FDA, 2012), and that can be used to help preserve food (Araújo, 2011; Ramalho and Jorge, 2006). Natural antioxidants (mainly polyphenols extracted from plants and fruits, and vitamins, Acosta-Estrada et al., 2014; Moo-Huchin et al., 2014; Viuda-Martos et al., 2014) are being preferred over synthetic ones due to the possible harmful effects of the latter ones to humans (Santana et al., 2013). Due to the high content of phenolic compounds, fruits (grape (Silva et al., 2013), jabuticaba (de Castro et al., 2014; Leite-Legatti et al., 2012), açaí (Del Pozo-Insfran et al., 2004), pomegranate (Fischer et al., 2011), carambola (Zainudin et al., 2014), blackberries (de Souza et al., 2014), passion fruit (de Assis et al., 2009; Spínola et al., 2015), cashews (Moo-Huchin et al., 2015), among others) and plants (black and green teas (Balasundram et al., 2006), essential oils (Regnier et al., 2012), *Pistacia lentiscus* (Dahmoune et al., 2014), kenaf leaves (Pascoal et al., 2015), thyme leaves (Lee et al., 2005; Vallverdú-Queralt et al., 2014), basil leaves (Lee et al., 2005), rosemary (Ribeiro-Santos et al., 2015 Vallverdú-Queralt et al., 2014), among others) are recognized as excellent sources of bioactive compounds that are able to stabilize free radicals, such as reactive oxygen substances (ROS), through the transfer of hydrogen molecules, reducing and stabilizing them, and consequently avoiding the oxidative process (Kolakowska and Bartosz, 2014). The oxidative processes, along with the microbiological growth, is one of the mechanisms of food quality deterioration (Araújo, 2011; Cushen et al., 2012; Kolakowska and Bartosz, 2014), being responsible for many important changes such as loss of nutritional values, texture modifications, development of undesirable compounds such as off-flavors, colored and even toxic substances to humans (Pazos and Medina, 2014). Therefore, antioxidant active packaging (Realini and Marcos, 2014) that can use natural compounds, is being highlighted as a new mechanism to prevent food oxidation (Barbosa-Pereira et al., 2014, 2013; Contini et al., 2014; López-de-Dicastillo et al., 2012; Moradi et al., 2012; Nerín et al., 2008; Siripatrawan and Noipha, 2012; Tian et al., 2013).

Although there are some studies on the properties of biofilms incorporated with natural antioxidants (Pola et al., 2016; Siripatrawan and Harte, 2010; Siripatrawan and Vitchayakitti, 2016), there are no relevant studies that compare the properties of biofilms incorporated with different natural antioxidants. Thus, the aim of this work was to develop an active packaging based on chitosan, a biodegradable biopolymer from natural resource, incorporated with several natural antioxidants extracted from different plant crops (five essential oils and six hydro-alcoholic extracts) and to characterize its physical properties. All extracts and EO's used have proven antioxidant activity due to their rich phenolic content being suitable to be used as oxidation preservatives by the food processing industry. As active extracts may interfere on the final properties of the material, resulting in a film either with improved or worsened characteristics (Sarantópoulos et al., 2002), this comparative study will allow to categorize the antioxidant active biofilms for future application.

2. Materials and methods

2.1. Materials and reagents

Chitosan with high molecular weight was purchased from Sigma Aldrich, Germany. Five different food grade essential oils from Biover (Belgium) were purchased in local market: Ginger EO (*Zingiber officinale* Roscoe); Rosemary EO (*Rosmarinus officinalis* L. ct. camphor); Sage EO (*Salvia lavandulifolia* Vahl); Tea tree EO (*Melaleuca alternifolia* (Maiden & Betche) Cheel); and Thyme EO (*Thymus zygis* Loefl. ex L. ct. linolol). Six different dried plants were used to obtain the hydro-alcoholic extracts (HAE): Black and Green tea (*Camellia sinensis* (L.) Kuntze) from Azores Island (Gorreana, Portugal); Rosemary (*Rosmarinus officinalis* L.); Ginger (*Zingiber officinale* Roscoe); and Sage (*Salvia officinalis* L.) were purchased from local market; and leaves of Kenaf (*Hibiscus cannabinus* L.) variety Everglades 41, were harvested in September 2005, before flowering (14–17th of October), from kenaf pilot fields at Caparica (latitude 38°40′03″N, longitude 9°12′8″W, altitude of 50 m), Portugal.

Glacial acetic acid, glycerol and tween 80 were purchased from Alfa Aesar (Germany). Water used was purified using a Milli-Q system (Millipore, Bedford, MA, USA) and all chemicals were of analytical reagent grade.

2.2. Sample preparation

2.2.1. Hydro-alcoholic extracts and essential oils

Commercial essential oils were used as acquired from producers. Hydro-alcoholic extraction was carried out according to Pascoal et al. (2015) and Turkmen et al. (2006) with modifications. All the plants used were in dry conditions, and previous to the extraction, were grounded into powder using an electric blender (ProfiCook model KSW 1021, Germany), in order to enhance the contact with the solvent extractor and to optimize the bioactive compounds content on the extracts. For each plant extract, 5 g of powder was weighed in analytical balance Mettler Toledo (Model AB204, Switzerland) and milled in 50 mL of ethanol 50% (v/v) using an electric mill ultra-turrax® (Model IKA®T18, Germany). The mixture was kept refrigerated in dark at 7 °C ± 2 °C for 24 h and then submitted to ultrasonication for 30 min/50 Hz in ultrasonic bath (Selecta, Barcelona, Spain) at room temperature (20 °C ± 2 °C). The system was centrifuged for 30 min at 4 °C with 10000*g* force (Sigma model 4K15, Germany), the supernatant was removed, and the extraction was repeated once more. The combined supernatants from both extractions were filtered through Whatman n° 4 filter paper, and the volume was corrected to 100 mL with ethanol 50%. To avoid degradation, the extracts were stored at −18 °C until analysis and use.

2.2.2. Film preparation

Chitosan film-forming solution was prepared according to Siripatrawan and Harte (2010) with modification on the concentration of chitosan according to preliminary experiments conducted (data not shown).

Film form solution was prepared by dissolving 1.5% (w/v) of chitosan in 1% (v/v) of glacial acetic acid solution with constant agitation using a magnetic stir plate during 24 h at room temperature. As plasticizer, glycerol was added in the proportion of 30% (w/w) to chitosan powder, and the system was agitated for 5 min to complete homogenization. For the films incorporated with the essential oils, tween 80 at a level of 0.2% w/v of essential oil was used as emulsifier (Abdollahi et al., 2012). EO or hydro-alcoholic extracts were incorporated to the system at the level of 1% (v/v) of film form solution and homogenized in magnetic stir plate during more 5 min at room temperature, obtaining the active antioxidant chitosan solution. The amount of EO or HAE was chosen based on previous works (Mayachiew and Devahastin, 2010; Perdones et al., 2014; Sánchez-González et al., 2010) and it was decided to use the same percentage in both EO and HAE in order to enable the comparison between treatments. After complete homogenization, the mixture was degassed using an ultrasonic bath for 5 min 140 mL of the resulting solution was casted in glass mold (18 × 25 cm) and dried for 72 h at room temperature. Dried films