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Active wheat gluten films obtained by thermoplastic processing

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ABSTRACT

Active films based on glycerol-plasticized wheat gluten protein containing different thyme oil concentrations (0–15 wt.%) were prepared by a thermoplastic process involving relatively high temperature and pressure. A complete thermal, structural, mechanical, antimicrobial and antioxidant characterization of all formulations was carried out. Antimicrobial activity tests showed that neat thyme oil presented a meaningful antimicrobial activity. The addition of thyme essential oil to formulations based on gluten protein allowed to prepare biodegradable and edible films with increased *in vitro* antioxidant and antimicrobial properties for the most concentrated samples. However, increasing essential oil concentration led to a continuous decrease in the tensile and storage modulus but to an increase in the deformability of the films. Only films containing 10 and 15 wt.% thyme oil showed reduced moisture sorption with respect to the control film (0% thyme oil), while the water vapor permeability and total soluble matter were not markedly affected by its addition. These changes were attributed to the interference of the oil in the formation of the protein molecular network, and were corroborated by the analysis of the films microstructures by SEM.

1. Introduction

Microbial growth and oxidation reactions occurring on food surface are two of the main causes of deterioration and loss of fresh and processed food products (Türe, Gällstedt, & Hedenqvist, 2012). Direct application of antimicrobial and/or antioxidant substances on the food surface to limit the undesirable microorganisms and oxidative reactions may result in the inactivation or evaporation of active agents and rapid migration into the bulk of foods (Quintavalla & Vicini, 2002; Türe et al., 2012). A proposed way of overcoming this problem and providing a sustainable packaging is to incorporate the antimicrobial agent into bio-based edible packaging materials (Cha & Chinnan, 2004; Türe et al., 2012).

Protein based materials have been explored as potential packaging materials because of their good barrier properties against oxygen and aroma compounds (Cuq, Gontard, & Guilbert, 1998; Türe et al., 2012). Among them, wheat gluten has been taken into account due to its interesting viscoelastic properties, ability to cross-link upon heating, low water solubility, low cost and availability as a co-product of the wheat starch industry (Zubeldía, Ansorena, & Marcovich, 2015). Moreover, wheat gluten films can be obtained by thermoplastic processing, which consists of mixing proteins and plasticizer by a combination of heat and shear (Hernandez-Izquierdo & Krochta, 2008) followed by an additional stage involving further thermo-mechanical treatments (e.g. compression molding) (Pommet, Redl, Guilbrt, & Morel, 2005; Sun, Song, & Zheng, 2008), which, from economical and environmental viewpoints, is the most viable way to produce rigid gluten-based materials since it is fast and requires no solvent (Gällstedt, Mattozzi, Johansson, & Hedenqvist, 2004; Jansens, Lagrain, Rombouts, Smet, & Delcour, 2011).

Film packaging can improve food storage, mainly as a result of their ability to act as barriers to water, preventing dehydration, and to oxygen and light, reducing lipid oxidation. Furthermore, a variety of compounds, including organic acids, enzymes and spices have been proposed for active food packaging (Martínez, Partal, García-Morales, Guerrero & Gallegos, 2013; Tharanathan, 2003). Essential oils (EO's) extracted from plants are rich sources of biologically active compounds such as terpenoids and phenolic that also have antimicrobial and antioxidant properties (Burt, 2004; Lamber, Skandamis, Coote, & Nychas, 2001). In particular, the antioxidant activity and antimicrobial properties of thyme essential oil have been demonstrated and attributed to its major components, thymol and carvacrol (Kuorwel, Cran, Sonneveld, Miltz, & Bigger, 2011).

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Despite the great potential of essential oils, their use in food preservation remains limited mainly due to their intense aroma and possible changes in the organoleptic properties of the food. The use of edible films to carry essential oils could minimize the required doses by the encapsulation effect in the polymer matrix, which limits their volatilization and controls the compounds release, reducing the negative impact of these ingredients. However, only a few studies have been reported on the development of antimicrobial and antioxidant biodegradable films using thermoplastic processes such as compression molding (Dawson, Carl, Acton, & Han, 2002), probably because of the inactivation of the incorporated active agents due to the high temperature and pressure associated with the process (Del Nobile et al., 2009).

The present work was performed in order to find an antimicrobial-antioxidant/material system that would respond to the compression molding; i.e. a material that would remain antimicrobial and antioxidant while having sufficient mechanical integrity. Thus, results related with the characterization of films obtained by intensive mixing followed by compression molding based on wheat gluten plasticized with glycerol and thyme essential oil are presented.

2. Materials and methods

2.1. Materials

Wheat gluten, food grade, protein content 77.8% (dry matter) according to the manufacturer and moisture content of 9.08 ± 0.10% (Zubeldía et al., 2015) provided by Dietetics Los Pinos (Mar del Plata, Argentina); glycerol, technical grade (DEM Chemicals, Mar del Plata, Argentina) and thyme essential oil (TO), obtained from the plant Thymus vulgaris (Las Boticarias, Buenos Aires, Argentina) were used to produce the films.

2.2. Preparation of films

Wheat gluten (WG) powder was mixed with glycerol (20 wt.% referred to the total mass) and with the appropriate amounts of thyme essential oil, leading to films containing 0–15 wt.% of the active component in a laboratory intensive mixer operated at 50 rpm and 80 °C during 5 min. The paste was then removed from the mixer, cut manually into pellets and formed into films by pressing them at 100 °C and 100 kg/m^2 for 10 min in a hydraulic heated press (Zubeldía et al., 2015). The visual appearance of the films was completely homogenous.

2.3. Extraction and quantification of thymol and carvacrol by HPLC

The average concentration of thymol and carvacrol in the wheat gluten films was determined by means of the method described by (Torres, Romero, Macan, Guarda, & Galotto, 2014) with some modifications. Extraction was performed by a shaker holding 0.5 gr of each film in flasks filled with 20 mL of methanol at room temperature for 24 h in dark conditions. Afterwards, these flasks were sonicated in an ultrasound chamber (PS-30A, RoHs, China) during 20 min at room temperature; methanol was recovered and filtered to be analyzed by HPLC. Quantification of thymol and carvacrol was determined by High Performance Liquid Chromatography (HPLC, Agilent 1200 series, Santa Clara, CA, USA) equipped with a high pressure pump, automatic injector and a UV–visible diode array detector. The analytical column was a Kromasil 100-5C18 (Eka Chemical, Stockholm, Sweden). The mobile phase was an acetonitrile: distilled water (40:60 v/v) mixture with a flow rate of 1.0 mL/min and an injection volume of 10 μL. The detection of thymol and carvacrol was performed with a wavelength of 274 nm. The extracts and standard compounds were analyzed under the same conditions.

2.4. Characterization techniques

Antimicrobial activity was evaluated by the agar diffusion method, determining the sensitivity of the native microflora of lettuce (Ponce, Aguero, Roura, Del valle, & Moreira, 2008) and broccoli (Moreira, Ponce, Ansorena, & Roura, 2011), and pure cultures of *Escherichia coli* (ATCC 25922), *Listeria innocua* (CIP 8011), *Pseudomona aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) to thyme oil and active WG films as described in Ponce, Fritz, del Valle, and Roura (2003) and Ramos et al. (2012). WG film disks without incorporation of TO and distilled water were used as control. Native microflora of lettuce and broccoli were prepared according to Ponce et al. (2003).

The microbial growth without seeding with bacterial culture was evaluated using unsterilized WG films discs and placing them on BHI agar plate. Samples were incubated at 37 °C for one week.

The antioxidant activity and the Total Phenolic (TP) content were evaluated by using hydrophilic extracts obtained according to Shivashankara, Isobe, Al-Haq, Takenaka, and Shiina (2004). The antioxidant capacity was analyzed using the stable radical 2,2-diphenil-1-picrylhydrazyl (DPPH) as previously reported by Brand-Williams, Cuvelier, and Berset (1995). Trolox was used as the standard of the measurement and the antioxidant activity was reported in mg Trolox/g. TP was determined spectrophotometrically using the Folin-Ciocalteu reagent (FCR) as described in Singleton and Rossi (1965). Results were expressed as mg gallic acid equivalents (GAE)/g.

Tensile and Dynamic Mechanical tests, Water vapor permeability (WVP), Total soluble matter (DRY and WET methods) and Equilibrium Moisture Content measurements, as well as Scanning Electron Microscopy (cryo-fractured film cross-section) and Statistical analysis were performed as described in Zubeldía et al. (2015).

Thermogravimetric (TGA) measurements were carried out on a Shimadzu TGA- 50 thermogravimetric analyzer. Thermal degradation was performed under air atmosphere, using heating ramps of 10 °C min^{-1} in the temperature range of 25–750 °C and samples of 4–10 mg.

3. Results and discussion

3.1. Antimicrobial and antioxidant properties

Total phenolic content, antioxidant capacity and thymol and carvacrol content in thyme essential oil are listed in Table 1. Some

Table 1
Thyme essential oil characterization (thymol and carvacrol concentration, total phenolic content and antioxidant capacity).

Pure thyme oil characterization	
Compounds	(% of TO)[a]
Thymol	65.8
Carvacrol	6.1
Others	28.1
Total phenolic content and antioxidant capacity	
TP (mg GAE/g)	74.54 ± 2.2
DPPH (mg trolox/g)	10.97 ± 0.4

TP and DPPH reported values were measured in triplicate and correspond to the mean ± standard deviation.

[a] Expressed as percentage of the total peak area of the chromatograms (referred to the total thyme oil concentration).