



Properties of wheat starch film-forming dispersions and films as affected by chitosan addition

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ABSTRACT

In order to evaluate the impact of chitosan on the physical properties of wheat starch–glycerol films, part of the wheat starch was replaced by chitosan, and the effect of composition on the properties of both the films and the film-forming dispersions was studied. The latter became more stable and viscous as the chitosan proportion was increased in the mixture. Both polymers appeared to integrate homogeneously in the film matrix. The combined effect of the glycerol and chitosan proportions affected the mechanical and barrier properties of the films. The tensile strength and elastic modulus of the films were improved as chitosan ratio increased. The oxygen and water vapor permeability slightly increased in line with the amount of chitosan in the blend although the induced differences were very small. Chitosan ratio directly affected the antimicrobial properties of the films, which showed a significant bactericide activity when the chitosan–starch ratio in the film was 50%. Nevertheless, at a starch:chitosan ratio of 80:20, counts of coliforms did not exceed the initial value in the meat after 7 storage days.

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1. Introduction

The continuously increasing interest of consumers in the quality, convenience and safety of food has encouraged research into edible films and coatings. The application of edible films permits very diverse objectives, such as the control of moisture loss, gas permeability and microbial growth, the preservation of the structural integrity of the product or the gradual release of encapsulated flavours, antimicrobials and antioxidants into the food (Arvanityannis et al., 1996). Biodegradable polymers based on natural polysaccharides, particularly starch, can be produced at low cost and on a large scale (Parra et al., 2004). According to the research of Narayan (2001), starch based materials reduce the utilization of non-renewable resources, as well as the environmental impact of synthetic plastics. Native starch is one of the most naturally abundant biopolymers. The starch granule is essentially composed of two main polysaccharides, amylose and amylopectin, and some minor components such as lipids and proteins. Starch has attracted considerable attention as a biodegradable thermoplastic polymer and biodegradable particulate filler (Angellier et al., 2006). The application of starch-based films in food packaging is promising because of their environmental appeal, low cost, flexibility and transparency (Müller et al., 2009; Bilbao-Sáinz et al., 2010). However, several authors have pointed out that, despite their ease of

preparation, starch-based films present some drawbacks, such as their poor mechanical properties which make the addition of plasticizing compounds necessary (Peressini et al. 2003; Bravin et al. 2006). These can also be improved by blending with other polymers.

Chitosan is a cationic polysaccharide that derives from the deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp, and crawfish (No et al., 2002). It has an excellent film-forming ability with no need for plasticizing compounds (Domard and Domard, 2001; Kittur and Kumar, 1998), and has proven antimicrobial activity (Georgantelis et al., 2007; Roller et al., 2002; Kendra and Hadwiger, 1984; Sudarshan et al., 1992; Sekiguchi et al., 1994). The exact mechanism for the antibacterial activity is not yet fully understood, but it has been suggested that it involves cell lysis, the breakdown of the cytoplasmic membrane and the chelation of trace metal cations that could be necessary for the microorganism's growth (Liu et al., 2004).

Chitosan has been previously added to films prepared with starches from different origins, such as potato (Mathew and Abraham, 2008; Shen et al., 2010), tapioca (Vásconez et al., 2009); corn (Xu et al., 2005) and kudzu (Zhong et al., 2011). However, to our knowledge, blends of wheat starch and chitosan have not been used for the preparation of films. The aim of this work was to characterize the most relevant properties of wheat starch–chitosan film-forming dispersions and edible films, in order to evaluate the role of the chitosan addition on these properties. Likewise, the antimicrobial activity of the films was checked in minced pork meat.

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2. Materials and methods

2.1. Reagents and raw materials

High molecular weight chitosan (CH) (practical grade, Batch MKBB0585, degree of deacetylation >77%), viscosity (1220 cPs), Sigma–Aldrich Quimica, Madrid, Spain) and wheat starch (WS) (food grade, Batch E3748, protein content (0.35% maximum), lipids (0.1% maximum), minerals (0.25% maximum, amylose/amylopectin ratio 25:75), Roquette Laisa S.A., Valencia, Spain) and Millipore water were used to prepare the film-forming dispersions (FFD). Glycerol, acetic acid and $Mg(NO_3)_2$ were provided from Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain).

For the microbiology study, pork meat was purchased in a local supermarket and processed at the laboratory. Tryptone Phosphate Water (peptone buffered water), Violet Red Bile Agar (VRB agar), Plate Count Agar (PCA), Tryptic Soy Broth (TSB) and Agar Bacteriological were provided by Scharlau Microbiology.

2.2. Preparation of the film-forming dispersions and casting of the films

Chitosan (1% w/w) was dispersed in an aqueous solution of glacial acetic acid (1.0% v/w) under magnetic stirring at 40 °C for 12 h, to obtain a CH solution. Wheat starch was diluted at 1% (w/w) in water and then heated in a water bath for 40 min at 95 °C to promote starch gelatinization, according to other authors (Jiménez et al., 2012a,b). Glycerol was afterwards added to the wheat starch solution in a starch:glycerol ratio of 1:0.15. The film-forming dispersions (FFD) were obtained by mixing CH and WS solutions at different ratios, where part of the starch was gradually replaced by chitosan, up to 50%. Formulations were named as follows, the subscripts indicating the WS:CH ratio: WS_{100} , $WS_{90}:CH_{10}$, $WS_{80}:CH_{20}$, $WS_{70}:CH_{30}$, $WS_{60}:CH_{40}$, $WS_{50}:CH_{50}$ and CH_{100} . All film-forming dispersions were prepared by using a rotor–stator homogenizer (Ultraturrax DI 25 basic–Yellowline, Janke & Kunkel, Staufen, Germany) at 21,500 rpm for 4 min at room temperature. After homogenization, the formulations were degassed at 25 °C with a vacuum pump (at 50 mbar, for 1 h). The FFD were cast at 5.6 mg solids/cm² in a framed and leveled polytetrafluorethylene (PTFE) plate (15 cm diameter) and dried at room temperature and 60% relative humidity (RH). The films were peeled off from the casting plates and conditioned for at least one week at 5 °C–58% RH or 25 °C–53% RH in chambers containing saturated solutions of $Mg(NO_3)_2$ prior to all analyses.

2.3. Characterization of the film-forming dispersions

2.3.1. ζ -Potential

ζ -Potential was measured in triplicate using a Zetasizer nano-Z (Malvern Instruments, Worcestershire, UK). Formulation WS_{100} was measured without dilution. All formulations with chitosan were diluted to reach a final concentration of chitosan of 0.02% w/w. The electrophoretic mobility of the droplets was transformed into ζ -potential values using the Smoluchowsky model, as described by Kirby and Hasselbrink Jr. (2004).

2.3.2. Rheological behavior

The rheological behavior of FFD was analyzed in triplicate at 25 °C using a rotational rheometer (HAAKE Rheostress 1, Thermo Electric Corporation, Karlsruhe, Germany) with a sensor system of coaxial cylinders, type Z34DIN Ti. Samples were left to rest for 5 min before the measurements were taken. The shear stress (σ) was obtained as a function of shear rate ($\dot{\gamma}$) between 0 and 150 s⁻¹, taking 5 min for each (up and down) cycle. Experimental

data were fitted to the Ostwald de Waale model (Iliuta and Thyriou, 1997) to determine the consistency index (K) and the flow behavior index (n).

2.4. Characterization of the films

2.4.1. Film thickness

The film thickness was measured using a Palmer digital micrometer (Comecta, Barcelona, Spain) to the nearest 0.001 mm. Six to eight random positions in each film sample were considered.

2.4.2. Microstructure, roughness and gloss

SEM micrographs of the cross-sections of the films were obtained by scanning electron microscopy (SEM), using a JEOL JSM-5410 (Japan) electron microscope. Film specimens were cryofractured by immersion in liquid nitrogen and mounted on copper stubs perpendicularly to their surface. Samples were gold coated and observed, using an accelerating voltage of 10 kV.

The surface of films, previously equilibrated at 33% RH, was analyzed by Atomic Force Microscopy (AFM) using a Nanoscope III.a, Scanning Probe Microscope (Digital Instruments, Inc. Santa Barbara, California). The formulations observed were WS_{100} , $WS_{80}:CH_{20}$, $WS_{50}:CH_{50}$, CH_{100} . A three-dimensional image of a film surface area (50 × 50 μm) was obtained in each test, and three images were captured per formulation. Three statistical parameters related with sample roughness (method ASME B46.1, 1995), were calculated, namely the average roughness (R_a : average of the absolute value of the height deviations from a mean surface), the root–mean–square roughness (R_q : root–mean–square average of height deviations taken from the mean data plane) and the Image Surface Area Difference (%ISAD: relative difference between the real and the geometric surface area).

The gloss of the films was measured at 60° incidence angle, according to the ASTM standard D523 (ASTM, 1999) using a flat surface gloss meter (Multi. Gloss 268, Minolta, Germany). Ten replicates were taken per formulation and angle. Results were expressed as gloss units, relative to a highly polished surface of black glass standard with a value near to 100.

2.4.3. Tensile properties

According to ASTM standard method D882 (ASTM, 2001), an Universal Testing Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to obtain the true stress–Hencky strain curves. From these curves elastic modulus (EM), tensile strength (TS) and elongation (% E) at break of the films. Rectangular samples (25 × 100 mm) were cut and stored at 25 °C, 58% RH, in cabinets containing $Mg(NO_3)_2$ saturated solution (Panreac quimica, S.A., Castellar del Vallés, Barcelona). Equilibrated film specimens were mounted in the film-extension grips and stretched at 50 mm min⁻¹ until breakage. The relative humidity and temperature (25 °C) of the testing environment were registered with a thermohygrometer (testo 60-H2, Testo GmbH & Co., Lenzkirch, Germany). Measurements were carried out (eight replicates of each formulation) after the film drying and equilibration (7 days) and after 90 days of storage at 25 °C–58% RH. Tests in pure starch films were also carried out immediately after 48 h drying.

2.4.4. Equilibrium moisture content and water vapor permeability

The moisture content (MC) of films equilibrated in cabinets containing $Mg(NO_3)_2$ saturated solution, at two temperatures (5 and 25 °C), was obtained gravimetrically. Films were weighed before and after drying in a vacuum oven at 60 °C for 48 h (in duplicate). MC (%) was calculated as the percentage of mass of water with respect to the mass of the dry film (DB).

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