



## The effects of Chitosan and grape seed extract-based edible films on the quality of vacuum packaged chicken breast fillets

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### ABSTRACT

The effect of grape seed extract (GSE) (at 5, 10 and 15%) incorporated into chitosan (CH) film was evaluated by considering its physico-mechanical properties, antioxidant and antimicrobial activities in order to show improved shelf life for vacuum-packed food under refrigerated conditions. GSE-incorporated films showed higher water vapor permeability, elastic modulus, opacity, and  $a^*$  and  $b^*$  values ( $p < 0.05$ ). Increasing the GSE concentration lowered the transmittance and  $L^*$  values. The addition of GSE provided antioxidant activity to the CH films and the addition of 15% GSE into CH films inhibited the oxidation of chicken breast fillets during refrigerated storage ( $p < 0.05$ ). GSE-incorporated CH films inhibited *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* more efficiently than CH films alone. CH films incorporating 15% GSE inhibited total mesophilic aerobic bacteria (TMAB) and coliforms in chicken breast fillets ( $p < 0.05$ ) and provided a 1.50–2.33 log reduction in TMAB and coliforms, respectively, during storage. These results reveal that the inclusion of GSE into CH films has the potential to help develop antioxidant/antimicrobial packaging for food products.

### 1. Introduction

The use of bio-based food packaging materials has been increasing due to the environmental problems induced by conventional food packaging materials (Tharanathan, 2003). Bio-based polymers, such as proteins, lipids and polysaccharides have emerged as alternative packaging materials to solve the municipal solid waste problem resulting from the use of conventional plastics (Cazon et al., 2017; Koshy et al., 2015).

Among various biopolymers, chitosan (CH), a linear cationic (1, 4)-2-amino-2-deoxy-d-glucan, has great potential in food packaging applications with its good film-forming ability and biodegradability (Aider, 2010; Duan & Zhang, 2013; Fan et al., 2009). In addition, it exhibits antimicrobial activity, (Kong et al., 2010) and the cationic property of CH allows electrostatic interactions with other compounds (Rubilar et al., 2013). By contrast, CH has poor water vapor permeability and mechanical properties (Bourbon et al., 2011; Elsabee & Abdou, 2013; Wan et al., 2013). Thus, to improve the properties of CH, various compounds have been incorporated, such as nanofillers (Hu et al., 2015; Rubenthaler et al., 2015), antimicrobials (Hosseini et al., 2009; Rubilar et al., 2013) and antioxidant agents (Ferreira et al., 2014; Friesen et al., 2015; Kanatt et al., 2012; Moradi et al., 2012).

Among different kinds of additives, phenolic compounds are often

selected because these compounds show various biological effects that include antimicrobial and antioxidant activity (Furiga et al., 2009). In recent years, the effects of many different phenolic compounds – such as curcumin, ferulic acid, gallic acid, quercetin and tannic acid – on CH-based films have been reported (Liu et al., 2016; Mathew & Abraham, 2008; Rivero, García, & Pinotti, 2010a; Rubenthaler et al., 2015; Souza et al., 2015; Sun et al., 2014). Some researchers have suggested that phenolic compounds could be used as a cross-linking agent to enhance mechanical strength (Mathew & Abraham, 2008; Rivero, García, & Pinotti, 2010b) or a plasticizer to eliminate the brittleness problems of CH films (Sun et al., 2014).

Active packaging systems containing such substances offer an improved shelf life for food products. Meat has a high nutritional value, which makes it sensitive to physical, chemical and microbiological changes (Nisa et al., 2015). Therefore, there is a growing interest in the preparation of active packaging films to control microbial growth and lipid oxidation, mainly for meat products. Antimicrobial packaging films can be used to reduce microbial contamination occurring on the surface of meat products (Khan et al., 2016). Moreover, lipid oxidation, which is considered to be the main reason for the deterioration in meat quality during refrigeration, has been successfully retarded with antioxidant packaging films by researchers (Khan et al., 2017; Qin et al., 2013; Rahman et al., 2017).

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Grape seed extract (GSE) includes polyphenolic compounds such as procyanidins, gallic acid, epicatechin and catechin (Monagas et al., 2003). Therefore, GSE, which has the ability to delay oxidation and microbial spoilage due to its antioxidant and antimicrobial activity, could be the ideal choice to be added to CH films to improve quality and extend shelf life in various foods. Alves et al. (2018) showed that CH films incorporating GSE and carvacrol increased the shelf life of refrigerated salmon by 4–7 days of storage due to the antimicrobial effect of the natural agents. There are reports on the development and characterization of CH films incorporating antioxidant/antimicrobial agents such as GSE (Moradi et al., 2012; Rubilar et al., 2013). However, the applications of these films to food products have been studied to a lesser extent (Alves et al., 2018). Thus, the aim of this study was to combine the antimicrobial properties of CH and the antioxidant properties of GSE, and characterize them by physico-mechanical analysis. After obtaining the films, chicken breast fillets were used to study the effectiveness of these film samples against microbial growth and lipid oxidation under refrigerated conditions.

## 2. Materials and methods

### 2.1. Materials

Chitosan (CH) was supplied by Sigma-Aldrich (St. Louis, Missouri, USA) with an acetylation degree of 75 to 85%. Sigma-Aldrich (St. Louis, Missouri, USA) also supplied 2,2-diphenyl-1-picrylhydrazyl (DPPH), magnesium nitrate 6-hydrate, acetic acid, trichloroacetic acid and 2-thiobarbituric acid. Grape seeds were supplied from a local winery (Denizli, Turkey). Distilled water was used continuously for 6 h at 25 °C to help extract the phenolic compounds from ground grape seeds (10 g ground grape seed/100 g). Later, the extracted solution was vacuum filtered and freeze dried (BW-100 F, Bluewave Industry Co., Ltd. Shanghai, China) to concentrate the final phenolic compounds (Grape seed extract, GSE).

### 2.2. Film preparation

Chitosan films were prepared by a casting method described by Sánchez-González, Cháfer, Chiralt, and González-Martínez, (2010). Briefly, CH (1.5%, w/w) was dissolved in acetic acid solution (1%, w/w). The film solution was obtained by adding glycerol at 0.3% (w/w). GSE (at 5, 10 and 15%, w/w, in dry CH-based film) was added to the CH film solution and homogenized (DAIHAN HG-15 A, Korea) for 5 min. The preliminary studies indicated that CH films with a GSE content lower than 5% (based on dry CH powder) showed poor antioxidant and antimicrobial activities, while concentrations over 15% resulted in a loss of structure (data not shown). Thus, we selected concentrations of 5 and 15% as the upper and lower limits that show antioxidant and antimicrobial properties even inside the film matrix while maintaining the integrity of the film samples. CH film solutions were then degassed to avoid the formation of air bubbles during casting. After degassing, 50 g of the solution was cast onto a Teflon<sup>®</sup> coated plate ( $\varnothing = 150$  mm) and spread uniformly. The casted films were dried in ambient conditions for 48 h. The resulting films were conditioned at 25 °C and 50% relative humidity (RH) for one week. A digital micrometer (Digimatic Micrometer QuantuMike IP65, Mitutoyo, Japan) was used to measure the film thickness at six random positions. The moisture content of the film samples after the preparatory steps ranged between  $21.90 \pm 0.40\%$  and  $29.71 \pm 0.01\%$ . Film samples including 5, 10 and 15% GSE were coded as CH5G, CH10 G and CH15 G, respectively.

### 2.3. Characterization of the film samples

#### 2.3.1. Scanning electron microscopy

Cross-sectional images of film samples were taken with a scanning electron microscope (FEI Quanta 250 FEG, Oregon USA) under a low

vacuum pressure (with an accelerating voltage of 10 kV). The microstructural analysis of each film sample was scanned at 500–2000 times magnification.

#### 2.3.2. Water vapor permeability (WVP) measurement

The water vapor permeability (WVP) of films were determined according to the ASTM standard method E96-95 (ASTM, 1995). Film samples were exposed to 100% RH, and the permeability measurements were carried out gravimetrically at 25 °C. The results were expressed as g mm of water vapor per kPa m<sup>2</sup> h.

#### 2.3.3. Tensile properties

The tensile properties of the film samples were determined by the ASTM standard method D882 (ASTM, 2001). The elastic modulus (EM), tensile strength (TS) and elongation ( $\epsilon$ ) at break point values were determined from the stress–strain curves using force–distance data for each film. A universal testing machine Lloyd LR5 (London, UK) was used by pulling the film samples at 50 mm/min until they broke. At least eight replications were carried out for each sample.

#### 2.3.4. Optical properties

Film transmittance values were measured by taking the percentage transmittance at a wavelength of 450 nm with a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The color of the films was determined by a Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) with a white standard calibration plate ( $Y = 92.7$ ,  $x = 0.3160$ ,  $y = 0.3321$ ) as a background. Results were expressed as CIE  $L^*$ ,  $a^*$  and  $b^*$  (lightness 'L', red–green 'a' and yellow–blue 'b') coordinates. The absorption spectrum of the film samples (1 × 4 cm) was scanned over wavelengths of 400–800 nm with a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). Film opacity was expressed as absorbance depth per unit thickness (AU nm/mm) (Friesen et al., 2015).

#### 2.3.5. Total phenolic compound content and antioxidant activity of film samples

All film samples were dissolved in acetic acid solution (1% w/w) with a final concentration of 0.1 g of film sample/10 ml before the analysis. The phenolic content in the dissolved film samples was determined by a method described by Singleton, Orthofer, and Lamuella-Raventos, (1999). All solutions were mixed with Folin-Ciocalteu reagent (0.2 N) and sodium carbonate (7.5% w/v), and measured by reading the absorbance of samples at a wavelength of 765 nm using a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). The antioxidant activity of dissolved film samples was measured with regard to its radical scavenging ability with the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sanchez-Moreno, 2002). All solutions were treated with DPPH solution (0.1 mM) before an absorbance measurement at a wavelength of 517 nm. The total activity of each sample was expressed as the reduced percentage of DPPH. The total phenolic content and the antioxidant activity values were measured for the GSE concentration included in a 1 g film sample.

#### 2.3.6. Antimicrobial activity of film samples

The antimicrobial effect of film samples was determined against *Escherichia coli* (ATCC 26922), *Listeria monocytogenes* (ATCC 19115), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) in a liquid medium (ASTM, 2014, E2149-10). Test microorganisms were grown in brain heart infusion (BHI) broth at 37 °C for 18 h. The cells were diluted to a concentration of between  $10^5$  and  $10^6$  CFU/ml. Pre-weighed 5 cm<sup>2</sup> film samples were transferred aseptically into test tubes containing 10 ml of culture medium and incubated at 37 °C for 24 h. Serial dilutions were then made in peptone water and plated on BHI solid medium. The colonies were counted after incubation at 37 °C for 24 h.

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