EURASIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

Development of Antioxidant Chitosan Films Incorporated with Quinoa Extract

Ece SÖĞÜT[1*], Atıf Can SEYDİM[1]

[1] Department of Food Engineering, Faculty of Engineering,
Süleyman Demirel University, Isparta/Turkey

*Corresponding Author: ececagdas@sdu.edu.tr

Abstract

In this study, the antioxidant chitosan (CH) films were prepared by incorporating quinoa extract (QE) (5, and 10%, wt) into CH film solutions. The effect of QE incorporation into CH film was evaluated by physicomechanical, and active properties (antioxidant, and antimicrobial activity). Active compounds were extracted from QE by distilled water at 60°C during 6 hours. The total phenolic content and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of QE were found as 34.35±0.27 mg GAE/100 g (dry weight) and 25.61±1.36 %, respectively. QE included CH films showed higher water vapor permeability values (WVP) and moisture content ($p<0.05$). The transmittance and L^* values of film samples decreased with the increasing QE content whereas opacity and a^* values increased ($p<0.05$). The elastic modulus and tensile strength of CH films did not change significantly by the incorporation of QE, however, the elongation at break values of films increased with the increasing amount of QE. CH films incorporated with QE extract exhibited 15.60±0.68 and 17.92±1.2% of DPPH radical scavenging activity for 5% QE and 10% QE incorporation, respectively. All film samples showed antimicrobial activity against *Escherichia coli*, and *Listeria monocytogenes* but QE incorporation did not promote the antimicrobial activity of CH films. CH films with active properties could have a potential to be used as active food packaging films along with conventional packaging films that could reduce the amount of film used.

Keywords: antimicrobial, antioxidant, chitosan, quinoa extract

INTRODUCTION

Consumption of plastic materials derived from petroleum sources and their poor degradation generate a massive accumulation of plastic waste that has been disposed of in the environment. The use of natural polymers has emerged as an alternative to face up to this problem. Thus, increasing attention has been paid to the development of edible and biodegradable food packaging films as alternatives to the non-biodegradable petrochemical-based plastics. The matrices of edible and biodegradable films are frequently chosen from food grade biopolymers, such as proteins, lipids, and polysaccharides (Mei et al. 2012; Kanmani & Rhim, 2014; Pagno et al. 2015).

Chitosan (CH) is an environmentally friendly material and can be used for edible film production due to its non-toxicity, biocompatibility, biodegradability and film-forming ability (Duan, & Zhang, 2013). In addition, the cationic property of CH offers an opportunity to establish electrostatic interactions with other compounds (Dutta et al. 2004). Besides adding plasticizing agents, disadvantages of the pure CH films can be improved by the incorporation of additional fillers (Rubentheren et al. 2015; Jahed et al. 2017) antioxidant compounds

(Moradi et al. 2012; Friesen et al. 2015), and antimicrobial agents (Hosseini et al. 2009; Ojagh et al. 2010; Rubilar et al. 2013).

Phenolic compounds are also an essential part of the human diet with several valuable biological activities, including antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, anticancer and metabolic regulation properties (Działo et al. 2016). It has been demonstrated that the incorporation of phenolic compounds into CH can improve the physical, mechanical and biological properties of composite films (Ferreira et al. 2014).

Quinoa extract (QE), includes high protein content with a better-balanced amino acid composition, dietary fiber, unsaturated fats, vitamins, and minerals (Alvarez-Jubete et al. 2010). Quinoa seeds also represent a potentially rich source of phenolic compounds, particularly flavonoids (Hirose et al. 2010).

Some researchers suggested that phenolic compounds could be used as a cross-linking agent to enhance the mechanical strength (Rivero et al. 2010) or a plasticizer to eliminate brittleness of CH films (Sun et al. 2014). Therefore, QE with its antioxidant ability could be ideal choice to be added to CH films for the improvement of film properties. The aim of this study was to combine the antimicrobial properties of CH and the antioxidant properties of QE and to characterize the physical properties of films as well as their active properties.

MATERIAL AND METHODS

Materials

Chitosan (CH) (acetylation degree of 75-85%), 2,2-diphenyl-1-picrylhydrazyl (DDPH), Folin-Ciocalteu reagent, magnesium nitrate 6-hydrate, and acetic acid were supplied from Sigma-Aldrich (St. Louis, Missouri, USA). Quinoa seeds (*Chenopodium quinoa* Willd.) were supplied from a local market (Isparta, TURKEY).

Phenolic extraction from quinoa seeds

Distilled water, preheated at 60°C, was added to the quinoa seeds at a ratio of 1:10 (w/w) and the mixture was stirred for 6 h (Carciochi et al. 2015). The obtained solution was filtered under vacuum and freeze-dried (BW-100F, Bluewave Industry Co., Ltd., China) to obtain phenolic compounds (Quinoa seed extract, QE).

Film preparation

Chitosan films were prepared by a casting method. CH film solutions of 1.5% (w/w) were prepared by dissolving the CH in 1 % (w/w) aqueous acetic acid solution with stirring at room temperature for 18 h. Afterward, glycerol was added at 0.3 (w/w), a plasticizer. QE at different concentration (5, and 10%, w/w) was added to CH film solutions followed by homogenization with a homogenizer (DAIHAN HG-15A, Korea) for 5 min. The preliminary studies inferred that CH films with QE lower than 5% showed poor active properties while higher concentration than 10% contributed to the loss of structure. The solutions were then filtered under vacuum and degassed. CH film solutions (50 g) was transferred into a Teflon coated Petri plates (Ø=15 cm). The film solutions were dried at ambient temperature and then dried film samples were conditioned at 25°C for one week. A micrometer (QuantuMike IP65, Mitutoyo, Japan) was used to measure the film thickness at six random positions. Film samples including 5, and 10% QE were coded as CH-5QE, and CH-10QE, respectively.

Characterization of film samples

Water vapor permeability, solubility in water, and water uptake values of film samples

The water vapor permeability (WVP) of films were determined by ASTM E96-95 standard method (ASTM, 1995). Film samples were exposed to 100% RH, and the permeability measurements were carried out gravimetrically at 25°C.

The solubility of film samples in water was determined according to the method described by Pereda et al. (2014). Briefly, film samples (2 × 2 cm) were placed in a beaker

containing 30 mL of distilled water to measure the percentage of dry matter solubilized after 1 h of immersion in distilled water. The undissolved dry matter was determined by removing the film pieces from the beakers and then drying at 105°C for 24 hours.

The water uptake values of film samples (2 × 2 cm) were measured as the ratio of the initial weight of pre-dried samples (at 105°C) to the change in weight after immersion (30 mL distilled water/1 h) (Rubentheren et al. 2016).

Tensile properties of film samples

Tensile properties of film samples were determined by ASTM D882 standard method (ASTM, 2001). Elastic modulus (EM), tensile strength (TS) and elongation (ε) at break point values were determined by a universal testing machine (Lloyd Instruments LR5, London, UK) by stretching the film samples at 50 mm/min. At least eight replicates were carried out for each sample.

Optical properties of film samples

Film opacity values were measured by taking the absorption spectrum of the film samples (1x4 cm) in a range of 400-800 nm with a UV-Visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan). Film opacity was then expressed as absorbance unit per thickness (AU nm/mm) (Friesen et al. 2015).

Transmittance values of the films were measured as percent transmittance at 450 nm determined by a UV-visible spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan).

The color of the films was taken with a white standard calibration plate (Y=92.7, x=0.3160, y=0.3321) as a background by a Minolta Chroma Meter (CR-400, Konica Minolta, Inc., Japan) Results were expressed as CIE *L**, *a** and *b** (lightness 'L', red–green 'a' and yellow–blue 'b') coordinates.

Total phenolic content and antioxidant activity of film samples

All film samples were dissolved in acetic acid solution (1% w/w) before the analysis. The amount of phenolic content in dissolved film samples was determined according to Singleton et al. (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 765 nm using a UV–vis spectrophotometer (Shimadzu, UV-1601, Tokyo, Japan).

The potential antioxidant activity of dissolved film samples was measured based on a 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) for 40 min at dark and then the absorbance was taken at 517 nm. The total activity of each sample was expressed as the percentage of reduced DPPH.

The antioxidant activity and total phenolic content of QE were also determined by the same method.

Antimicrobial activity of film samples

The antimicrobial effect of film samples was tested against *Escherichia coli* (ATCC 26922), and *Listeria monocytogenes* (ATCC 19115) with the zone of inhibition assay on solid media. Test microorganisms were grown in brain heart infusion (BHI) broth at 37°C for 18 h. The cells were diluted to a concentration of 10^5-10^6 CFU/mL. Film samples (Ø=15 mm) were placed aseptically on the Petri dishes inoculated with bacterial strains. The plates were incubated at 37°C for 24 h and were then examined for antimicrobial activity.

Statistical analysis

An analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare the different treatments at the 95 % confidence level. The statistical analysis was performed by the Minitab 17 software (Minitab Inc., Brandon, UK). Three observations were performed for each sample, and each experiment was replicated three times.

RESULTS AND DISCUSSION

Water vapor permeability, solubility in water, and water uptake values of film samples

The thickness, WVP, water solubility, and water uptake values of film samples are presented in Table 1.

Table 1. WVP, solubility in water, and water uptake values of film samples

Film sample	Thickness (µm)	WVP (g mm/kPa h m^2)	Solubility (%)	Water uptake (%)
CH	46±4[b]	31.53±3.78[b]	21.93±1.92[a]	61.23±7.43[a]
CH-5QE	47±1[b]	66.70±6.40[a]	22.37±0.21[a]	65.36±4.77[a]
CH-10QE	62±5[a]	69.21±5.06[a]	25.27±0.09[a]	79.89±7.31[a]

[a-b] Different letters in the same column indicate significant differences ($p<0.05$)

QE incorporated CH films exhibited higher thicknesses than CH film. The thickness of CH-QE films gradually increased with the increase in QE content. The CH-10QE film showed the highest thickness ($p<0.05$) as an indication that the thickness of CH-phenolic composite film was greatly affected by the amount of phenolic compound added. Similarly, the higher thickness was reported by other studies for CH films including gallic acid (Sun et al. 2014).

As shown in Table 1, QE incorporated CH films showed higher WVP values than CH film ($p<0.05$). The presence of QE scattered in the film could disrupt the inner network of CH film and increase the free volume and segmental motions, leading to the increase of water vapor permeability (Liu et al. 2017).

The water solubility, expressed as the percent of the water-soluble matter in the film, is frequently used to indicate the resistance of film towards the water. The addition of QE into CH film resulted in the elevation of water solubility. The water solubility of CH films increased with the increase of QE content ($p>0.05$). The increase in the water solubility of CH films should be attributed to the hydrophilic property of QE molecules (Kurek et al. 2012). As shown in Table 1, QE added CH films presented higher water uptake values ($p>0.05$) similar to WVP and solubility values. QE caused an increase in both water solubility and water uptake values but there are no significant differences between the film samples. This behavior could be explained by the hydrophilic nature of QE which might offer more free hydrophilic positions to water molecules for hydrogen bonding (Kurek et al. 2012). These results are in agreement with Rubilar et al. (2013) who studied CH films including carvacrol-grape seed extract.

Tensile properties of film samples

The EM, TS, and ε (%) values of film samples are shown in Table 2.

Table 2. Tensile properties of film samples

Film sample	EM (MPa)	TS (MPa)	ε (%)
CH	247.28±87.79	21.29±0.67	24.65±0.82
CH-5QE	493.24±47.33	27.35±0.94	23.59±1.52
CH-10QE	610.13±33.67	30.75±1.50	16.15±2.15

Tensile strength is required to maintain the structural integrity and barrier property of films. In addition, appropriate flexibility is also desired for easy handling of films. With the

increase of QE content, the TS values of CH films increased which could be attributed to the strong molecular interactions between QE molecules and CH chains. However, the elongation at break values of CH-QE films decreased with the increase of QE content. This indicated that the motion of CH film matrices was greatly restricted after incorporation of QE molecules (Ferreira et al. 2014). EM values of CH films, a measure of stiffness and the degree of deformation, increased with the increase in QE concentration. Similar increases in EM and TS values were reported for CH films including cinnamaldehyde (López-Mata et al. 2018).

Optical properties of film samples

Color parameters and optical properties of films including CIE *L**, *a**, *b**, transmittance (T, %) and opacity are summarized in Table 3. With the addition of QE, the *L** and % T values of CH film decreased while the opacity tends to increase, indicating CH film became darker when QE was incorporated into the film. Besides, *a** and *b** values of CH-QE films were higher than that of CH film, suggesting the tendency of composite films toward redness.

Table 3. Optical properties of film samples

Film sample	Transmittance (%)	Opacity (AU nm/mm)	*L**	*a**	*b**
CH	86.30±0.14a	401.04±22.47a	96,50±0.14a	-0.30±0.03a	3.50±0.06b
CH-5QE	83.10±2.12ab	504.96±46.71a	95,99±0.04b	-0.33±0.04a	3.85±0.27b
CH-10QE	80.35±0.21b	594.12±80.00a	95,29±0.26c	-0.45±0.04b	4.74±0.36a

[a-c] Different letters in the same column indicate significant differences ($p<0.05$)

Similarly, other researchers have found a decrease in transparency, and lightness in accordance with higher opacity values upon the addition of tea extract (Wang et al. 2013), carvacrol-grape seed extract (Rubilar et al. 2013), and protocatechuic acid (Liu et al. 2017) into CH films. This could be attributed to the impenetrable matrix created by phenolic interactions which promoted the light scattering through the film.

Total phenolic content and antioxidant activity of film samples

Active packaging such as antioxidant packaging is a very promising technique for extending the shelf life of foods without affecting the integrity of them. The total phenolic content and DPPH radical scavenging activity of film samples are shown in Figure 1. QE has a potential to be used as a natural antioxidant agent with its high phenolic content (Carciochi et al. 2015).

The total amount of phenolic componds and antioxidant activity of QE (aqueous solution) were 34.3±0.3 mg GAE/ml, and 35.6±0.7% respectively. However, film samples including QE showed lower total phenolic content and antioxidant activity. The degree of antioxidant capacity and total phenolic content of CH film was generally proportional to the amount of the QE added ($p<0.05$). Their inclusion in the films confers them antioxidant activity which can be attributed to its hydrogen donating ability due to the formation of the stable end-product by giving hydrogen from the phenolic hydroxyl groups (Carciochi et al. 2015).

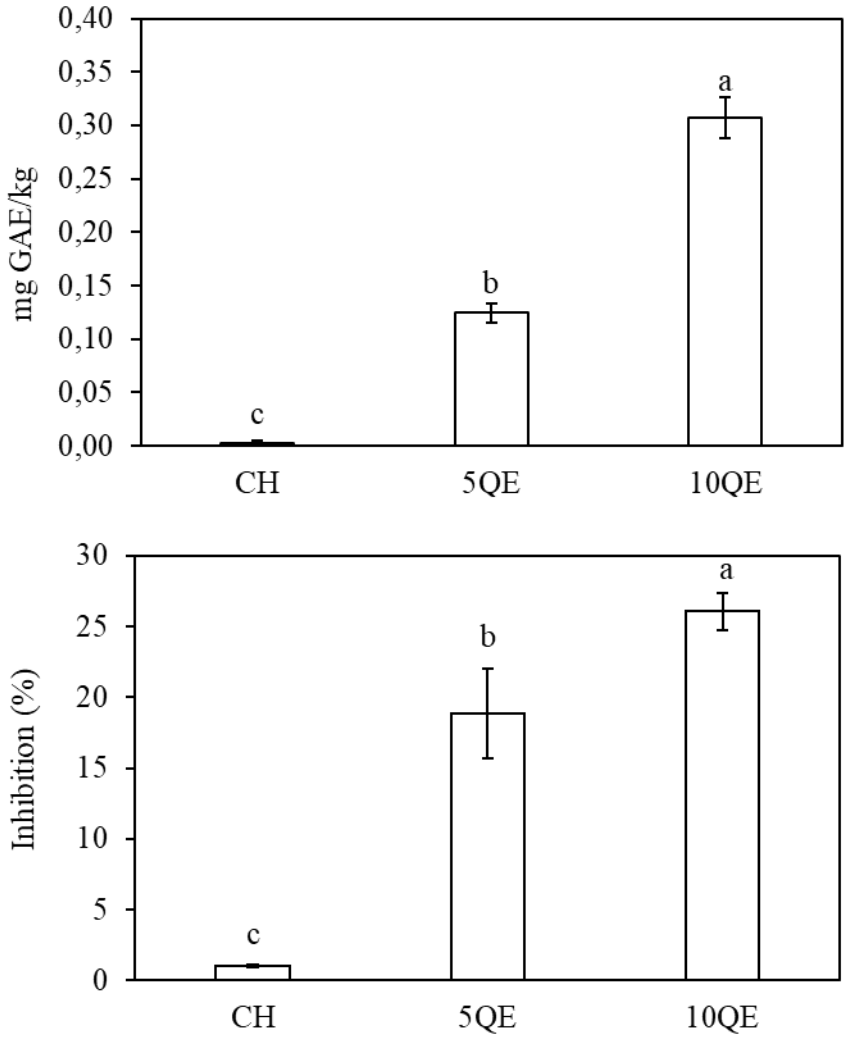

Figure 1. Total phenolic content and DPPH radical scavenging activity of film samples

Antimicrobial activity of film samples

The antimicrobial activity of film samples was tested with zone inhibition by controlling the growth under films and taking the area of clear zones (Figure 2). Although QE includes a high amount of phenolic compounds, results indicated high potential of QE for antimicrobial activity. All film samples showed inhibition against selected bacteria (Figure 2) but distinct clear inhibition zones did not appear when the agar diffusion method was used for determination of CH and CH-QE films activity. This is because of the limitation of CH diffusion in the of film form to diffuse through the agar medium (Coma et al. 2002). Although inhibition zones were not present, the growth of bacteria under CH films was inhibited. It was stated that the effectiveness of antimicrobial activity of film samples is strongly related to retention and diffusivity mechanism of active compounds in the matrix (Fernandez-Pan et al. 2012).

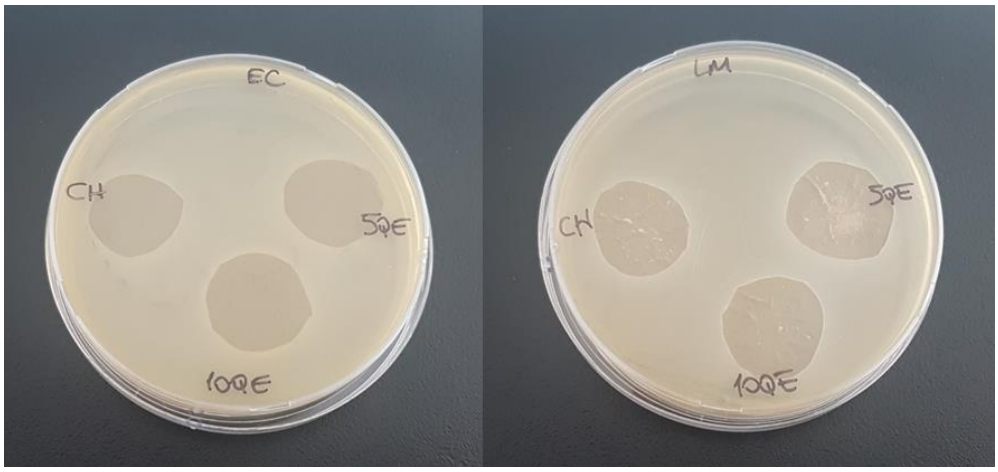

Figure 2. Antimicrobial effect of film samples (EC=*E. coli*, and LM=*L. monocytogenes*)

CONCLUSIONS

The addition of QE significantly affected the physicomechanical, and active properties of CH films. QE promoted an increase in water vapor permeability, solubility, and water uptake values due to its highly hydrophilic nature. QE incorporated CH films showed higher opacity, and lower lightness. QE improved the tensile properties of film samples while causing a decrease in elasticity of film samples. QE included CH films also showed antioxidant activity, but QE did not contribute to the antimicrobial activity of CH films. CH films with active properties could have a potential to be used as active layers along with conventional packaging films that could reduce the amount of film used.

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