Effect of Gallic acid on mechanical and water barrier properties of zein-oleic acid composite films

Kingsley Masamba^{1,2} · Yue Li¹ · Joseph Hategekimana¹ · Fei Liu¹ · Jianguo Ma¹ · Fang Zhong¹

Revised: 16 November 2015 / Accepted: 30 December 2015 / Published online: 10 June 2016
© Association of Food Scientists & Technologists (India) 2016

Abstract In this study, the effect of gallic acid on mechanical and water barrier properties of zein-oleic acid 0–4 % composite films was investigated. Molecular weight distribution analysis was carried out to confirm gallic acid induced cross linking through change in molecular weight in fraction containing zein proteins. Results revealed that gallic acid treatment increased tensile strength from 17.9 MPa to 26.0 MPa, decreased water vapour permeability from 0.60 (g mm m⁻² h⁻¹ kPa⁻¹) to 0.41 (g mm m⁻² h⁻¹ kPa⁻¹), increased solubility from 6.3 % to 10.2 % and marginally increased elongation at break from 3.7 % to 4.2 % in zein films only. However, gallic acid treatment in zein-oleic composite films did not significantly influence mechanical and water barrier properties and in most instances irrespective of oleic acid concentration, the properties were negatively affected. Results from scanning electron

microscopy showed that both gallic acid treated and untreated zein films and composite films containing 3 % oleic acid had a compact and homogeneous structure while those containing 4 % oleic acid had inhomogeneous structure. The findings have demonstrated that gallic acid treatment can significantly improve mechanical and water barrier properties especially in zein films only as opposed to when used in composite films using zein and oleic acid.

Keywords Zein · Oleic-acid · Gallic acid · Mechanical properties · Water barrier properties

Introduction

Considerable advocacy has been made and continues to gain momentum about the environmental related benefits of using biodegradable films as opposed to synthetic films. However, this momentum in advocacy for promotion of the biodegradable films is understandably weakened by the consensus that biodegradable films are mechanically weak and poor barriers to water as compared to synthetic films which has correspondingly necessitated the need for use of different approaches in improving film properties.

Among the biopolymers used in edible and/or biodegradable films technology, proteins have been highlighted since they allow chemical modifications that can contribute to the production of films with better physical properties (Makishi et al. 2013). Chemical treatment, especially the use of aldehydes, has been one of the widely used methods in improving biodegradable film properties. However, although the aldehydes are highly reactive, these components have a major

Highlights: •Mechanical and water barrier properties of zein-oleic acid composite films investigated •Gallic acid induced cross-linking confirmed by molecular weight increase •Gallic acid improved tensile strength and water vapour permeability in zein films •Solubility increased in control gallic acid zein treated films
•No improvement in both mechanical and barrier properties in zein-oleic acid films

✉ Fang Zhong
fzhong@jiangnan.edu.cn

¹ Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Food Science and Technology, Jiangnan University, 1800 Lihu Road, 214122 Wuxi, Jiangsu Province, People's Republic of China

² Bunda College Campus, Department of Food Science and Technology, Lilongwe University of Agriculture and Natural Resources (LUANAR), P.O. Box 219, Lilongwe, Malawi

disadvantage: toxicity. On the other hand, the use of other chemical agents such as gallic acid brings major advantages over aldehydes since they are non-toxic and entirely biodegradable (Orliac et al. 2002).

Gallic acid (2,3,4-trihydroxybenzoic acid) is one of the natural phenolic compounds with lower molecular weight, widely available in the plant kingdom and showing pharmacological properties, e.g. strong antimutagenic, anticarcinogenic, and antioxidant activities (Wang et al. 2007). Gallic acid is both soluble in water and in organic solvents such as ether or acetone and this characteristic makes it a very suitable cross-linking agent for hydrocolloids like zein which is soluble in organic solvents. Although zein possesses excellent film forming and gas barrier properties, poor water barrier properties, brittleness and flexibility problems associated with films produced from zein limit their use or application. Over the years, a number of approaches such as enzymatic and chemical treatment continue to be applied in the production of hydrocolloid based biodegradable films to improve film properties. In addition, composite based films are being promoted with an aim of minimizing the disadvantages of individual components while at the same time making use of strength in their properties to improve film properties. In our previous unpublished work, we demonstrated that proper selection of concentrations of oleic acid incorporated in zein films cross linked by transglutaminase can improve both mechanical and water barrier properties of zein-oleic acid composite films. Other authors working with different lipids have previously reported improvements in film properties after the lipids were incorporated into the films (Teixeira et al. 2014; Monedero et al. 2010). Although several studies have been carried in cross-linking of proteins using various cross-linkers such as chemical agents, it is as observed by Ustunol and Mert (2004) difficult to compare results from different studies because of different experimental conditions.

The main objective of this current study was to determine the effect of gallic acid on mechanical and water barrier properties of zein-oleic acid composite films. In this study, the oleic acid concentration in the composite film ranged from 0 to 4 % based on zein weight giving a final concentration of 6 % for both zein and oleic acid (6 g/100 mL 95 % ethanol). Gallic acid at a concentration of 40 mg/g zein was used and this concentration was found to provide better results from our previous preliminary work.

Materials and methods

Materials

Zein (with supplier's provided information as follows: approx molecular weight (35,000), nitrogen content (13.1–17.0), fat (<2 %), heavy metal (<0.002 %), lead (≤2 ppm) was

purchased from Hangzhou Showland Technology Company Limited, Hangzhou, People's Republic of China. Gallic acid was purchased from Shanghai Canspec Scientific Instruments Co. Ltd., Shanghai, People's Republic of China. All the other chemicals and reagents used were of analytical grade.

Preparation of films

Film preparation for control and Gallic acid treated film samples without oleic acid

Six grams zein was dissolved in 100 ml of 95 % purity ethyl alcohol and heated at 80 °C in a hot plate provided with a magnetic stirrer for 30 min. After 30 min, ethylene glycol at 27 % based on protein zein weight was added and the mixture was further heated for 30 min. For the control samples, the solutions were further heated to boiling point using a hot plate and were left to boil for 15–20 s. While still hot, the solutions were filtered using a polyester screen mesh with mesh opening size of 110 µm to remove any foreign materials and undissolved components. The filtered solution was later homogenized using FJ 200-S homogenizer (Shanghai Biaoben Mould Factory Company, Shanghai, China) at 10,000 rpm for 3 min. The film forming solution was degassed using ultrasonic cleaning machine for 30 min and the solution was cast in square plastic plates (10 cm by 10 cm) by weighing 15 g of the film forming solution. The films were dried for 40 h at 30 °C. The drying time was extended beyond 24 h after it was observed that the films were not well dried. The delay in drying may be due to the high humidity prevailing in the atmospheric conditions during the study period. For the gallic acid treated samples, the same procedure as outlined for the control samples was followed. After 30 min from addition of plasticizer, the solution was left to cool before gallic acid at the concentration of 40 mg/g zein was added to the film forming solution. The gallic acid was first dissolved using luke warm distilled water (50 °C). After stirring for 1–2 min using a magnetic stirrer, the gallic acid treated film forming solution was incubated at 50 °C for 30 min. After the incubation, the solutions were further heated to boiling point using a hot plate and were left to boil for 15–20 s and thereafter the same procedure as applied for control samples was followed.

Gallic acid- treated zein-oleic acid composite films The same procedure as outlined for gallic acid treated samples without oleic acid was followed. Soon after the film solutions were filtered, the solutions were allowed to cool before different concentrations of oleic acid were added ((1 %, 2 %, 3 % and 4.0 % based on protein zein weight). The solutions were homogenized at 13,500 rpm for 1 min followed by 20, 500 rpm for 3 min. Thereafter, the film solutions were degassed for 30 min using ultrasonic cleaning machine and

the same procedure outlined for gallic acid treated films was followed.

Molecular weight distribution

The molecular weight distribution of the samples was carried out to confirm cross linking by the gallic acid treatment. The molecular weight (MW) distribution profiles of the samples were estimated by high-performance gel-filtration chromatography. Waters 600 liquid chromatography system (Waters Co., Milford, MA, USA) equipped with 2487UV detector and Empower work station was used for this experiment. The column used was a TSK gel 2000 SWXL7.8 i.d $\times$ 300 mm (Tosoh Co., Tokyo, Japan), while the mobile phase consisted of acetonitrile/water/trifluoroacetic acid (40/60/0.1, v/v/v) which was delivered at a flow rate of 0.5 mL/min. The column temperature was 30 °C and 10 μ L of sample was injected into the HPLC system. A MW calibration curve was obtained from the following standards from Sigma: cytochrome C (12,500 Da), aprotinin (6500 Da), bacitracin (1450 Da), tetrapeptide GGYR (451 Da), and tripeptide GGG (189 Da). The results were obtained using UV detector (220 nm), and data analysis was performed using gel-permeation chromatography software.

Film thickness

After the films were conditioned for 48 h at 50 % relative humidity and 25 °C, film thickness was measured using a hand held micrometer caliper (Guanglu Digital Caliper Co., Ltd., China) by averaging five readings taken randomly on each of the film sample.

Moisture content

Film pieces measuring 3 by 3 cm were oven dried at 105 °C to constant weight for 24 h and the moisture content was determined as percentage of mass lost during the 24 h drying period as shown in the equation below:

$$\text{Moisture content percent} = (W1 - W2)/W1 \times 100$$

Where W1 and W2 are initial and final mass (dry) of films respectively. A total of four measurements were used to get a mean value.

Solubility

Film solubility was determined according to the method of Shen et al. (2010) with minor modifications. Film pieces (2x2cm) were oven dried to constant weight at 105 °C for 24 h. The film sample was then placed into a 50 mL plastic centrifuge tube containing 20 mL of distilled water and placed

in a shaking water bath set at a shaking speed of 95 rpm and a temperature of 25 °C for 24 h. After collecting the undissolved film, its dry weight was determined after drying in an oven at 105 °C for 24 h. The film water solubility was calculated as follows:

Film solubility (%) = (Initial dry weight-final dry weight)/initial dry weight $\times$ 100. A total of four measurements were used to get a mean value.

Water vapour permeability

Water vapour permeability tests were conducted according to ASTM method E96-95 (1995) with minor modifications. Special bottles with a measured diameter of 2.7 cm and a depth of 5 cm were used to determine the WVP of the films. Films (4.5 $\times$ 4.5 cm) were cut to ensure that they adequately cover the mouth area of the bottles (5.73cm²). 10 mL of distilled water was placed in the special bottles used and the films were mounted on to the bottles and subsequently sealed using super glue previously applied on the mouth to ensure that films firmly stuck to the bottles. A small load was placed on the films for 5–10 min to ensure a good seal is achieved as a result of the action of the applied super glue. The other portions of the sample extending beyond the mouth area of the bottles were carefully cut off to avoid errors resulting from sample or specimen area being larger than the exposed mouth area. The whole assembly was placed in a dessiccator containing silica and the water vapour transmission rate and water vapour permeability were calculated based on the equations (1) and (2):

$$\text{WVTR} = \text{Slope/Film area (g m}^{-2} \text{ h}^{-1}) \quad (1)$$

$$\text{WVP} = (\text{WVTR} \times L)/(\text{PA}_1 - \text{PA}_2)(\text{g mm m}^{-2} \text{ h}^{-1} \text{ kPa}^{-1}) \quad (2)$$

PA_1 = Vapor partial pressure at film outer surface in the dessicator = 0 kPa.

PA_2 = Vapor partial pressure at film inner surface in bottle = 3.169 KPa.

ΔP = -3.169 kPa.

L = The average film thickness (mm).

The dessicator containing the samples was thereafter placed in an incubator at 25 °C and the bottles (whole assembly, bottle with film sample) were first weighed every 2 h and then measurements were carried out every 12 h for two days. Water vapour transport was determined by weight changes of the bottle. A total of four measurements were used to get a mean value.

Tensile strength and elongation at break

Tensile strength (TS) and percentage elongation at break (EAB) were performed in accordance with ASTM standard method D882 (ASTM 1996) using a texturometer (Stable

Microsystems, model TAXT2i, Surrey, UK) fitted with a 25 kg load cell. The initial grip separation was set at 50 mm and crosshead speed at 1 mm/s. The tested film strips were cut into 80 x20mm sizes. Tensile strength was calculated by dividing maximum force with film cross-section (thickness x width) and percentage elongation at break was calculated by dividing the film elongation at rupture by initial gauge length or the difference in distance between the grips holding the film specimen before and after the break. A total of six measurements for tensile strength and elongation at break were used to get a mean value.

Scanning electron microscopy (SEM)

The morphology of the films was analyzed by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). Samples were attached to double-sided adhesive tape and then mounted on the specimen holder. The samples were sputter coated with 10 µm thickness of gold under vacuum and scanned with an accelerating beam voltage of 2 kV.

Statistical analysis

Statistical analysis was performed using IBM-SPSS Inc. software (version 16.0). One-way analysis of variance (ANOVA) was used to determine significant differences between means, with the significance level taken at ($p < 0.05$). Duncan multiple range test was used to analyse significant difference in different mean values and differences were considered to be significant at $p < 0.05$.

Results and discussions

Molecular weight distribution

The molecular weight distribution analysis was specifically carried out to confirm cross linking by gallic acid. Results of the molecular weight distribution analysis are presented in Table 1. Although there were 8 molecular weight fractions from the gel-permeation chromatography software analysed data, the focus was on the fraction with the average molecular weight greater than 10,000 Da. The choice of fraction >10,000 Da was based on the fact this was the fraction with the

highest molecular weight and this fraction covered the zein proteins although it is generally known that the molecular weight of zein protein can range as low as 19,000 Da to as high as ranging from 20,000–24,000 Da or even above as reported by other authors (Dauzer and Rees 1976; Cabral et al. 2005). Our assumption was also based on the fact that all the other components in the film forming solution which was analysed (95 % ethanol Mw = 46.07, gallic acid Mw = 170 and ethylene glycol MW = 62.07) had molecular weights less than 100 Da and therefore any component contributing to molecular weight above 10,000 Da was coming from zein proteins. Results from Table 1 showed the molecular weight increased from 11,203 Da to 11,528 Da after gallic acid treatment confirming that gallic acid induced cross linking took place and this was further reflected in the improvement of the mechanical and water barrier properties in gallic acid treated films. However, although the increase in the molecular weight was as low as 2.9 %, we previously in our unpublished preliminary work using enzymatic cross linking agents found out that a higher increase in molecular weight negatively affected other barrier properties such as water vapour permeability suggesting probably that the lower molecular weight increase reflected the effective cross linking. This observation on the increase of molecular weight as an indication of cross linking has previously been reported by other authors (Renzetti et al. 2012; Vehvilainen et al. 2010).

Effect of Gallic acid on thickness, moisture content and solubility

Results on film thickness, moisture content and solubility for both the control and gallic acid treated zein-oleic acid composite films are presented in Figs. 1, 2 and 3. Film thickness was significantly affected ($p < 0.05$) by gallic acid treatment (Fig. 1). The incorporation of oleic acid increased thickness and the gallic acid treated samples registered the lowest thickness of 0.08 mm. Moisture content was significantly ($p < 0.05$) affected by gallic acid treatment. Low moisture content of 14.5 % was registered in gallic acid treated films as compared to 18.0 % registered in control films. Some authors have previously reported that there is a decrease in moisture uptake for cross linked protein amino groups especially those of lysinyl residues since they are not available to bind water by hydrogen bonding (Tang et al. 2005). However, when oleic acid in different concentrations was incorporated into the zein films, moisture content correspondingly increased with increasing oleic acid concentration. This increase in moisture content as a result of oleic acid incorporation is contrary to findings from various authors who reported a decrease in moisture content after addition of lipid based

Table 1 Average molecular weights (Da) of molecular weight fraction (>10,000 Da) of control and gallic acid treated zein film forming solutions

Molecular weight fraction	Control	Gallic acid treated
> 10,000 (Da)	11, 203	11,528

Average molecular weights of molecular weight fraction (>10 000 Da) for control and gallic acid treated film forming solutions obtained as analysed data from gel-permeation chromatography software

Fig. 1 Thickness(mm) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 5$) and bars with different letters indicate significant difference ($p < 0.05$)

substances such as oleic acid (Valenzuela et al. 2013; Kowalczyk and Baraniak 2014). The authors further reported that the decrease in moisture content was attributed to fewer sites available for water sorption in the polymeric matrix due to an increase in number of hydrophobic chains. However, it would be too speculative to predict or suggest the reasons for the increase in moisture content after incorporation of oleic acid and probably there is need to further investigate the reasons for the increase in moisture content for zein-oleic acid composite films in future or subsequent studies.

The use of gallic acid significantly increased solubility by 62 % when compared with the control. Solubility increased from 6.3 % for the control to 10.2 % for the gallic acid treated films (Fig. 2). The increase in the solubility for the gallic acid treated films can be attributed to the high number of hydroxyl groups in gallic acid as well as the inherent property of gallic acid of being soluble in water. Some authors have previously reported that depending on incorporated gallic acid concentration, 60–80 % was released from the films, while the remaining gallic acid was trapped in the film matrix (Alkan et al. 2011). It was observed (data not shown) that the fraction less than 180 Da which covered the gallic acid had the highest

concentration after gallic acid treatment (56 % for the gallic acid treated film solution against 22 % for the control) suggesting that gallic acid was retained and considering the hydrophilic nature of gallic acid, this might probably be the reason for the increased solubility from the presumed trapped gallic acid in the film matrix. However, some authors working with chemical agents such as formaldehyde reported a reduction in solubility after cross linking (Ustunol and Mert 2004) suggesting probably that the chemical composition and properties of the chemical cross linkers apart from their action in formation of covalent bonds through cross-linking can also influence other film properties in different ways. The incorporation of oleic acid in the zein films significantly reduced solubility and the trend was that the increased oleic acid concentration resulted in further reduction in solubility and this was attributed to the hydrophobic nature of oleic acid. Solubility was reduced from 10.2 % for the gallic acid treated films to 6.3 % for the gallic acid treated zein-oleic acid composite films incorporated with 3 % oleic acid representing 62 % reduction in solubility as compared to gallic acid treated films without oleic acid. These results are in agreement with previously reported findings by other authors who similarly

Fig. 2 Moisture content (%) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 4$) and bars with different letters indicate significant difference ($p < 0.05$)

Fig. 3 Solubility (%) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 4$) and bars with different letters indicate significant difference ($p < 0.05$)

reported reductions in solubility after incorporation of lipids in different protein based films (Kowalczyk and Baraniak 2014; Teixeira et al. 2014; Chiumarelli and Hubinger 2014).

Effect of Gallic acid on water vapour permeability

Results on water vapour permeability of the films are presented in Fig. 4. Cross linking by gallic acid significantly reduced water vapour permeability. Water vapour permeability of the films after gallic acid treatment was reduced from 0.60 ($\text{g mm}^{-2} \text{h}^{-1} \text{kPa}^{-1}$) for the control films to 0.41 ($\text{g mm}^{-2} \text{h}^{-1} \text{kPa}^{-1}$). This reduction can be attributed to the formation of cross-linkages induced by the gallic acid resulting into a more compact film structure restricting water movement. A number of authors have also reported reductions in water vapour permeability after chemical treatments with various cross linking chemical agents (Sun et al. 2014; Rivero et al. 2010; Hernandez-Munoz et al. 2004; Carvalho and Grosso 2004; Carvalho et al. 2008; Chiou et al. 2008; Pires et al. 2013). However, when oleic acid in different concentrations was introduced into the gallic acid treated films, there was an increase in water vapour permeability (Fig. 4). It was further observed that for the oleic acid incorporated films, the

films incorporated with 3 % oleic had the lowest water vapour permeability value and this fitted well with results from scanning electron microscopy where the 3 % oleic acid incorporated films had a smooth, homogeneous and compact microstructure. Lowest solubility value for the oleic acid incorporated films was also registered in the 3 % oleic acid incorporated films. On the other hand, the water vapour permeability increased in the 4 % oleic acid incorporated films and results from scanning electron microscopy (Fig. 4) revealed presence of pores and white spots suggesting heterogeneity in the film matrix and this might have explained why both solubility and water vapour permeability increased in the 4 % oleic acid incorporated films. This was unexpected considering that the hydrophobic nature of oleic acid was expected to reduce water vapour permeability. However, considering that this was a composite system, there might have been some factors which this study did not look at which might have contributed to the increase in water vapour permeability when the oleic acid was introduced. Our findings are contrary to previously reported findings by various authors who reported reduction in water vapour permeability of different types of films after lipids based substances were incorporated (Zahedi et al. 2010; Valenzuela et al. 2013; Yang and Paulson 2000). The authors

Fig. 4 Water vapour permeability ($\text{g mm}^{-2} \text{h}^{-1} \text{kPa}^{-1}$) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 4$) and bars with different letters indicate significant difference ($p < 0.05$)

further reported that the reduction in water vapour permeability was attributed to the hydrophobic interactions and presence of clusters of hydrophobic masses on the surfaces of these films (Valenzuela et al. 2013).

Effect of Gallic acid on mechanical properties

Results on tensile strength and elongation at break of the films are presented in Figs. 5 and 6. Gallic acid treatment significantly increased tensile strength while elongation at break was not significantly affected. Tensile strength was increased from 17.9 MPa for the control samples to 26.0 MPa for the gallic treated samples representing an increase of 45 %. This increase can be attributed to the formation of a strong and compact polymer network as a result of the gallic acid induced cross-linkages as evidenced by the increase in molecular weight (Table 1). Our results are in agreement with findings from other authors who have reported an increase in tensile strength after chemical treatment using different chemical cross linking agents (Rivero et al. 2010; Orliac et al. 2002; Poole and Church 2015; Ou et al. 2005). Elongation at break was not significantly affected though it was increased marginally from 3.7 % for the control samples to 4.2 % for the gallic acid treated films representing an increase of 14 %. This was unexpected considering the general belief that cross linked films with improved tensile strength are always accompanied by reduced elongation at break values mainly due to the resultant rigid compact structure. Our findings are contrary to observations made by some authors who found that either there were no significant changes in elongation at break or the elongation at break was reduced after chemical cross-linking (Rivero et al. 2010; Hernandez-Munoz et al. 2004; Orliac et al. 2002; Cao et al. 2007). The incorporation of oleic acid into the zein films reduced tensile strength (Fig. 5) and the reduction has mainly been attributed to the heterogeneity of the film matrix resulting from the addition of lipid based substances which probably led to weakened intermolecular

interactions and result in consequent decrease in tensile strength of emulsified films (Zahedi et al. 2010). On the other hand, the incorporation of different oleic acid concentration into the zein films slightly increased elongation at break with the highest registered value of 4.4 %. However, there was one exception where elongation at break was reduced to 3.3 % after 2 % oleic acid was incorporated into the films. Contrary to our findings where there were no significant differences with respect to gallic acid treated and oleic acid incorporated films, other authors have reported significant increase in elongation at break after incorporation of lipid based substances such as oleic acid and attributed the increase to the plasticizing effect of the lipids (Monedero et al. 2010; Fabra et al. 2008).

Effect of Gallic acid on microstructure of the films

Results on the effect of gallic acid on the morphology or microstructure of the films as determined by scanning electron microscopy are presented in Fig. 7. There were no noticeable morphological differences for the control, gallic acid treated and the 3 % incorporated films as they all showed a smooth and homogeneous surface structure (Fig. 7, a, b, c). However, the micrographs obtained for the 4 % oleic acid incorporated films (Fig. 7, d) showed a rough and heterogeneous surface characterized by presence of pores and white spots. However, although morphologically, the films for control and 3 % oleic acid incorporated films are not different from the gallic acid treated films, we can assume that the gallic acid treatment resulted in a more compact structure as confirmed by the increase in molecular weight and subsequent low water vapour permeability. The heterogeneous surface and presence of white spots in the 4 % oleic acid incorporated films might probably suggest that beyond a certain concentration of oleic acid, there might have been some form of heterogeneity in the film matrix due to presumed phase separation. Some authors have previously reported that the problem of incorporating

Fig. 5 Tensile strength (MPa) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 6$) and bars with different letters indicate significant difference ($p < 0.05$)

Fig. 6 Elongation at break (%) of control and gallic acid treated zein-oleic acid composite films. GA and OA represents Gallic acid treated and oleic acid incorporated samples respectively. Error bars represents standard deviations ($n = 6$) and bars with different letters indicate significant difference ($p < 0.05$)

lipids into a hydrocolloid in a homogeneous way has still to be solved (Bertan et al. 2005). The presence of the white spots or linings on the film surface suggests heterogeneity and this seems to be in line with what other authors have previously reported that the appearance of a white spot suggests some heterogeneity in the chitosan matrix when gallic acid was incorporated (Sun et al. 2014).

Conclusions

In this study, the effect of gallic acid on mechanical and water barrier properties of zein-oleic acid composite films was investigated. The results demonstrated that cross linking of the zein proteins in films with gallic acid improved tensile strength and water vapour permeability while elongation at break was not significantly

affected. However, the gallic acid did not significantly improve the mechanical and water barrier properties in the zein-oleic acid composite films and in most instances, the properties were negatively affected suggesting probably that the incorporated oleic acid took prominence in influencing the overall effect on both the mechanical and water barrier properties in the zein-oleic acid composite films. Results from scanning electron microscopy showed that a more compact and homogeneous structure was formed as a result of gallic acid induced cross linking in the films as reflected in the low water vapour permeability. Furthermore, the results have also shown that the concentration of oleic acid in the zein films should not exceed 3 % as higher concentration would result in a heterogeneous structure probably due to phase separation. It can therefore be concluded that gallic acid treatment can be used to improve mechanical and water barrier properties of zein films and compatibility of the film constituents in zein-oleic acid composite based films needs to be further investigated.

Fig. 7 Scanning electron micrographs of control and gallic acid treated zein-oleic acid composite films. Different letters on the micrographs represent the type of the zein-oleic acid composite films (a = control (untreated zein only films), b = gallic acid treated films without oleic acid, c = zein-oleic composite films containing 3 % oleic acid, d = zein-oleic composite films containing 4 % oleic acid)

Acknowledgments This work was financially supported by National 125 Program2011BAD23B02, 2013AA1022207, NSFC31171686; NSFJiangSU-BK2012556; 111 Project B07029 and PCSIRT0627.

References

- Alkan D, Aydemir LY, Arcan I, Yavuzdurmaz H, Atabay HI, Ceylan C, Yemenicioglu A (2011) Development of flexible antimicrobial packaging materials against *Campylobacter jejuni* by incorporation of Gallic acid into zein-based films. *J Agric Food Chem* 59(20): 11003–11010
- ASTM D882-91 (1996) Standard test methods for tensile properties of thin plastic sheeting. Annual Book of ASTM. American Society for Testing and Materials, Philadelphia
- ASTM E96-95 (1995) Standard test methods for water vapour transmission of material. Annual Book of ASTM. American Society for Testing and Materials, Philadelphia
- Bertan LC, Tanada-Palmu PS, Siani AC, Grosso CRF (2005) Effect of fatty acids and ‘Brazilian elemi’ on composite films based on gelatin. *Food Hydrocoll* 19:73–82
- Cabral V, Arrequin R, Galvez A, Quirasco M, Vazquez-Duhalt R, Farres A (2005) Characterisation of a 19Dka alpha-zein of high purity. *J Agric Food Chem* 53(3):725–729
- Cao N, Fu Y, He J (2007) Mechanical properties of gelatin films cross-linked, respectively, by ferulic and tannin acid. *Food Hydrocoll* 21(4):575–584
- Carvalho RA, Grosso CRF (2004) Characterisation of gelatin based films modified with transglutaminase, glyoxal and formaldehyde. *Food Hydrocoll* 18(5):717–726
- Carvalho RA, Grosso CRF, Sobral PJA (2008) Effect of chemical treatment on the mechanical properties, water vapour permeability and sorption isotherms of gelatin-based films. *Packag Technol Sci* 21: 165–169
- Chiou B, Avena-Bustillos RJ, Bechtel PJ, Jafri H, Narayan R, Imam SH, Glenn GM, Orts WJ (2008) Cold water fish gelatin films: effects of cross-linking on thermal, mechanical, barrier and biodegradation properties. *Eur Polym J* 44(11):3748–3753
- Chiumarelli M, Hubinger MD (2014) Evaluation of edible films and coatings formulated with cassava starch, glycerol, carnauba wax and stearic acid. *Food Hydrocoll* 38:20–27
- Dauzer LA, Rees ED (1976) Molecular weight of an extremely hydrophobic protein, zein, in dimethylformamide and in formamide. *Can J Biochem* 54(2):196–199
- Fabra MJ, Talens P, Chiralt A (2008) Effect of alginate and λ -carrageenan on tensile properties and water vapour permeability of sodium caseinate-lipid based films. *Carbohydr Polym* 74(3):419–426
- Hernandez-Munoz P, Villalobos R, Chiralt A (2004) Effect of cross-linking using aldehydes on properties of glutenin-rich films. *Food Hydrocoll* 18(3):403–411
- Kowalczyk D, Baraniak B (2014) Effect of candelilla wax on functional properties of biopolymer emulsion films- a comparative study. *Food Hydrocoll* 41:195–209
- Makishi GLA, Lacerda RS, Bittante AMQB, Chambi HNM, Costa PA, Gomide CA, Carvalho RA, Sobral PJA (2013) Films based on castor bean (*Ricinus communis* L.) proteins cross linked with glutaraldehyde and glyoxal. *Ind Crop Prod* 50:375–382
- Monedero FM, Fabra MJ, Talens P, Chiralt A (2010) Effect of calcium and sodium caseinates on physical characteristics of soy protein isolate-lipid films. *J Food Eng* 97 (2): 228–234
- Orliac O, Rouilly A, Silvestre F, Rigal L (2002) Effects of additives on the mechanical properties, hydrophobicity and water uptake of thermo-moulded films produced from sunflower protein isolate. *Polymer* 43(20):5417–5425
- Ou S, Wang Y, Tang S, Huang C, Jackson MG (2005) Role of ferulic acid in preparing edible films from soy protein isolate. *J Food Eng* 70: 205–210
- Pires C, Ramos C, Teixeira B, Batista I, Nunes ML, Marques A (2013) Hake proteins edible films incorporated with essential oils: physical, mechanical, antioxidant and antibacterial properties. *Food Hydrocoll* 30:224–231
- Poole AJ, Church JS (2015) The effects of physical and chemical treatments on Na_2S produced feather keratin films. *Int J Biol Macromol* 73:99–108
- Renzetti S, Behr J, Vogel RF, Barbiroli A, Lametti S, Bonomi F, Arendt EK (2012) Transglutaminase treatment of brown rice flour: a chromatographic, electrophoretic and spectroscopic study of protein modifications. *Food Chem* 131(4):1076–1085
- Rivero S, Garcia MA, Pinotti A (2010) Cross linking capacity of tannic acid in plasticized chitosan films. *Carbohydr Polym* 82:270–276
- Shen XL, Wu JM, Chen Y, Zhao G (2010) Antimicrobial and physical properties of sweet potato starch films incorporated with potassium or chitosan. *Food Hydrocoll* 24(4):285–290
- Sun X, Wang Z, Kadouh H, Zhou K (2014) The antimicrobial, mechanical, physical and structural properties of chitosan-Gallic acid films. *LWT-Food Science and Technology* 57(1):83–89
- Tang CH, Jiang Y, Wen QB, Yang XQ (2005) Effect of transglutaminase treatment on properties of cast films of soy protein isolates. *J Biotechnol* 120(3):296–307
- Teixeira B, Marques A, Pires C, Ramos C, Batista I, Saraiva JA, Nunes ML (2014) Characterisation of fish protein films incorporated with essential oils of clove, garlic and origanum: physical, antioxidant and antibacterial properties. *LWT-Food Science and Technology* 59(1):533–539
- Ustunol Z, Mert B (2004) Water solubility, mechanical, barrier, and thermal properties of cross-linked whey protein isolate-based films. *J Food Sci* 69(3):129–133
- Valenzuela C, Abugoch L, Tapia C (2013) Quinoa protein-chitosan-sunflower oil edible film: mechanical, barrier and structural properties. *LWT-Food Science and Technology* 50(2):531–537
- Vehvilainen M, Kamppuri T, Nousiainen P, Kallioinen A, Siika-Aho M, Christoffersson KE, Rom M, Janicki J (2010) Effect of acid and enzymatic treatments of TCF dissolving pulp on the properties of wet spun cellulosic fibres. *Cellul Chem Technol* 44(4–6):147–151
- Wang X, Wang J, Yang N (2007) Flow injection chemiluminescent detection of Gallic acid in olive fruits. *Food Chem* 105:340–345
- Yang L, Paulson AT (2000) Effects of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Res Int* 33(7):571–578
- Zahedi Y, Ghanbarzadeh B, Sedaghat N (2010) Physical properties of edible emulsified films based on pistachio globulin and fatty acids. *J Food Eng* 100(1):102–108