Carbohydrate Polymers 112 (2014) 24–31

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Thermo-mechanical and hydrophilic properties of polysaccharide/gluten-based bioplastics

L.S. Zárate-Ramírez [a], A. Romero [b], C. Bengoechea [b], P. Partal [c], A. Guerrero [b,*]

[a] Universidad Pedagógica y Tecnologica de Colombia UPTC, Facultad de Ingeniería, Escuela de Metalurgia, Avenida Central del Norte, Tunja, Colombia
[b] Departamento de Ingeniería Química, Universidad de Sevilla, Escuela Politécnica Superior, 41011 Sevilla, Spain
[c] Departamento de Ingeniería Química, Centro de Investigación en Tecnología de Productos y Procesos Químicos (Pro²TecS) – Campus de Excelencia Internacional Agroalimentario, ceiA3, Universidad de Huelva, Campus el Carmen, 21071 Huelva, Spain

ARTICLE INFO

Article history:
Received 17 December 2013
Received in revised form 30 April 2014
Accepted 16 May 2014
Available online 29 May 2014

Keywords:
Bioplastics
Dynamic mechanical thermal analysis
Polysaccharide
Tensile strength tests
Water absorption capacity
Wheat gluten

ABSTRACT

The influence of adding different polysaccharides (locust bean gum, LBG; methyl cellulose, MC; and carboxymethyl cellulose, CMC) to gluten-based biodegradable polymeric materials was assessed in this work. Gluten/polysaccharide/plasticiser bioplastics were prepared at different polysaccharide concentrations (0–4.5%) and pH values by mixing in a two-blade counter-rotating batch mixer (at 25 °C under adiabatic conditions) and thermomoulding at 9 MPa and 130 °C. Bioplastic probes were evaluated through dynamic mechanical thermal analysis, tensile strength and water absorption capacity tests. Results pointed out that a moderate enhancement of the network structure may be achieved by adding polysaccharide at a pH close to the protein isoelectric point (pH 6), which also conferred a further thermosetting capacity to the system. Moreover, the addition of MC and CMC was found to significantly enhance material elongation properties. However, the presence of charges induced by pH leaded to a higher incompatibility between the polysaccharide and protein domains forming the composite. The pH value played a relevant role in the material water absorption, which significantly increased under acidic or basic conditions (particularly at pH 3).

1. Introduction

The global consumption of plastic in 2011 was almost 200 million tonnes with an annual growth of approximately 5% (Raphael & Yang, 2013). In this context, conventional plastics continue to dominate the market since, among other reasons, petroleum-based production benefits from large economies of scale and mature technologies. However, although bioplastic only constitutes a small part of the plastic consumption, production capacity will triple from 3.5 million tonnes in 2011 to nearly 12 million tonnes in 2020. With an expected total polymer production of about 400 million tonnes in 2020, the bio-based production should increase from 1.5% in 2011 to 3% in 2020 (Dammer, Carus, Raschka, & Scholz, 2013). Moreover, there exists a great interest in the utilisation of renewable biomass for the production of goods manufactured as an alternative to synthetic polymers, in order to reduce consumption of petrochemical feedstock and diminish environmental pollution. Therefore, bioplastics (biobased products) are marketed as offering potential environmental advantages over conventional plastics, along with some economic benefits (Iles & Martin, 2013).

Proteins, lipids and polysaccharides have been proposed as biopolymers sources to obtain biodegradable plastic materials for many years (Averous, 2004; De Graaf, 2000; Hernandez-Izquierdo and Krochta, 2008; Irissin-Mangata, Bauduin, Boutevin, & Gontard, 2001; Siracusa, Rocculi, Romani, & Dalla Rosa, 2008). Most studies based on proteins have used plant proteins (i.e. from corn, wheat gluten or soy) to manufacture bioplastics since they are relatively inexpensive and environmentally friendly (Cuq, Boutrot, Redl, & Lullien-Pellerin, 2000; Jerez, Partal, Martinez, Gallegos, & Guerrero, 2005; Kim, 2008; Pommet, Redl, Morel, Domenek, & Guilbert, 2003; Reddy & Yang, 2013; Zheng, Tan, Zhan, & Huang, 2003). In particular, wheat gluten is a vegetable protein obtained as an agricultural by-product, which is considered a promising material due to their abundance, relatively low cost, good biodegradability and suitable material properties (Bengoechea, Arrachid, Guerrero, Hill, & Mitchell, 2007). In addition, gluten proteins show fast degradation rates and can be processed by thermoplastic methods with the aid of plasticisers (Chen, Reddy, Wu, & Yang, 2012;

* Corresponding author at: Departamento de Ingeniería Química, Universidad de Sevilla, Facultad de Química, 41012 Sevilla, Spain. Tel.: +34 954557179; fax: +34 954556447.
E-mail address: aguerrero@us.es (A. Guerrero).

http://dx.doi.org/10.1016/j.carbpol.2014.05.055
0144-8617/

Zarate-Ramirez, Martinez, Romero, Partal, & Guerrero, 2011; Zhang & Mittal, 2010). Without such plasticising agents, gluten films are brittle and difficult to process and handle (Jerez et al., 2005). The most common plasticisers, among a wide variety, are water and glycerol. Previous results on glycerol/gluten-based bioplastics have pointed out a relevant effect of both processing and formulation on material properties, yielding a wide range of swelling and release behaviours (Gomez-Martinez, Partal, Martinez, & Gallegos, 2009, 2013; Lagrain, Goderis, Brijs, & Delcour, 2010). Furthermore, the addition of polysaccharides to protein/plasticiser blends also may play a role, due to the relevance of non-covalent protein–polysaccharide interactions that can be used to design and fabricate materials with novel or improved properties. In fact, due to the difference between the elemental composition of proteins (covalent bonds between hundreds of amino acids) and polysaccharides (covalent bonds between monosaccharides with ramifications), their mixtures can evidence a wide variety of two- and three-dimensional structures with different physicochemical and rheological properties (Schmitt, Aberkane, & Sanchez, 2009).

Regarding polysaccharides, starch is widely used as a packaging material, usually mixed with biodegradable polyesters (Siracusa et al., 2008). However, there are other works that study the combination between these two different kinds of biopolymers, protein and polysaccharide, in order to improve the properties of the film due to their different physical properties and interactions. Several authors found that using a polysaccharide in combination to protein leaded to improved barrier properties by reducing permeability. This included films consisting of starch and gelatin (Aguilar-Mendez, Martin-Martinez, Tomas, Cruz-Orea, & Jaime-Fonseca, 2008), starch/alginate and milk proteins (Ciesla, Salmieri, & Lacroix, 2006), as well as whey protein-based films containing pectin or alginate (Parris, Coffin, Joubran, & Pessen, 1995), methylcellulose (Erdohan & Turhan, 2005) and pullulan (Gounga, Xu, & Wang, 2007). In addition to this enhancement in barrier properties, Coughlan, Shaw, Kerry, and Kerry (2004) also found higher tensile properties (tensile strength, elasticity modulus and elongation at break) by adding polysaccharide (particularly alginate) to whey protein. Lee, Shim, and Lee (2004) also found an improvement in tensile strength but at the expense of a reduction in elongation, by adding gellan gum to gelatin. On the other hand, Oses et al. (2009) found the opposite effect by adding mesquite gum to whey protein, which may be regarded as a typical plasticising effect that leads to a reduction of the interaction between protein molecules, thereby increasing flexibility (i.e. extensibility). Moreover, some authors have found improved elongational properties and water vapour permeability in film formulated by gluten proteins and chitosan (Park & Bae, 2006), cellulose acetate phthalate (Fakhouri, Tanada-Palmu, & Grosso, 2004; Zuo, Song, & Zheng, 2009) or methylcellulose (Zuo et al., 2009).

Most of the above-mentioned contributions are based on films processed by casting, thereby presenting some limitations related to its commercial applications as compared to other processing techniques such as extrusion or injection moulding. In any case, further research attention should be paid to the application of such thermomechanical processing techniques to obtain improved biodegradable polymeric materials.

It is generally regarded that incorporation of polysaccharides into protein matrices may extend their functional properties (Coughlan et al., 2004; Turgeon & Beaulieu, 2001; Zaleska, Ring, & Tomasik, 2000). Schmitt, Sanchez, Desobry-Banon, and Hardy (1998) have shown that protein–polysaccharide complexes gel more effectively than polysaccharides or proteins alone, which has been attributed to the simultaneous presence of the two biopolymers, as well as to the structure of the complexes. Likewise, as indicated by Zaleska et al. (2000) it is possible to manipulate combinations of polysaccharide and protein component films to adjust water-vapour resistance or structural strength.

On these grounds, the overall objective of this work has been to evaluate the influence of the addition of different polysaccharides (locust bean gum, LBG; methyl cellulose, MC; and carboxymethyl cellulose, CMC) on the rheological and mechanical properties of WG/GL/polysaccharide/water blends subjected to thermoplastic compression moulding. Likewise, the influence of polysaccharide concentration and pH was analysed. The properties of the bioplastics obtained were evaluated by dynamic mechanical thermal analysis, tensile strength tests and water absorption capacity. This study would contribute to evaluate the potentials of adding a polysaccharide to gluten/plasticiser systems in order to develop bioplastics with enhanced tensile mechanical properties and tailored hygroscopic characteristics.

2. Material and methods

2.1. Materials

Wheat gluten, WG (83 wt% protein, 3 wt% lipid, 1 wt% ash, 8 wt% moisture and 10 wt% starch), was provided by Productos Riba S.A. (Granollers/Barcelona, Spain). Glycerol (GL), from Panreac Química S.A.U. (Castellar del Vallés/Barcelona, Spain), and distilled water were used as protein plasticisers. Polysaccharides such as locust bean gum, methyl cellulose and carboxymethyl cellulose (respectively, referred to as LBG, MC and CMC) were provided by Sigma–Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

The WG/GL/polysaccharide/water systems consisted of 50 wt% gluten, 18 wt% glycerol, 0–4.5 wt% polysaccharide and 32–27.5 wt% water, depending on the percentage of polysaccharide. All the components were mixed in a two-blade counter-rotating batch mixer turning at a 3:2 differential speed (Rheomix 3000p; ThermoHaake, Karlsruhe, Germany). Mixing process was carried out at 25 °C and 50 rpm for 20 min under adiabatic conditions. Each polysaccharide was properly dispersed by the standard protocol described by Garnier et al. (1995) and Murray (2009) prior its addition. Native pH was generally found around 6, which corresponds to the isoelectric point (IEP) of gluten proteins. Furthermore, different pH values were used by adding NaOH or HCl 2 M solutions. The pH value was measured by a Crison pH 25 pH meter using a puncture electrode (Crison Instruments S.A., Barcelona, Spain).

Bioplastic probes were prepared from the dough-like materials obtained after the mixing process through thermo-moulding at 9 MPa and 130 °C for 10 min. Two types of moulds were used to prepare the probes: a 50 mm × 10 mm × 3 mm rectangular shape mould and a type IV probe defined by ASTM D638-99 (American Society for Testing Materials, 2005). All the probes (rectangular and type IV) were transferred into recipients at 53% relative moisture and allowed to reach moisture equilibrium at room temperature for at least 2 weeks before testing. The desired relative humidity was achieved by means of a desiccator, using a saturated solution of $Mg(NO_3)_2$. The values of the actual moisture of each sample were determined by evaporating the water at 105 °C to constant weight (Association of Official Analytical Chemists, 2005).

2.3. Dynamic mechanical temperature analysis (DMTA)

DMTA tests were carried out with a RSA3 (TA Instruments, New Castle, DE, USA), on rectangular probes using dual cantilever bending. All the experiments were carried out at constant frequency (1 Hz) and strain (between 0.01 and 0.3%, within the linear

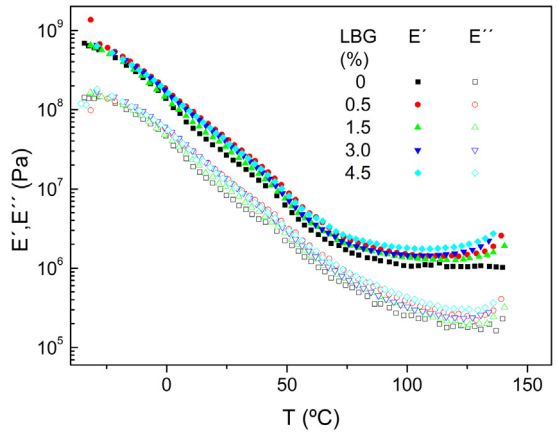

Fig. 1. Temperature dependence of the storage modulus, E', and loss modulus, E'', for WG/GL/LBG/water bioplastics with different LBG concentrations (0–4.5%) obtained from DMTA measurements.

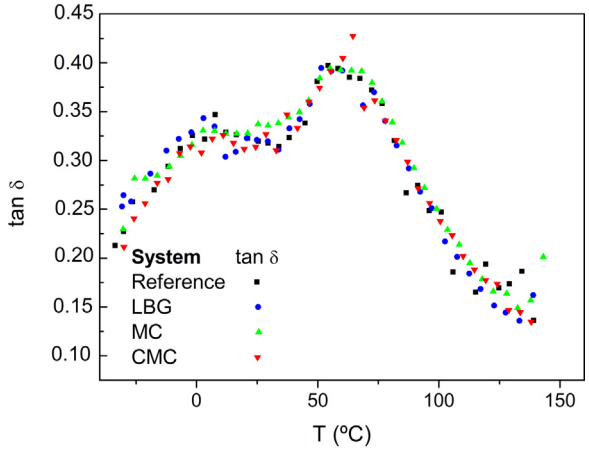

Fig. 2. Temperature dependence of the loss tangent, tan δ, for WG/GL/LBG/water bioplastics with different LBG concentrations (0–4.5%) obtained from DMTA measurements.

viscoelastic region). The selected heating rate was 5 °C min^{-1}. All the samples were coated with silicone oil to minimise water loss.

2.4. Tensile strength measurements

Tensile tests were performed by using the Insight 10 kN Electromechanical Testing System (MTS, Eden Prairie, MN, USA), according to ASTM D638 (American Society for Testing Materials, 2005). Tensile stress and elongation at break were evaluated from at least four replicates for each product using type IV probes and an extensional rate of 20 mm min^{-1} at room temperature.

2.5. Water uptake capacity and loss of soluble matter

Water uptake of bioplastics was determined following the ASTM D570 norm (ASTM D570-98, Standard test Method for Water Absorption of Plastics) (American Society for Testing Materials, 2005) using at least three 50 mm × 10 mm × 3 mm specimens. Initially, specimens were put into an oven at 50 ± 2 °C for 24 h in order to determine their dry weights (w_0). After that, specimens were immersed in distillate water for 2 and 24 h at room temperature. Finally, each specimen was periodically weighed (w_t) or dried under the same conditions before weighing again (w_f). According to this method, water uptake and loss of soluble mater were calculated as follows:

$$\text{Water update (wt\%)} = \frac{w_t - w_0}{w_0} \times 100 \quad (1)$$

$$\text{Loss of soluble matter (wt\%)} = \frac{w_t - w_f}{w_0} \times 100 \quad (2)$$

2.6. Statistical analysis

At least three replicates of each measurement were carried out. Statistical analysis was performed using t-test and one-way analysis of variance (ANOVA, $p < 0.05$) by means of the statistical package SPSS 18. Standard deviations from some selected parameters were calculated.

3. Results and discussion

3.1. Effect of the polysaccharide addition at pH 6

3.1.1. DMTA behaviour

Fig. 1 shows storage (E') and loss (E'') moduli curves obtained from bioplastics formulated with varying concentrations of a selected polysaccharide, LBG. It is worth noting that the polysaccharide content used in Fig. 1 is expressed as weight percentage of polysaccharide (wt% polysaccharide) with respect to the initial gluten content. As may be seen, no matter the LBG concentration, all samples behave like the reference system, a polysaccharide-free bioplastic containing 50 wt% gluten and 18 wt% glycerol. All materials exhibit a predominantly elastic character, being E' higher than E'' between −30 and 140 °C. As the temperature rises, both moduli (E' and E'') decrease towards a plateau region, which is observed above 75 °C. Subsequently, this plateau is followed by a high-temperature increase of both moduli, which evidences that LBG-containing bioplastics still have a remaining thermosetting potential, even after being moulded at 140 °C. The values of both moduli are slightly higher for LBG bioplastics than for the reference in the whole temperature range, being significant with some exceptions in the low temperature regime (see Table 1). Such differences turn out more noticeable at temperatures higher than 50 °C. Similar DMTA profiles have been obtained for the other polysaccharides. In this sense, Table 1 shows the values of E', at two different temperatures, for a polysaccharide-free (used as reference system) and all polysaccharide/gluten-based bioplastics manufactured in this work. As may be seen, polysaccharide concentration does not significantly affect bioplastic modulus values but comparing polysaccharide-containing samples, CMC leads to bioplastic with lower values of E'. In any case, all bioplastics containing polysaccharides exhibit higher thermosetting potentials and moduli values than the reference system. It is interesting to indicate that addition of gums also contributes to an increase in moisture content, but the increase is so moderate that the only significant difference has been found between the reference system and the rest of gum-containing specimens. The moisture content values for specimens containing 0, 1.5, 3 and 4.5% LBG are 13.7 ± 0.5, 17.3 ± 0.9, 16 ± 1 and 16.8 ± 0.7, respectively. However, as mentioned above, the viscoelastic moduli always increase after the incorporation of any of the three polysaccharides studied. In other words, addition of polysaccharide always induces an enhancement in viscoelastic properties, although it seems to be partially dampened by the increase in moisture content, also explaining why the differences in moduli are so small.

Fig. 2 shows the curves of tan δ from DMTA measurements performed on different WG/polysaccharide/GL/water bioplastics for a selected concentration of 1.5 wt% polysaccharide. Furthermore, the reference system is included for sake of comparison. As may be observed, tan δ profiles at 1 Hz display two well defined peaks at temperatures close to 0 °C and 60 °C. These two peaks were

Table 1
Storage modulus values at 0 and 20 °C (E'_0 and E'_{20}), at the minimum of E' (value of E_{min} at T_{min}) and characteristic temperatures of loss tangent peaks ($T_{\alpha1}$, $T_{\alpha2}$) obtained from polysaccharide-free and polysaccharide/gluten-based gluten bioplastics.

	Concentration (%)	E'_0 (MPa)	E'_{20} (MPa)	E'_{min} (MPa)	T_{min} (°C)	$T_{\alpha1}$ (°C)	$T_{\alpha2}$ (°C)
	0	125.1 ± 6.5	35.4 ± 2.5	1.1 ± 0.1	110 ± 2	6	60
LBG	0.5	175.2 ± 8.9	56.5 ± 2.3	1.4 ± 0.1	109 ± 3	8	63
	1.5	132.5 ± 6.2	43.0 ± 5.1	1.3 ± 0.2	113 ± 2	4	61
	3	159.9 ± 5.8	50.1 ± 3.0	1.4 ± 0.1	103 ± 4	3	59
	4.5	171.2 ± 3.2	51.9 ± 2.1	1.8 ± 0.3	106 ± 2	3	61
MC	0.5	159.4 ± 1.2	56.9 ± 7.1	1.6 ± 0.2	114 ± 5	−1	62
	1	173.2 ± 0.8	56.9 ± 6.0	1.5 ± 0.1	117 ± 3	2	61
	1.5	163.5 ± 1.9	53.2 ± 2.5	1.5 ± 0.1	119 ± 2	5	60
CMC	0.5	138.0 ± 1.5	40.7 ± 4.6	1.4 ± 0.1	106 ± 2	4	58
	1	132.3 ± 3.2	39.6 ± 0.8	1.3 ± 0.1	109 ± 1	3	57
	1.5	139.5 ± 4.9	41.2 ± 2.5	1.2 ± 0.1	113 ± 3	2	60

identified in a previous work for gluten-based bioplastics containing a glycerol/water blend as plasticiser Zarate-Ramirez et al. (2011). The first peak, at $T_{\alpha1}$ located close to 0 °C, would be mainly related to the glass transition of the plasticiser blend affected by some fractions of gluten isolate (Zarate-Ramirez et al., 2011), whereas the second peak, at $T_{\alpha2}$, was attributed to a glass transition of the plasticised gluten (Jerez, Partal, Martinez, Gallegos, & Guerrero, 2007; Sun, Song, & Zheng, 2007, 2008). As may be seen in Fig. 2 and Table 1, $T_{\alpha1}$ and $T_{\alpha2}$ are hardly modified by polysaccharide addition at pH 6.

According to these results, polysaccharide addition would not produce a compatibilising effect between components or phases, at least under the experimental conditions and concentrations studied. Bioplastic microstructure seem to be dominated by the protein matrix and the inner spaces would be occupied with disperse particles of polysaccharide (Matveev, Grinberg, & Tolstoguzov, 2000). Therefore, DMTA results suggest that polysaccharide would play the role of fillers, which contribute to the moderate increase in E' and E'' values, whilst their profiles remain nearly unperturbed.

3.1.2. Tensile tests

Fig. 3A shows some typical stress vs. strain curves obtained from tensile tests conducted on the reference system and WG/GL/polysaccharide/water bioplastic probes prepared at pH 6 and containing 1.5 wt% polysaccharide. Stress curves exhibit a linear elastic region with a marked increase in strength at low strain values, characterised by high values of the Young's Modulus (E). This zone is followed by an extensive plastic region showing a decrease in the stress–strain slope towards a constant value. Eventually, the rupture of samples takes place once a maximum stress (σ_{max}) and strain (ε_{max}) are reached. As seen in Fig. 3A, bioplastics with polysaccharides exhibit higher values of stress for an applied strain, as well as improved elongation at break, if compared to the probe without polysaccharide.

Fig. 3B shows the tensile parameters (σ_{max}, ε_{max} and Young's Modulus) obtained from the specimens studied. As may be observed, the presence of polysaccharide generally yields an apparent enhancement of tensile properties, particularly for MC and CMC-containing probes. All the three parameters follow the same general trend: Reference < LBG < MC < CMC. However, no significant differences were found for σ_{max}, and ε_{max} between the reference and LBG-containing probes. These results are in a good agreement with those reported by Zhou, Zheng, Wei, Huang, and Chen (2008) for thermomechanically processed soya-based bioplastic containing 5 wt% MC. According to these authors, the miscibility between protein components and polysaccharide were responsible for the improvement of the strength and even resulted in the simultaneous enhancement of strength and elongation. However, an increase in MC (above 5 wt%) produces a decrease in ε_{max} and σ_{max}. These authors explained such behaviour in terms of strong aggregations between crystalline domains inside the protein matrix and, subsequently, outside of this.

Under the experimental conditions selected in this work, both biopolymers (MC and CMC) have a synergistic effect on the mechanical properties of the protein based bioplastic materials. Polysaccharide molecules seem to interact with protein chains altering the rigid structure of gluten and causing a reduction of hydrogen bonds among protein molecules, if compared to the polysaccharide-free system, replaced by protein/polysaccharide interactions.

Even though MC and CMC seem to act as fillers, as may be deduce from the higher Young's Modulus values obtained, they are able to exhibit more compatibility with the protein matrix altering hydrogen bonds and preventing the formation of strong bonds after material thermo-moulding, which could explain the observed increase in ε_{max}. In this sense, previous studies have reported that after heat treatment the electrical charges of the components may lead to different microstructures within the protein–polysaccharide complex. A continuous protein network can be formed with polysaccharide inclusions; thereby strengthening the composite, if they are electrically compatible and the attractive forces between the protein and anionic polysaccharide prevail (Zaleska et al., 2000). On the other hand, as reported by Parris et al. (1995) or Coughlan et al. (2004) an electrical incompatibility between both components may lead to a decrease in intermolecular interaction between the components (protein and polysaccharide).

3.1.3. Water uptake capacity and loss of soluble matter

Fig. 4 shows the values of water uptake and water-soluble matter loss for the reference and WG/GL/polysaccharide/water bioplastic probes after being immersed for 2 and 24 h. Bioplastics were prepared at pH = 6, having 1.5 wt% of the different polysaccharides studied. The values of water uptake capacity are very similar in all cases, so that, all systems showed about 40 wt% water absorption after 2 h and this value increases up to 70 wt% after 24 h testing. On the other hand, the water-soluble matter loss (likely related to the plasticiser and some protein/polysaccharide molecules still soluble after processing) is approximately the same for all the samples, about 23 wt%. This concentration is close to the amount of glycerol initially added to form the bioplastic and confirms its release during the immersion test in water.

3.2. Effect of the pH

3.2.1. Dynamic mechanical temperature analysis (DMTA)

Fig. 5 shows the effect of pH on DMTA curves of bioplastics containing 1.5 wt% LBG and polysaccharide-free samples (i.e. reference

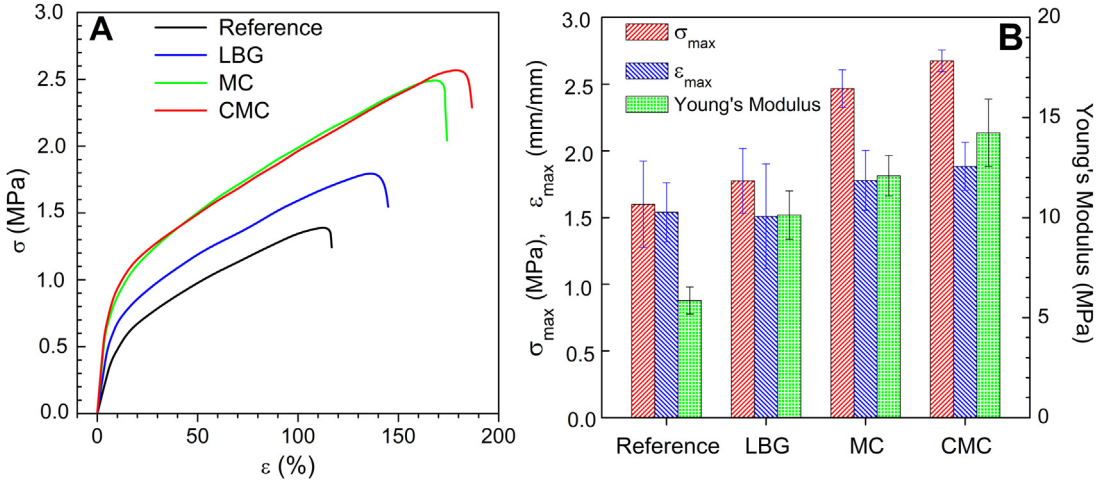

Fig. 3. Results from tensile strength measurements for WG/GL/polysaccharide/water bioplastics formulated with different types of polysaccharide: (A) stress–strain curves; (B) tensile strength parameters: maximum stress (σ_{max}), maximum strain (ε_{max}) and Young's Modulus.

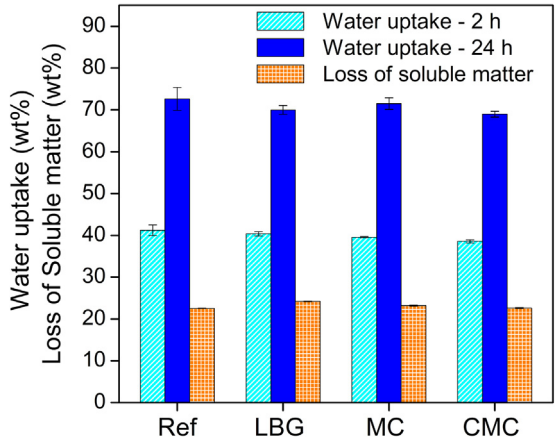

Fig. 4. Water absorption capacity results after immersion for 2 h and 24 h and loss of soluble matter for WG/GL/polysaccharide/water bioplastics with different types of polysaccharide.

systems). As may be seen, polysaccharide-free bioplastic probes prepared at pH 3 exhibit much lower values of E' (as well as, higher tan δ values) than those probes prepared at the native pH of gluten (i.e. at the protein IEP of pH 6) or alkaline pH values (pH 9). This behaviour reveals that under acidic conditions the presence of

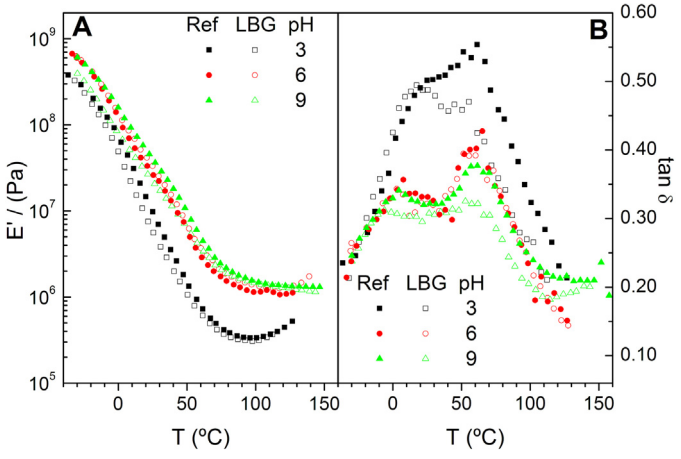

Fig. 5. Temperature dependence of the storage modulus, E', and loss modulus, E'', (A), and loss tangent, tan δ, (B), for the reference and LBG-containing bioplastics, as a function of pH from DMTA measurements.

positive charges on the protein surfaces would hinder the development of the bioplastic microstructure during the thermo-moulding process, leading to a softer material in accordance to the results already reported by Zhang, Song & Zheng (2008). Likewise, Jansens et al. (2013) demonstrated that this is indeed the case. Acid hinders cross-linking, but does not completely prevent it. Thus, by applying higher temperatures more cross-linking indeed occurs, explaining occurrence of a remaining thermosetting potential (as may be deduced from the modulus increase shown in Fig. 5 above 100 °C for pH 3). In other words, the protein matrix might require higher moulding temperatures in order to obtain higher values of E'. In contrast, alkaline conditions, where negative charges are predominant at the protein surface, slightly affect the viscoelastic properties over those of polysaccharide-free bioplastics. These results are also consistent with those shown by Jansens et al. (2013), who reported protein degradation but also enhanced cross-linking when processing gluten with high alkali concentration. Thus, alkaline conditions may induce protein degradation, likely caused by peptide bond hydrolysis, with the formation of low MW protein fragments. On the other hand, exposure to alkaline conditions, particularly when coupled to thermal processing, induces formation of nondisulphide covalent crosslinks, such as dehydroalanine, lysinoalanine and lanthionine (Gerrard, 2002). The resulting intra- and intermolecular crosslinks are stable such that, as stated by Jansens et al. (2013), replacement of disulphide bonds with other covalent bonds does not apparently affect the material properties of the end products. In addition, as suggested by these authors, alkaline conditions allow processing at lower moulding temperatures to bring about similar strength values, which explains the fact that no remaining thermosetting potential is found after being thermo-moulded at 140 °C. On the other hand, the effect of the LBG addition also depends on the pH conditions. Thus, a reduction in the viscoelastic modulus E' is generally found when the pH is far below or above the IEP. This result would suggest a higher incompatibility between charged proteins, at pH 3 or 9, and the non-ionic polysaccharide LBG.

However, under alkaline conditions this decrease losses significance at 50 °C and above. In contrast, close to the IEP (pH = 6), where the protein charge balance is zero, the presence of polysaccharide does not lead to any significant change in E' profile (Fig. 5A).

Interestingly, Fig. 5B shows the overlapping of peaks $T_{\alpha 1}$ and $T_{\alpha 2}$ at pH 3, which suggests an increase in compatibility among bioplastics microphases, so that, $T_{\alpha 2}$ shifts towards lower values in the LBG-free system, resulting in a lower glass transition temperature of plasticised proteins. On the contrary, the addition of LBG

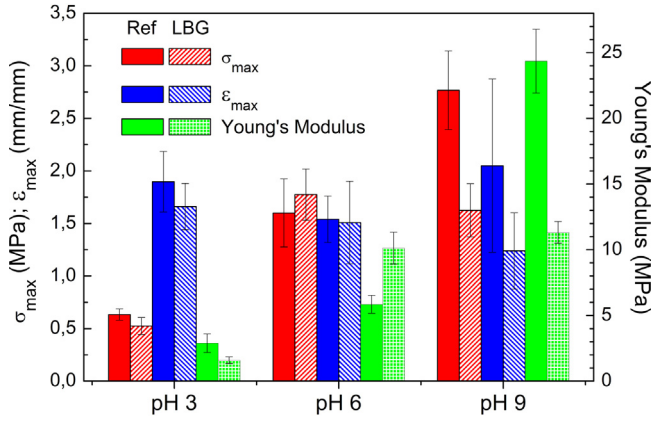

Fig. 6. Tensile strength parameters: maximum stress (σ_{max}), Maximum Strain (ε_{max}) and Young's Modulus; for the reference and LBG-containing bioplastics probes as a function of pH.

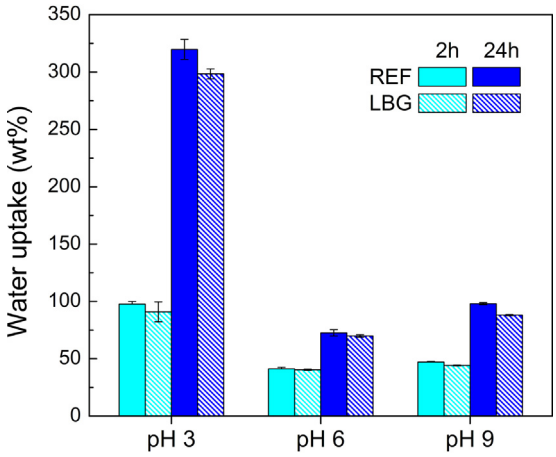

Fig. 7. Water absorption capacity results after immersion for 2 h and 24 h for the reference and LBG-containing bioplastics as a function of pH.

led to a peak separation, since $T_{\alpha 1}$ decreased whereas $T_{\alpha 2}$ remained essentially constant, at pH = 3. Conversely, both peaks shifted to lower temperatures at pH = 9 and no overlapping was observed. These results are in good agreement with the above-commented incompatibility between the charged proteins and the non-ionic polysaccharide. In any case, it is worth noting that, at both pH 3 and 9, bioplastics containing LBG (acting as filler) showed a higher elastic character than the polysaccharide-free systems in a wide range of temperatures, as may be deduced from the lower values of the loss tangent ($\tan\delta = G''/G'$) curves, recorded after LBG addition (Fig. 5B). Finally, $\tan\delta$ profile remains unaltered by the presence of LBG at the protein native pH of 6.

DMTA results point out that the presence of charges favours the incompatibility between the protein and LBG, probably due to the so-called segregative separation effect (McClements, 2006). Segregative separation is related to the relatively strong repulsion between the two different kinds of biopolymers. The molecular origin of this effect is usually the steric exclusion when one of the biopolymers, LBG in this case, has no charge whilst the protein matrix is positively (at pH = 3) or negatively (at pH = 9) charged.

3.2.2. Tensile tests

Fig. 6 shows the values of tensile parameters (σ_{max}, ε_{max} and Young's Modulus) obtained for the reference systems and for probes containing 1.5 wt% LBG prepared at different pH. If only polysaccharide-free bioplastics are compared, the presence of charges leads to apparent modifications of the tensile properties of the system. At low pH, charges prevent formation of protein cross-linking by thermo-moulding, which leads to a microstructure with higher mobility and ability to deform, due to presence of weak bonds (hydrogen bonds and van der Waals bonds), but showing lower modulus Jansens et al. (2013). This microstructure is responsible for the low values of σ_{max}, and Young's Modulus and the high material elongation (ε_{max}) found at pH 3 (Fig. 6). At pH 6, the non-charged proteins are able to establish stronger bonds (ionic bonds and covalent bonds such as peptide and disulfide bonds) after thermo-mechanical processing, which results in higher modulus values (σ_{max}, and Young's Modulus) and lower ε_{max}, if compared to pH 3. Finally, at pH 9, the resultant microstructure is affected by the negative charges and the formation of lysinoalanine, lanthionine and dehydroalanine-derived cross-links stimulated with alkali at moulding temperatures exceeding 130 °C Jansens et al. (2013). Thus, the highest values of both σ_{max}, Young's Modulus are found at pH 9. Furthermore, ε_{max} is higher than the value obtained at pH 6 but differences are not significant due to the data dispersion. Zhang et al. (2008) reported similar evolution of these tensile parameters as a function of acid or alkali content for compression-moulded gluten bioplastics.

As may be observed in Fig. 6, the same behaviour takes place when LBG is present such that the results found at pH 3 and pH 6 are similar to those of the polysaccharide-free specimens. However, the above mentioned enhancement of tensile properties reported for LBG-free samples at pH 9 is inhibited by the presence of LBG, which may be related to some crosslinking inhibition effects.

Consistent with DMTA results, the thermodynamic incompatibility between WG proteins and LBG, due to the segregative separation phenomenon, seems to induce a decrease in the bioplastic elongation at break (ε_{max}), if compared to their reference systems.

3.2.3. Water uptake capacity

Fig. 7 shows the values of water uptake capacity, as a function of pH, of the reference systems and bioplastics containing 1.5 wt% LBG that were immersed for 2 and 24 h. As may be seen, water uptake capacity is enhanced when the pH was far from the IEP. These results indicate that the presence of charges at protein surfaces must play an important role. Particularly at pH 3, bioplastic water absorption exceeded 300 wt% after 24 h. This result agrees with a microstructure formed by a higher number of hydrophilic bonds (i.e. hydrogen bonds) and larger available free spaces within the protein matrix (Gomez-Martinez et al., 2009), probably due to the smaller aggregation of the protein network after moulding (Pommet, Redl, Guilbert, & Morel, 2005). Instead, the lower water absorption of the resultant material thermo-moulded at pH 6 suggests an increase in the cross-link density after moulding. In this sense, different authors have confirmed such a decrease in the swelling ratio of a polymeric matrix as cross-linking degree increases (Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003; Felix, Martin-Alfonso, Romero, & Guerrero, 2014; Jerez et al., 2007; Zarate-Ramirez et al., 2011; Zárate-Ramírez et al., 2014; Zheng et al., 2003). On these grounds, water absorption obtained at pH 9, although higher than that obtained at pH 6, is much lower than the value found at pH 3 due to the aforementioned formation of lysinoalanine, lanthionine and dehydroalanine-derived cross-links, which takes place under alkaline conditions and the selected moulding temperature (130 °C).

However, the addition of polysaccharide yielded a slight reduction of water uptake capacity, which was more apparent at pH 3 and 9 after 24 h. LBG acting as filler would partially occupy the free spaces within the protein matrix, reducing water absorption. In agreement with this, Parris et al. (1995) reported that an increase in intermolecular hydrogen bonding between biopolymer

molecules reduces spacing between the macromolecules, giving rise to a reduction in WVP.

According to these results, water absorption capability strongly depends on the characteristics of the protein phase. The presence of charges at protein surfaces (and the protein network disaggregation) facilitates the retention of polar water molecules; regardless they were positively charged, at low pH, or negatively, at alkaline pH. In addition, the marked water uptake effect found at pH 3 seems to be also associated to its poorer DMTA and tensile strength properties. This weaker microstructure of the protein matrix at pH 3 indicates a lower level of association between the microdomains present in the system, which means that many active sites must be still available for hydrogen bonding with water molecules.

4. Concluding remarks

The results obtained suggest that polysaccharides generally play the role of fillers, which are able to interact with the protein molecules in different manners depending on the protein charge and their aggregation degree after bioplastic thermomoulding. However, biopolymers MC and CMC have a synergistic effect on the elongation properties of the protein based bioplastic materials, which suggests that polysaccharides might also play a plasticiser role. Interestingly, polysaccharide incorporation not only enhances elongation but also leads to higher Young's Modulus.

Nevertheless, pH is the most relevant parameter that affects these protein-based materials. Their properties (e.g. Young's Modulus, elongation, water absorption) strongly depend on the resultant protein microstructure after being prepared at different pH values. Thus, low pH hinders protein cross-linking and leads to materials with small moduli, high water absorption and enhanced elongation capability. A pH value of 6 leads to materials with high cross-linking degree (due to disulphide bonds formation) with high moduli and low elongation and water absorption ability. Finally, alkaline pH induces negative charges on proteins and also promotes the formation of lysinoalanine, lanthionine and dehydroalanine-derived cross-links stimulated at the selected moulding temperature (130 °C). Both of them influence protein aggregation and lead to materials with high moduli as well as lower elongation at break and lower water absorption than pH 3 but higher than those obtained at pH 6.

In addition, polysaccharide incorporation slightly affects material hydrophilic character. As a result, bioplastic formulation at pH 3 and pH 9 would lead to materials to be used in applications requiring enhanced water absorption capacity and suitable end-use mechanical properties.

Acknowledgement

Financial support by "Ministerio de Economía y Competitividad" from Spain (MICINN project Ref. MAT2011-29275-C02-02/01) is gratefully acknowledged.

References

Aguilar-Mendez, M. A., Martin-Martinez, E. S., Tomas, S. A., Cruz-Orea, A., & Jaime-Fonseca, M. R. (2008). Gelatine-starch films: Physicochemical properties and their application in extending the post-harvest shelf life of avocado (*Persea americana*). *Journal of the Science of Food and Agriculture*, *88*(2), 185–193.

American Society for Testing Materials. (2005). *Annual book of ASTM Standards*. ASTM International.

Association of Official Analytical Chemists. (2005). *Official methods of analysis of AOAC International*. Gaithersburg, MD: AOAC International.

Averous, L. (2004). Biodegradable multiphase systems based on plasticized starch: A review. *Journal of Macromolecular Science: Polymer Reviews*, *C44*(3), 231–274.

Bengoechea, C., Arrachid, A., Guerrero, A., Hill, S. E., & Mitchell, J. R. (2007). Relationship between the glass transition temperature and the melt flow behavior for gluten, casein and soya. *Journal of Cereal Science*, *45*(3), 275–284.

Buonocore, G. G., Del Nobile, M. A., Panizza, A., Corbo, M. R., & Nicolais, L. (2003). A general approach to describe the antimicrobial agent release from highly swellable films intended for food packaging applications. *Journal of Controlled Release*, *90*(1), 97–107.

Chen, L., Reddy, N., Wu, X., & Yang, Y. (2012). Thermoplastic films from wheat proteins. *Industrial Crops and Products*, *35*(1), 70–76.

Ciesla, K., Salmieri, S., & Lacroix, M. (2006). Modification of the properties of milk protein films by gamma radiation and polysaccharide addition. *Journal of the Science of Food and Agriculture*, *86*(6), 908–914.

Coughlan, K., Shaw, N. B., Kerry, J. F., & Kerry, J. P. (2004). Combined effects of proteins and polysaccharides on physical properties of whey protein concentrate-based edible films. *Journal of Food Science*, *69*(6), E271–E275.

Cuq, B., Boutrot, F., Redl, A., & Lullien-Pellerin, V. (2000). Study of the temperature effect on the formation of wheat gluten network: Influence on mechanical properties and protein solubility. *Journal of Agricultural and Food Chemistry*, *48*(7), 2954–2959.

Dammer, L., Carus, M., Raschka, A., & Scholz, L. (2013). Market developments of and opportunities for biobased products and chemicals. *Nova-Institute for Ecology and Innovation Report*.

De Graaf, L. A. (2000). Denaturation of proteins from a non-food perspective. *Journal of Biotechnology*, *79*(3), 299–306.

Erdohan, Z. O., & Turhan, K. N. (2005). Barrier and mechanical properties of methylcellulose-whey protein films. *Packaging Technology and Science*, *18*(6), 295–302.

Fakhouri, F. M., Tanada-Palmu, P. S., & Grosso, C. R. F. (2004). Characterization of composite biofilms of wheat gluten and cellulose acetate phthalate. *Brazilian Journal of Chemical Engineering*, *21*(2), 261–264.

Felix, M., Martin-Alfonso, J. E., Romero, A., & Guerrero, A. (2014). Development of albumen/soy biobased plastic materials processed by injection molding. *Journal of Food Engineering*, *125*, 7–16.

Garnier, C., Schorsch, C., & Doublier, J. L. (1995). Phase separation in dextran locust bean gum mixtures. *Carbohydrate Polymers*, *28*(4), 313–317.

Gerrard, J. A. (2002). Protein–protein crosslinking in food: Methods, consequences, applications. *Trends in Food Science & Technology*, *13*(12), 391–399.

Gomez-Martinez, D., Partal, P., Martinez, I., & Gallegos, C. (2009). Rheological behaviour and physical properties of controlled-release gluten-based bioplastics. *Bioresource Technology*, *100*(5), 1828–1832.

Gomez-Martinez, D., Partal, P., Martinez, I., & Gallegos, C. (2013). Gluten-based bioplastics with modified controlled-release and hydrophilic properties. *Industrial Crops and Products*, *43*, 704–710.

Gounga, M. E., Xu, S.-Y., & Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. *Journal of Food Engineering*, *83*(4), 521–530.

Hernandez-Izquierdo, V. M., & Krochta, J. M. (2008). Thermoplastic processing of proteins for film formation – review. *Journal of Food Science*, *73*(2), R30–R39.

Iles, A., & Martin, A. N. (2013). Expanding bioplastics production: Sustainable business innovation in the chemical industry. *Journal of Cleaner Production*, *45*, 38–49.

Irissin-Mangata, J., Bauduin, G., Boutevin, B., & Gontard, N. (2001). New plasticizers for wheat gluten films. *European Polymer Journal*, *37*(8), 1533–1541.

Jansens, K. J. A., Lagrain, B., Brijs, K., Goderis, B., Smet, M., & Delcour, J. A. (2013). Impact of acid and alkaline pretreatments on the molecular network of wheat gluten and on the mechanical properties of compression-molded glassy wheat gluten bioplastics. *Journal of Agricultural and Food Chemistry*, *61*(39), 9393–9400.

Jerez, A., Partal, P., Martinez, I., Gallegos, C., & Guerrero, A. (2005). Rheology and processing of gluten based bioplastics. *Biochemical Engineering Journal*, *26*(2–3), 131–138.

Jerez, A., Partal, P., Martinez, I., Gallegos, C., & Guerrero, A. (2007). Protein-based bioplastics: Effect of thermo-mechanical processing. *Rheologica Acta*, *46*(5), 711–720.

Kim, S. (2008). Processing and properties of gluten/zein composite. *Bioresource Technology*, *99*(6), 2032–2036.

Lagrain, B., Goderis, B., Brijs, K., & Delcour, J. A. (2010). Molecular basis of processing wheat gluten toward biobased materials. *Biomacromolecules*, *11*(3), 533–541.

Lee, K. Y., Shim, J., & Lee, H. G. (2004). Mechanical properties of gellan and gelatin composite films. *Carbohydrate Polymers*, *56*(2), 251–254.

Matveev, Y. I., Grinberg, V. Y., & Tolstoguzov, V. B. (2000). The plasticizing effect of water on proteins, polysaccharides and their mixtures. Glassy state of biopolymers, food and seeds. *Food Hydrocolloids*, *14*(5), 425–437.

McClements, D. J. (2006). Non-covalent interactions between proteins and polysaccharides. *Biotechnology Advances*, *24*(6), 621–625.

Murray, J. (2009). Cellulosics. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 710–723). Cambridge, UK: Woodhead Publishing Limited.

Oses, J., Fabregat-Vazquez, M., Pedroza-Islas, R., Tomas, S. A., Cruz-Orea, A., & Mate, J. I. (2009). Development and characterization of composite edible films based on whey protein isolate and mesquite gum. *Journal of Food Engineering*, *92*(1), 56–62.

Park, S. K., & Bae, D. H. (2006). Antimicrobial properties of wheat gluten-chitosan composite film in intermediate-moisture food systems. *Food Science and Biotechnology*, *15*(1), 133–137.

Parris, N., Coffin, D. R., Joubran, R. F., & Pessen, H. (1995). Composition factors affecting the water-vapor permeability and tensile properties of hydrophilic films. *Journal of Agricultural and Food Chemistry*, *43*(6), 1432–1435.

Pommet, M., Redl, A., Guilbert, S., & Morel, M. H. (2005). Intrinsic influence of various plasticizers on functional properties and reactivity of wheat gluten thermoplastic materials. *Journal of Cereal Science*, *42*(1), 81–91.

Pommet, M., Redl, A., Morel, M. H., Domenek, S., & Guilbert, S. (2003). Thermoplastic processing of protein-based bioplastics: Chemical engineering aspects of mixing, extrusion and hot molding. *Macromolecular Symposia*, *197*, 207–217.

Raphael, I., & Yang, A. (2013). Plastics production from biomass: Assessing feedstock requirement. *Biomass Conversion and Biorefinery*, *3*(4), 319–326.

Reddy, N., & Yang, Y. (2013). Thermoplastic films from plant proteins. *Journal of Applied Polymer Science*, *130*(2), 729–738.

Schmitt, C., Aberkane, L., & Sanchez, C. (2009). Protein–polysaccharide complexes and coacervates. In G. O. Phillips, & P. A. Williams (Eds.), *Handbook of hydrocolloids* (pp. 420–476). Cambridge, UK: Woodhead Publishing Limited.

Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and technofunctional properties of protein–polysaccharide complexes: A review. *Critical Reviews in Food Science and Nutrition*, *38*(8), 689–753.

Siracusa, V., Rocculi, P., Romani, S., & Dalla Rosa, M. (2008). Biodegradable polymers for food packaging: A review. *Trends in Food Science & Technology*, *19*(12), 634–643.

Sun, S., Song, Y., & Zheng, Q. (2007). Morphologies and properties of thermo-molded biodegradable plastics based on glycerol-plasticized wheat gluten. *Food Hydrocolloids*, *21*(7), 1005–1013.

Sun, S., Song, Y., & Zheng, Q. (2008). Thermo-molded wheat gluten plastics plasticized with glycerol: Effect of molding temperature. *Food Hydrocolloids*, *22*(6), 1006–1013.

Turgeon, S. L., & Beaulieu, M. (2001). Improvement and modification of whey protein gel texture using polysaccharides. *Food Hydrocolloids*, *15*(4–6), 583–591.

Zaleska, H., Ring, S. G., & Tomasik, P. (2000). Apple pectin complexes with whey protein isolate. *Food Hydrocolloids*, *14*(4), 377–382.

Zarate-Ramirez, L. S., Martinez, I., Romero, A., Partal, P., & Guerrero, A. (2011). Wheat gluten-based materials plasticised with glycerol and water by thermoplastic mixing and thermomoulding. *Journal of the Science of Food and Agriculture*, *91*(4), 625–633.

Zhang, H., & Mittal, G. (2010). Biodegradable protein-based films from plant resources: A review. *Environmental Progress & Sustainable Energy*, *29*(2), 203–220.

Zhang, Q., Song, Y., & Zheng, Q. (2008). Influences of acid and alkali on mechanical properties of compression-molded gluten bioplastics. *Cereal Chemistry*, *85*(3), 379–383.

Zheng, H., Tan, Z. A., Zhan, Y. R., & Huang, J. (2003). Morphology and properties of soy protein plastics modified with chitin. *Journal of Applied Polymer Science*, *90*(13), 3676–3682.

Zhou, Z., Zheng, H., Wei, M., Huang, J., & Chen, Y. (2008). Structure and mechanical properties of cellulose derivatives/soy protein isolate blends. *Journal of Applied Polymer Science*, *107*(5), 3267–3274.

Zuo, M., Song, Y., & Zheng, Q. (2009). Preparation and properties of wheat gluten/methylcellulose binary blend film casting from aqueous ammonia: A comparison with compression molded composites. *Journal of Food Engineering*, *91*(3), 415–422.

Zárate-Ramírez, L. S., Romero, A., Martínez, I., Bengoechea, C., Partal, P., & Guerrero, A. (2014). Effect of aldehydes on thermomechanical properties of gluten-based bioplastics. *Food and Bioproducts Processing*, *92*(1), 20–29.