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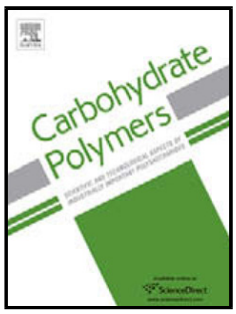

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INFLUENCE OF TRAGACANTH GUM IN EGG WHITE BASED BIOPLASTICS: THERMOMECHANICAL AND WATER UPTAKE PROPERTIES

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Highlights

- The presence of tragacanth gum in EW-bioplastics enhances their water uptake.
- A higher content of tragacanth gum results in higher water uptake values.
- Tragacanth gum influences the mechanical properties of EW-bioplastics.

Abstract

This study aims to extend the range of applications of tragacanth gum by studying its incorporation into bioplastics formulation, exploring the influence that different gum contents (0-20 wt.%) exert over the thermomechanical and water uptake properties of bioplastics based on egg white albumen protein (EW). The effect of plasticizer nature was also evaluated through the modification of the water/glycerol ratio within the plasticizer fraction (fixed at 40 wt.%). The addition of tragacanth gum generally yielded an enhancement of the water uptake capacity, being doubled at the highest content. Conversely, presence of tragacanth gum resulted in a considerable decrease in the bioplastic mechanical properties: both tensile strength and maximum elongation were reduced up to 75% approximately when compared to the gum-free system. Ageing of selected samples was also studied, revealing an important effect of storage time when tragacanth gum is present, possibly due to its hydrophilic character.

Keywords: *egg white; tragacanth gum; plasticizer; bioplastic; mechanical properties; ageing*

1. Introduction

Proteins and polysaccharides have been used as biopolymer sources for many years. A wide range of tailored functional properties may be achieved modulating the different interactions and intermolecular linkages that take place between and within heteropolymer molecules (Gómez-Martínez, Partal, Martínez, & Gallegos, 2009; Pommet, Redl, Morel, & Guilbert, 2003). Proteins like egg white or soy protein represent renewable materials that are produced massively. The suitability of some of these proteins for the manufacture of bioplastics has been shown by different studies (Kim, 2008; Mohanty et al., 2005; Tummala, Liu, Drzal, Mohanty, & Misra, 2006; Zheng, Tan, Ran Zhan, & Huang, 2003).

The processing of bioplastics generally requires the addition of plasticizers and, sometimes, disrupting agents in the formulation (Pommet, Redl, Guilbert, & Morel, 2005; Sothornvit, Krochta, & Han, 2005). A plasticizer is a component included to overcome brittleness and to avoid chipping and cracking of polymeric materials during subsequent handling and storage. Plasticizers, like glycerol or water, are molecules with low molecular weight and volatility, which reduce the intermolecular forces and increase the mobility of the polymeric chains, which results in a decrease in the material glass transition temperature, through modification of the three-dimensional structure of proteins (Matveev, Grinberg, & Tolstoguzov, 2000).

The barrier properties of biopolymeric films are important parameters in food packaging applications. Protein and polysaccharide films display generally good barrier properties against oxygen at low and intermediate relative humidity (RH) values, showing good mechanical properties, like elongation at break (Anker, Berntsen, Hermansson, & Stading, 2002). An adequate selection of composition and processing parameters may lead to materials with unique properties (Pommet et al., 2003). Recent works by Fernández-Espada et al. (2013) and González-Gutierrez et al. (2010; 2011) have revealed the feasibility of producing bioplastics

from egg white protein (albumen). Those bioplastics obtained through thermo-mechanical methods showed suitable values for Young's modulus, stress at break or elongation for biodegradable food packaging applications. This would represent a novel alternative for egg white when compared to the traditional use given by the food industry due to its functional properties, such as gelling, foaming, heat setting and binding adhesion.

Moreover, if modified atmosphere packaging (MAP) applications are pursued, a hydrophilic character may be desirable for the bioplastic material, as some processes leading to oxygen scavenging or CO_2 emitting have been proved to be moisture dependent. Thus, those processes would only take place after moisture has been absorbed from the food product or package atmosphere into the bioplastic material (Conte et al., 2013; Ščetar, Kurek, & Galić, 2010). Tragacanth gum is a hydrophilic and very complex heterogeneous anionic polysaccharide of high molecular weight, around 840 $kg \cdot mol^{-1}$ (Weiping & Branwell, 2000), consisting of two main fractions: a) a water-insoluble component called *bassorin*, which has the capacity to swell and form a gel; and b) a water-soluble component called *tragacanthin* (Balaghi, Mohammadifar, Zargaraan, Gavlighi, & Mohammadi, 2011). Tragacanth gum has been reported, when mixed with glycerol and water, to form a useful excipient to bind pill masses in the pharmaceutical or cosmetic industries (Phillips & Williams, 2000). Tragacanth gum has been accepted since 1961 as generally recognized as safe (GRAS), according to FDA, and used for many years as a stabiliser, thickener, emulsifier and suspending agent in the food, pharmaceutical, cosmetic, textile and leather industries as well as in technical applications (Anderson & Bridgeman, 1985). It presents high viscosity at low concentration, good suspending action, unusually high stability to heat and acidity and effective emulsifying properties. It is also pourable and has creamy mouth feel and good flavour-release properties (Weiping & Branwell, 2000) and very long shelf life (Levy & Schwarz, 1958).

Egg white based bioplastic materials have been previously studied, displaying water absorption capacities under 100% (Fernandez-Espada, Bengoechea, Cordobes, & Guerrero, 2016; Jerez, Partal, Martinez, Gallegos, & Guerrero, 2007). A potential way to improve the water uptake of egg white based bioplastic materials would be to include a hydrocolloid in its formulation. Tragacanth gum has proved to swell and form gels (Weiping & Branwell, 2000), having been used in food products due to its attractive functional properties (e.g. high ability to bind water or effective emulsifying properties). More recently, potential applications of tragacanth gum in the field of packaging or biomaterials have also been studied (Mostafavi, Kadkhodaee, Emadzadeh, & Koocheki, 2016; Ranjbar-Mohammadi, Prabhakaran, Bahrami, & Ramakrishna, 2016). However, just few studies have investigated the properties of tragacanth gum as part of moulded bioplastic materials (López-Castejón, Bengoechea, García-Morales, & Martínez, 2015).

The main objective of this work was to evaluate the effect of tragacanth gum on the water uptake capacity, linear viscoelasticity and tensile properties of egg white protein matrices when they were plasticized with mixtures of varying water/glycerol ratios. Bioplastics were obtained through a conventional thermo-mechanical process, keeping the biopolymer/plasticizer ratio constant but modifying the composition within each fraction.

2. Experimental

2.1 Materials and sample preparation

Materials

Spray-dried egg white albumen (designated EW; with 73 wt.% protein (dry basis), 6 wt.% ashes and 8 wt.% moisture) provided by OVOSEC S.A. (Spain) was used as base material for bioplastics manufacture. EW was produced by the automatic shelling of eggs, which prior to

their homogenisation and pasteurisation are desiccated by atomisation (Navarro, 2003). After this, the product is subjected to strict physico-chemical and bacteriological tests. On the other hand, tragacanth gum (designated T) (39-42% carbon content) was supplied by Sigma-Aldrich (USA). In relation with the plasticizers, glycerol, from Panreac Química, S.A. (Spain), and distilled water were designated G and W, respectively.

Sample preparation

Different egg white/tragacanth gum/glycerol/water (EW/T/G/W) compositions, always keeping a constant biopolymer (EW+T)/plasticizer (G+W) ratio of 60/40 have been studied, following a established protocol (López-Castejón et al., 2015). Three different plasticizer contents have been used: W40, with only water; G20W20, using both glycerol and water at a ratio equal to 1; and G40, containing only glycerol. Moreover, three different biopolymer contents have also been studied: EW60, with no tragacanth gum; EW50T10, with a tragacanth gum content of 10% (w/w); and EW40T20, with a gum content of 20% (w/w). A summary of the 9 compositions studied is shown in Table 1.

With regard to the bioplastics manufacture, this was accomplished by a thermo-mechanical process, which includes two stages:

a) Mixing of ingredients: it was carried out for 10 min in the kneading tool (Rheomix 600p) of a torque-rheometer (Polylab, Thermo Haake GmbH, Germany) equipped with two counter-rotating rollers turning at 50 rpm (Jerez, Partal, Martínez, Gallegos, & Guerrero, 2005). Temperature, starting at 25ºC, was allowed to naturally evolve over this period (no heating/cooling). The Polylab mixer used allowed the record of the torque and temperature along the mixing time. In every case, 100 g of blend was obtained.

b) Compression-moulding: the resulting dough-like material was subjected to pressure of 10 MPa and temperature of 120 ºC for 10 min in a hot-plate press, as described by Jerez et al.

(2007). Two types of moulds were used: one to obtain rectangular 3-mm-thick specimens for both DMTA and water uptake capacity measurements; and a second one to obtain type IV-dumbbell specimens (2003) for tensile tests.

After preparation and before testing, these samples were placed in desiccators at relative humidity of 53% with a saturated solution of $Mg(NO_3)_2 \cdot 6H_2O$ at room temperature (Nyqvist, 1983). Samples were always stored at least for 24 hours prior any test was conducted.

2.2. Testing

2.2.1. Water uptake capacity

Water uptake tests, according to ASTM D570 (2005), were carried out on rectangular probes (50×10×3 mm^3) immersed into distilled water for 24 hours. Three replicates were done for each sample, and the water uptake percentage calculated as:

$$Water\ uptake(wt.\%) = \frac{wet\ wt. - reconditioned\ wt.}{conditioned\ wt.}\ x\ 100 \quad \text{(Eq. 1)}$$

where: conditioned weight, is the initial weight of the probe; wet weight, refers to the weight of the probe just after 24 hours of water immersion; and reconditioned weight, is the final weight of the wet sample after 24 hours of drying in an oven at 50ºC.

2.2.2. Tensile properties

Tensile tests were performed with a MTS Insight 10 kN (USA), according to ASTM D638 (2003), with an extension rate of 5 mm/min, at room temperature. An extensometer was used in order to accurately register the sample elongation. At least, five tests were carried out for each dumb-bell shaped type IV sample (thickness: 3.2 mm).

2.2.3. Dynamic Mechanical Thermal Analysis (DMTA)

DMTA experiments were performed with a RSA3 rheometer (TA Instruments, USA) in dual cantilever bending mode on rectangular 50×10×3 mm^3 specimens, according to ASTM D5023-01 (2001). Dynamic temperature sweeps were performed at a constant frequency of 1 Hz and strains within the linear viscoelasticity (LVE) region. Strain sweep tests were performed to determine the LVE region. The heating rate was set at 3 ºC/min, within a temperature interval from -30 to 140 ºC. All tests were performed at least three times.

2.2.4. Statistical analysis

The data were presented as mean ± standard deviation (SD) of three (water uptake capacity and DMTA tests) or five (tensile tests) determinations. A probability value of $p<0.05$ was considered significant.

3. Results and Discussion

3.1. Torque and temperature profiles along the mixing stage

An intimate mixing of these ingredients is essential to obtain homogeneous dough-like materials that will eventually be processed into bioplastics through compression moulding. Figure 1 shows the evolution of both torque and temperature during this mixing process at 50 rpm for the different compositions studied. Mixing time was limited to 10 minutes based on analogous previous studies (Jerez, Partal, Martinez, Gallegos, & Guerrero, 2007), as longer times would produce a granular and heterogeneous material (Gómez-Martínez et al., 2009; Jerez et al., 2005) unsuitable for further thermo-mechanical treatments. According to Jerez et al. (Jerez et al., 2007), the evolution of torque displayed in Figure 1 would correspond to a first induction period that normally occurs at short mixing times, followed by a nearly steady value of torque, which seems to be greater for those samples that, independently of the gum content (0, 10 or 20 wt.%), contain a mixture of the plasticizers (G20W20). A meaningful parameter to consider in

the mixing stage is the Specific Mechanical Energy (SME) input, which is the energy provided by the mixer per mass unit, defined as (Redl et al., 1999):

$$SME = \frac{\omega}{m}\int_0^{t_{mix}} M(t)dt$$

where ω represents the rotational speed (in rad/s) of the mixer, m is the total sample mass that is introduced (in g), M (in N m) is the torque, and t_{mix} (in s) is the mixing time. The SME values for all the formulations studied are shown in Table 2. As observed, those systems including both plasticizers (G20W20) reach the highest SME values (250-310 kJ/kg). As was previously reported (Chen, Li, Song, & Yang, 2009; Gómez-Martínez et al., 2009; López-Castejón et al., 2015) systems which included both glycerol and water in their formulation displayed higher torque values, what might be the result of interactions between glycerol and water. The inherent hygroscopic character of glycerol may, at some point, lead to synergistic effects due to the formation of hydrogen bonds.

When observing the influence of the presence of the gum, it may be pointed out that those blends containing both biopolymers (EW50T10, EW40T20) display higher steady torque values and SME than those that contain only protein (EW60) for those samples including only one type of plasticizer. This increase when the gum is present may be related to its high hydrophilic character that may promote its capacity to hold water, even when the tragacanth content was as low as 10 wt.% (Maier et al., 1993).

Figure 1 also shows the evolution of temperature along the mixing process for the EW/T/G/W blends, as a consequence of mechanical energy dissipation that takes place along the induction stage. The highest increase in temperature was found for the systems containing both plasticizers, reaching a final temperature of 41±1ºC and 56±0.5ºC for EW60 and EW40T20, respectively. This is probably due to the interactions among plasticizers commented above.

However, it should be noticed how the system with 10 wt.% of tragacanth gum (EW50T10/G20W20) remains around 25 ºC for the whole induction period. In any case, it may be observed how temperature is always lower than the reported albumen denaturation temperature (~ 65ºC (conalbumin) and 84ºC (ovalbumin) (Donovan, Mapes, Davis, & Garibaldi, 1975)).

3.2. Compression moulded bioplastics

3.2.1. Water uptake capacity

Water uptake values for compression moulded EW-based bioplastics are shown in Figure 2 for different plasticizers (W40, G20W20, G40) and biopolymers (EW60, EW50T10, EW40T20).

When observing the influence of the plasticizer nature, it is noticeable that samples including a mixture of plasticizers (G20W20) always display lower water uptake capacity than those samples with only one plasticizer (W40 or G40), for which very similar absorption degrees were found. The reason of this may lie on water migration that may take place along equilibration at RH 53%. In a previous work it was reported that bioplastics containing only glycerol or water were more affected by the storage under RH control than those samples including a mixture of both plasticizers. Thus, it was proved that RH promotes gaining of water from the surrounding saturated atmosphere when only glycerol was present, while water desorption is favoured when water is the only plasticiser in the formulation, significantly altering their water uptake capacity (López-Castejón et al., 2015). Under those conditions, probes with the highest glycerol contents were observed to absorb water from the surroundings during equilibration, while probes with the highest water contents seemed to lose water. If compared to samples that were not subject to RH control, equilibration would result in a decrease, for the G40 sample, and an increase, for the W40 sample, with respect to the expected absorption values measured with the standardized test. As a consequence, the referred samples presented

analogous absorption degrees, but always higher than that corresponding to G20W20, which did not experience any water content variation during its equilibration.

Analysing the influence of tragacanth gum, a higher content in the bioplastic formulation always conferred a greater water uptake capacity after 24h immersion (EW40T20 > EW50T10 > EW60). This is attributed to the well-known water-holding capacity of gums, which makes them suitable thickening agents for food products (Mirhosseini & Amid, 2012). This result suggests that tragacanth gum content could be adjusted conveniently so that materials with tailored water uptake capacities are obtained, which may be of great importance in several applications in the fields of biomedical, pharmaceutical, environmental and agricultural engineering (Buchholz & Graham, 1997; Castro, Panilaitis, & Kaplan, 2008). However, Zárate-Ramírez et al. (2014) found no differences in the water uptake capacities of wheat gluten-based bioplastics when adding different gums like locust bean gum (LBG), methyl cellulose (MC) or carboxymethyl cellulose (CMC) to their formulation. It should be pointed out that water uptake values obtained when no gum was present are similar to those found with egg white bioplastics (Jerez et al., 2007). Thus, current and reported results evidence the importance of the balanced combination of the appropriate biopolymers in the bioplastic formulation targeted at a material with improved water uptake properties.

3.2.2. Tensile properties

Figure 3 shows values obtained from tensile tests for Young's modulus, E, maximum elongation, ε_{max}, and tensile strength, σ_{max} for EW-based bioplastics with different plasticizer (G20W20, G40) and biopolymer (EW60, EW50T10, EW40T20) contents. All the stress-strain curves show a similar qualitative response (Figure 3, inset): a monotonic increase in tensile stress that becomes slower as strain increases, until it reaches a maximum stress value at break (characterized by tensile strength at break, σ_{max}, and percentage elongation at break,

ε_{max}). Those probes containing only water (W40) could not be properly tested due to a hardening process that takes place during the equilibration under controlled RH conditions, as they tend to dry partially (López-Castejón et al., 2015).

It also seems plausible to conclude, in view of the results in Figure 3, that when there is no gum in the formulation, the influence of the nature of the plasticizer fraction seems to be negligible. On the other hand, a higher glycerol content results in samples with lower Young's modulus and tensile strength values for those systems where tragacanth gum is present. Those samples with a higher glycerol content (G40) also displayed a lower steady torque and SME values along mixing (Figure 1, Table 2), which was related to the lower extent of hydrogen bonding when water was absent in the formulation. Furthermore, it is probably related to greater water absorption upon equilibration at 53% RH for the G40 sample compared to G20W20, which eventually results in a softening of the structure (López-Castejón et al., 2015). This may be connected to the well-known hygroscopic character of glycerol (Adeodato Vieira, da Silva, dos Santos, & Beppu, 2011). So, water absorbed does clearly have a different effect on the bioplastic response than that initially included in the bioplastic formulation.

A decrease in Young's modulus, E, maximum elongation, ε_{max}, and tensile strength, σ_{max}, when the tragacanth gum is present may be generally observed (EW60 > EW50T10 > EW40T20), especially for the sample with only glycerol (G40). Zhou, Zheng, Wei, Huang and Chen (Zhou, Zheng, Wei, Huang, & Chen, 2008) obtained similar results when increasing the methyl cellulose content above 5 wt.% for thermomechanically processed soy-based bioplastics. Such behaviour was explained in terms of strong aggregations between crystalline domains inside the protein matrix and, subsequently, outside of this. In addition, other authors have also related this effect with the presence of heterogeneities in the protein matrix. These heterogeneities acted as stress concentration points that eventually might induce cracking and result in lower values of strength and elongation when polysaccharides were added to the

formulation (González-Gutiérrez et al., 2011). However, Zárate-Ramírez et al. (2014) reported an increase of the same tensile parameters for gluten-based bioplastics when a gum was present. This was explained on the basis of a synergistic interaction between the protein and the polysaccharide.

Thus, an adequate selection of the bioplastic formulation and its processing conditions may yield a wide spectrum of materials with varying desired mechanical responses (Rouilly, Jorda, & Rigal, 2006).

3.2.3. Dynamic Mechanical Thermal Analysis (DMTA)

The evolution of both the storage and loss flexural moduli (E', E'') with temperature is displayed in Figure 4 for EW-based bioplastics with different plasticizer (G20W20, G40) and biopolymer contents (EW60, EW50T10, EW40T20). The sample containing water as the only plasticizer (W40) possesses a remarkable brittleness that does not assure reliable results (data not shown), being this effect more apparent as gum content increases. Table 2 includes values for E' with their corresponding standard deviations at two reference temperatures: 20ºC and 100ºC.

All samples studied show similar qualitative results when increasing the temperature, as both E' and E'' generally decrease down to a plateau region at the highest temperatures tested. Therefore, the moulding temperature selected for the compression process seems to be appropriate, as in every case the rubbery-like plateau region was reached at temperatures above 100ºC, no observing generally any increase in E' and E'' for the samples at this temperature. These results would imply that the thermosetting of the protein has already taken place along the moulding stage at 120ºC. Hence, previously other authors associated an increase in the moduli of a protein-based bioplastic sample at high temperatures to a thermosetting potential due to a relatively low moulding temperature (Zarate-Ramirez, Martinez, Romero, Partal, & Guerrero, 2011). In addition, a similar plateau region has been reported

previously for EW-based bioplastics processed at temperatures above 120ºC (González-Gutiérrez et al., 2011; Jerez et al., 2007).

When looking at the effect of the glycerol content, it may be noticed that a higher content of glycerol leads to lower values for both E' and E'' mainly at higher temperatures (e.g. 100ºC), although at relatively low temperatures (e.g. 20ºC) no relevant differences are found. This, once more, may be attributed to the double plasticizing effect exerted by glycerol: one due to its mere presence in the film, and another due to its intensively hygroscopic character, which tends to incorporate additional water into the matrix during the controlled-RH equilibration (López-Castejón et al., 2015). Coupland et al. (2000) suggested that the latter effect might be the main responsible for the plasticizing properties of glycerol. Similar results revealing the plasticising effect of glycerol were reported in previous studies (Antoniou, Liu, Majeed, Qazi, & Zhong, 2014; Lavorgna, Piscitelli, Mangiacapra, & Buonocore, 2010; López-Castejón et al., 2015).

The values for E' and E'' of probes containing 20 wt.% of tragacanth gum (EW40T20), independently of the plasticizer content, are generally lower than the corresponding to samples containing 10% of gum or no gum at all (EW50T10 or EW60, respectively), which display similar E' and E'' values. Thus, it seems that a greater presence of gum in the formulation (20%) results in a softening of the samples, possibly due to its hydrophilic character that promotes water absorption (López-Castejón et al., 2015).

3.2.4. Ageing

Ageing-related changes, either physical (migration of additives and crystallization and rearrangements of amorphous regions) or chemical (oxidation), may induce changes in the mechanical behaviour of the bioplastics (Fabra, Lopez-Rubio, & Lagaron, 2015).

In the present study, the effect of ageing of EW-based bioplastics was studied through DMTA tests. Thus, results after 1 day and 15 days of storage at constant relative humidity (RH) of 53%

were compared in the absence (EW60, Figure 5A) and in the presence of 20% of tragacanth gum (EW40T20, Figure 5B). Table 2 includes values for E' with their corresponding standard deviations at two reference temperatures: 20ºC and 100ºC.

Regardless the samples composition, the temperature-dependency of E' (in terms of qualitative evolution) remains practically unaffected by the storage time. Moreover, samples show little changes with storage time, although a slight decrease seems to takes place, most probably related to the hydrophilic character of glycerol. The presence of glycerol promote water absorption along storage, which eventually would result in an increase of the plasticiser fraction, that would turn out into the observed lowering of E' and E''. In samples containing gum (EW40T20), the softening of its structure with storage, which results in a decrease of its flexural moduli, might be additionally related to the hydrophilic character of tragacanth gum. The presence of gum would favour an enhanced absorption of water along the storage time, as found by other authors (Fabra et al., 2015).

4. Conclusions

The inclusion of tragacanth gum in the formulation of egg white based bioplastics influenced their water uptake capacities, tensile properties and linear rheology. Thus, the hydrophilic character of tragacanth gum resulted in a marked enhancement of the water uptake capacities of those bioplastics that included gum in their composition. For example, an additional 100% of absorption with respect to the plain egg white protein was always exhibited when the tragacanth gum content was of 20wt.%. Also, with this gum content (EW40T20), a softening of the structure denoted by a lowering in the values of both the main tensile properties (E, ε_{max}, and σ_{max}) and the flexural moduli (E', E'') takes place.

The nature of the plasticizer also determined the water uptake capacity, obtaining higher capacities when only one plasticizer is used, either glycerol or water, though it might be

additionally conditioned by the fact that samples were subjected to RH of 53% before testing. In any case, an enhanced capacity to absorb water was always accompanied by a decrease of both the tensile properties and the flexural moduli of egg white based bioplastics. This might be related to inclusion of water that does not interact with the bioplastic components in the same way as it does during its compounding stage and so, leads to a softer material. The above properties worsen as water/glycerol ratio decreases for those samples including tragacanth gum, as the plasticizing efficiency is generally expected to be proportional to the molecular weight and inversely proportional to the percent of hydrophilic groups of the plasticizer.

Thus, from these results, it is clear how a rightful selection of ingredients and their content is of extreme importance to obtain matrixes with appropriate functional properties for certain applications.

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6. References

Adeodato Vieira, M. G., da Silva, M. A., dos Santos, L. O., & Beppu, M. M. (2011). Natural-based plasticizers and biopolymer films: A review. *European Polymer Journal, 47*(3), 254-263.

Anderson, D. M. W., & Bridgeman, M. M. E. (1985). The composition of the proteinaceous polysaccharides exuded by astragalus microcephalus, A. Gummifer and A. Kurdicus—The sources of turkish gum tragacanth. *Phytochemistry, 24*(10), 2301-2304.

Anker, M., Berntsen, J., Hermansson, A.-M., & Stading, M. (2002). Improved water vapor barrier of whey protein films by addition of an acetylated monoglyceride. *Innovative Food Science & Emerging Technologies, 3*(1), 81-92.

Antoniou, J., Liu, F., Majeed, H., Qazi, H. J., & Zhong, F. (2014). Physicochemical and thermomechanical characterization of tara gum edible films: Effect of polyols as plasticizers. *Carbohydrate Polymers, 111*, 359-365.

ASTM (2001). Standard test method for plastics: dynamic mechanical properties: In flexure (three-point bending). Designation D5023-01. In Annual book of ASTM standards Philadelphia. PA: American Society for Testing and Materials.

ASTM (2003). Standard Test Method for Tensile Properties of Plastic. Designation D638-2003. In Annual book of ASTM standards Philadelphia. PA: American Society for Testing and Materials.

ASTM (2005). Standard Test Method for Water Absorption of Plastics. Designation: D570-98(2005). In Annual book of ASTM standards Philadelphia. PA: American Society for Testing and Materials.

Balaghi, S., Mohammadifar, M. A., Zargaraan, A., Gavlighi, H. A., & Mohammadi, M. (2011). Compositional analysis and rheological characterization of gum tragacanth exudates from six species of Iranian Astragalus. *Food Hydrocolloids, 25*(7), 1775-1784.

Buchholz, F. L., & Graham, T. (1997). *Modern Superabsorbent Polymer Technology* (L. Wiley & Sons Ed. 1st ed.). New York.

Castro, G. R., Panilaitis, B., & Kaplan, D. L. (2008). Emulsan, a tailorable biopolymer for controlled release. *Bioresource Technology, 99*(11), 4566-4571.

Chen, C., Li, W. Z., Song, Y. C., & Yang, J. (2009). Hydrogen bonding analysis of glycerol aqueous solutions: A molecular dynamics simulation study. *Journal of Molecular Liquids, 146*(1–2), 23-28.

Conte, A., Longano, D., Costa, C., Ditaranto, N., Ancona, A., Cioffi, N., Del Nobile, M. A. (2013). A novel preservation technique applied to fiordilatte cheese. *Innovative Food Science & Emerging Technologies, 19*, 158-165.

Coupland, J. N., Shaw, N. B., Monahan, F. J., Dolores O'Riordan, E., & O'Sullivan, M. (2000). Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *Journal of Food Engineering, 43*(1), 25-30.

Donovan, J. W., Mapes, C. J., Davis, J. G., & Garibaldi, J. A. (1975). A differential scanning calorimetric study of the stability of egg white to heat denaturation. *Journal of the Science of Food and Agriculture, 26*(1), 73-83.

Fabra, M. J., Lopez-Rubio, A., & Lagaron, J. M. (2015). Effect of the film-processing conditions, relative humidity and ageing on wheat gluten films coated with electrospun polyhydryalkanoate. *Food Hydrocolloids, 44*, 292-299.

Fernández-Espada, L., Bengoechea, C., Cordobés, F., & Guerrero, A. (2013). Linear viscoelasticity characterization of egg albumen/glycerol blends with applications in material moulding processes. *Food and Bioproducts Processing, 91*(4), 319-326.

Gonzalez-Gutierrez, J., Partal, P., Garcia-Morales, M., & Gallegos, C. (2010). Development of highly-transparent protein/starch-based bioplastics. *Bioresource Technology, 101*(6), 2007-2013.

González-Gutiérrez, J., Partal, P., García-Morales, M., & Gallegos, C. (2011). Effect of processing on the viscoelastic, tensile and optical properties of albumen/starch-based bioplastics. *Carbohydrate Polymers, 84*(1), 308-315.

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

Jerez, A., Partal, P., Martinez, I., Gallegos, C., & Guerrero, A. (2007). Egg white-based bioplastics developed by thermomechanical processing. *Journal of Food Engineering, 82*(4), 608-617.

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.

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

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

Lavorgna, M., Piscitelli, F., Mangiacapra, P., & Buonocore, G. G. (2010). Study of the combined effect of both clay and glycerol plasticizer on the properties of chitosan films. *Carbohydrate Polymers, 82*(2), 291-298.

Levy, G., & Schwarz, T. W. (1958). The effect of certain additives on the gel point of methylcellulose. *Journal of the American Pharmaceutical Association, 47*(1), 44-46.

López-Castejón, M. L., Bengoechea, C., García-Morales, M., & Martínez, I. (2015). Effect of plasticizer and storage conditions on thermomechanical properties of albumen/tragacanth based bioplastics. *Food and Bioproducts Processing, 95*, 264-271.

Maier, H., Anderson, M., Karl, C., Magnuson, K., Whistler, R. L., & Bemiller, J. N. (1993). CHAPTER 8 - GUAR, LOCUST BEAN, TARA, AND FENUGREEK GUMS *Industrial Gums (Third Edition)* (pp. 181-226). London: Academic Press.

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.

Mirhosseini, H., & Amid, B. T. (2012). A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Research International, 46*(1), 387-398.

Mohanty, A. K., Tummala, P., Liu, W., Misra, M., Mulukutla, P. V., & Drzal, L. T. (2005). Injection Molded Biocomposites from Soy Protein Based Bioplastic and Short Industrial Hemp Fiber. *Journal of Polymers and the Environment, 13*(3), 279-285.

Mostafavi, F. S., Kadkhodaee, R., Emadzadeh, B., & Koocheki, A. (2016). Preparation and characterization of tragacanth-locust bean gum edible blend films. *Carbohydrate Polymers, 139*, 20-27.

Nyqvist, H. (1983). Saturated salt solutions for maintaining specified relative humidities. *International Journal of Pharmaceutical Technology Product Manufacture*, 4(2), 47-48

Phillips, G. O., & Williams, P. A. (2000). *Handbook of Hydrocolloids* (C. Press Ed.).

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., & Guilbert, S. (2003). Study of wheat gluten plasticization with fatty acids. *Polymer, 44*(1), 115-122.

Ranjbar-Mohammadi, M., Prabhakaran, M. P., Bahrami, S. H., & Ramakrishna, S. (2016). Gum tragacanth/poly(l-lactic acid) nanofibrous scaffolds for application in regeneration of peripheral nerve damage. *Carbohydrate Polymers,* 140, 104-112.

Redl A, Morel MH, Bonicel J, Guilbert S and Vergnes B, Rheological properties of gluten plasticized with glycerol: dependence on temperature, glycerol content and mixing conditions. *Rheologica Acta* **38**:311-320 (1999).

Rouilly, A., Jorda, J., & Rigal, L. (2006). Thermo-mechanical processing of sugar beet pulp. II. Thermal and rheological properties of thermoplastic SBP. *Carbohydrate Polymers, 66*(1), 117-125.

Sothornvit, R., Krochta, J. M., & Han, J. H. (2005). 23 - Plasticizers in edible films and coatings *Innovations in Food Packaging* (pp. 403-433). London: Academic Press.

Tummala, P., Liu, W., Drzal, L. T., Mohanty, A. K., & Misra, M. (2006). Influence of plasticizers on thermal and mechanical properties and morphology of soy-based bioplastics. *Industrial and Engineering Chemistry Research, 45*(22), 7491-7496.

Weiping, W., & Branwell, A. (2000). Tragacanth and karaya. In G. O. a. W. Phillips, P.A. (Ed.), *Handbook of Hidrocolloids* (pp. 231-246). Cambridge: Woodhead Pub.

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.

Zarate-Ramirez, L. S., Romero, A., Bengoechea, C., Partal, P., & Guerrero, A. (2014). Thermo-mechanical and hydrophilic properties of polysaccharide/gluten-based bioplastics. *Carbohydrate polymers, 112*, 24-31.

Zheng, H., Tan, Z. a., Ran Zhan, Y., & 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.

Ščetar, M., Kurek, M., & Galić, K. (2010). Trends in Fruit and Vegetable Packaging – a Review. *Croatian Journal of Food Technology, Biotechnology and Nutrition, 5*(3-4), 17.

Figure Captions

Figure 1. Evolution of torque and temperature during the mixing process of different bioplastic formulations

Figure 2. Water uptake values for albumen-tragacanth gum bioplastics with different glycerol/water ratios (W40; G20W20 and G40) and different albumen-tragacanth gum composition (EW60, EW50T10 and EW40T20).

Figure 3. Tensile properties for different albumen-tragacanth gum compositions for bioplastics with different glycerol/water ratio. (A) G20W20; (B) G40. Inset: stress-strain (σ-ε) relationship

Figure 4. Evolution of E' and E'' with temperature, at 1 Hz, in thermo-mechanically processed bioplastics with different albumen-tragacanth compositions (EW60, EW50T10 and EW40T20), for two different glycerol/water ratios: (A) G20W20; (B) G40

Figure 5. Evolution of E' with temperature, at 1 Hz, in thermo-mechanically processed bioplastics, subject to controlled RH environment for 1 or 15 days, at two different glycerol/water ratios (G20W20 and G40), for two different compositions: (A) EW60; (B) EW40T20

Figure 1

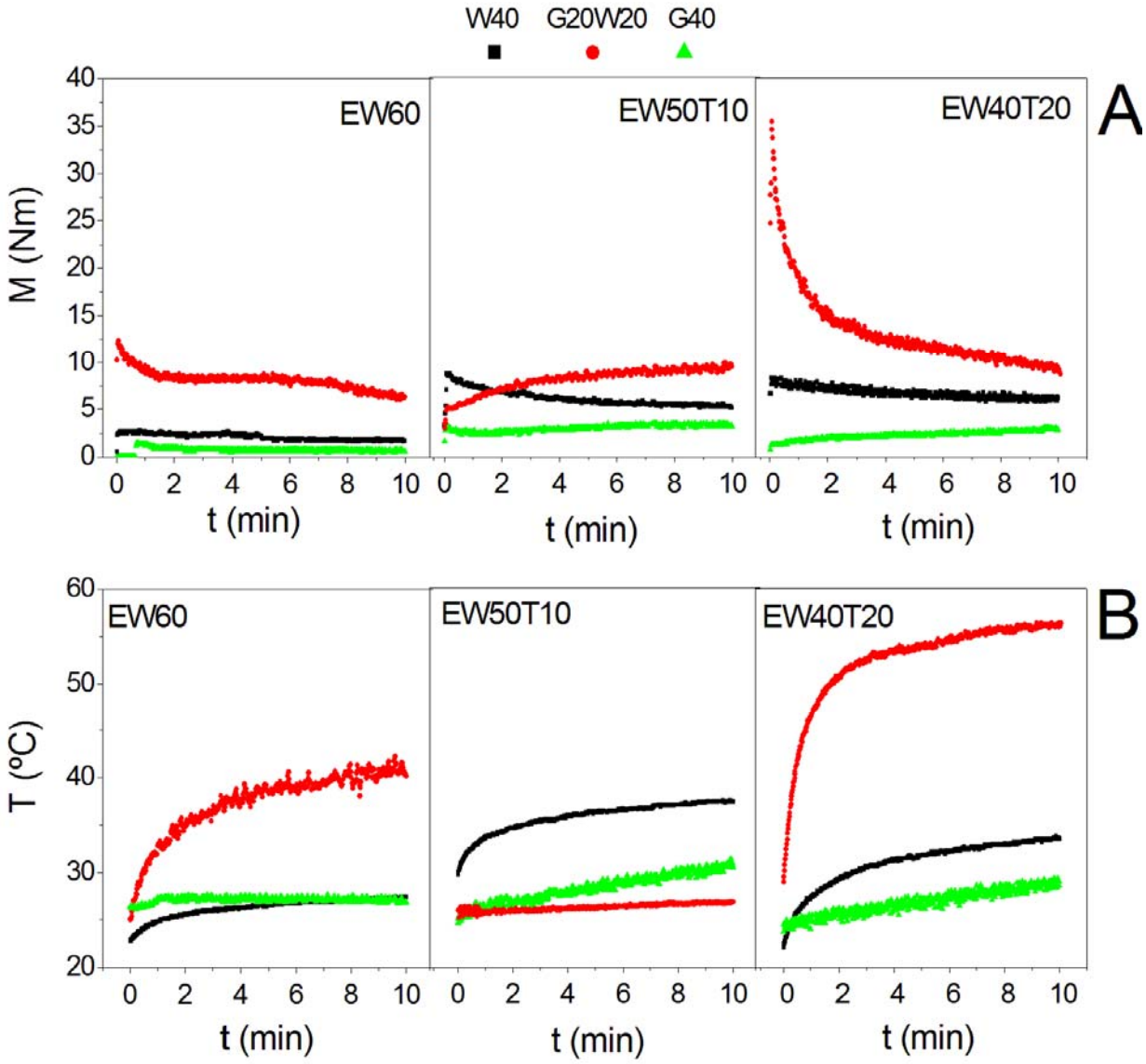

Figure 2

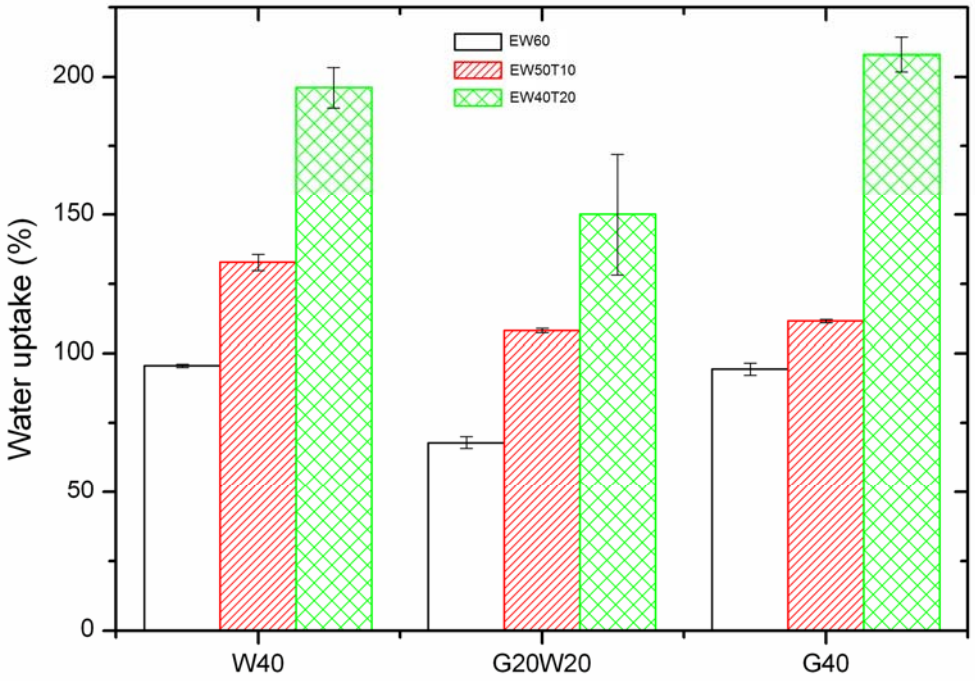

Figure 3

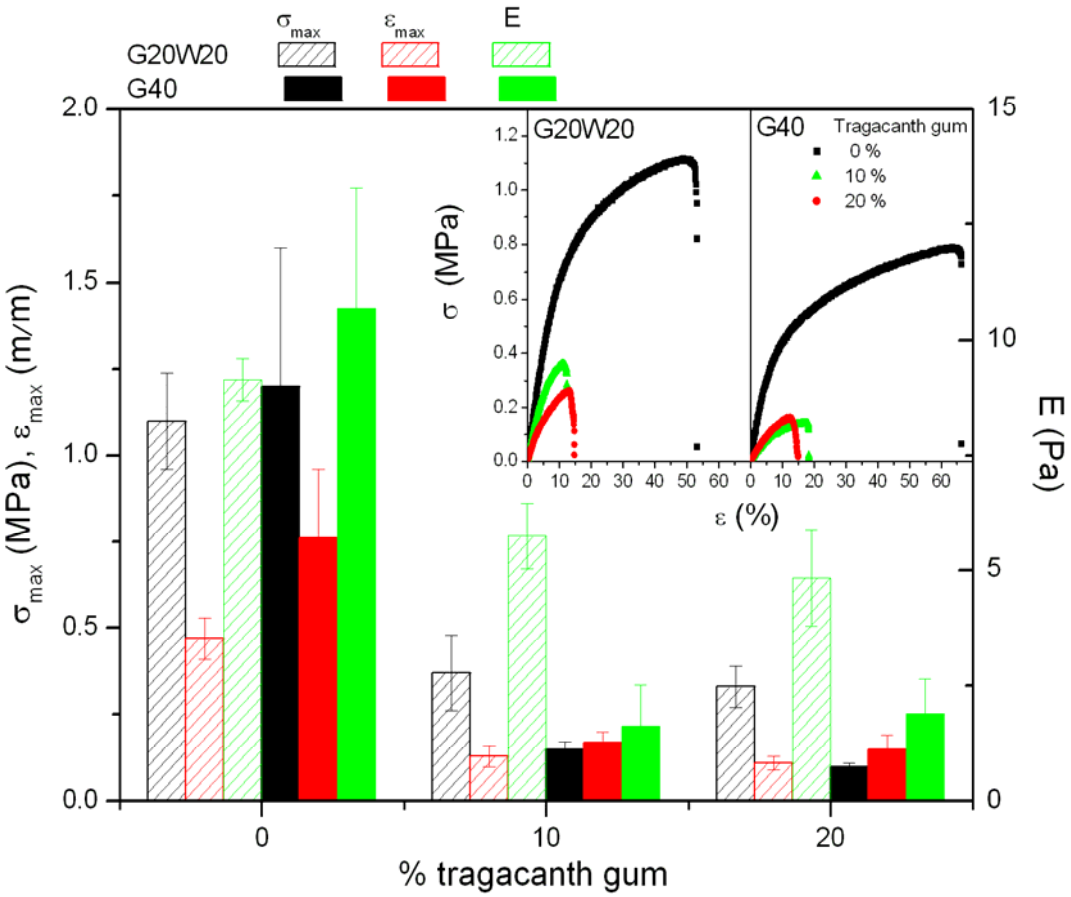

Figure 4

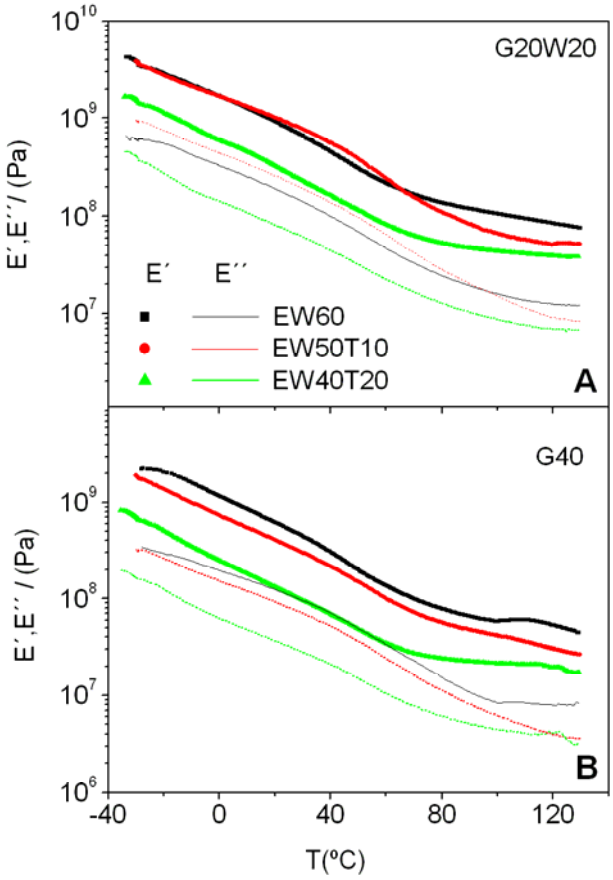

Figure 5

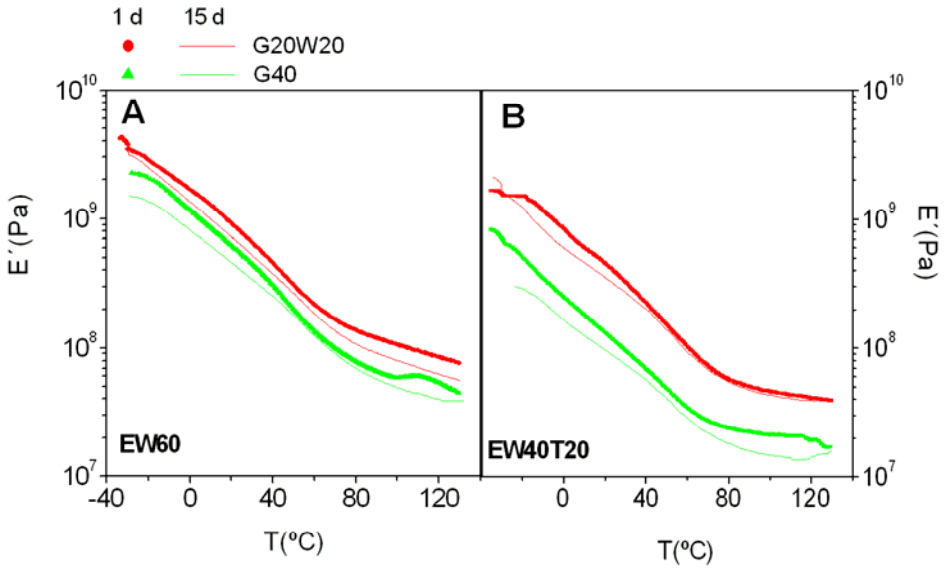

Table 1. Formulation of the protein-based bioplastics (EW: egg white albumen; T: tragacanth gum; G: glycerol; W: water)

	Ingredients (% (w/w) wet basis)				Plasticizer ratio
BIOPOLYMER/PLASTICIZER	**EW**	**T**	**G**	**W**	**G:W**
EW60/G40	60	0	40	0	1:0
EW60/G20W20	60	0	20	20	1:1
EW60/W40	60	0	0	40	0:1
EW50T10/G40	50	10	40	0	1:0
EW50T10/G20W20	50	10	20	20	1:1
EW50T10/W40	50	10	0	40	0:1
EW40T20/G40	40	20	40	0	1:0
EW40T20/G20W20	40	20	20	20	1:1
EW40T20/W40	40	20	0	40	0:1

All contents are expressed as weight percentages (wet basis)

Table 2. Specific Mechanical Energy (SME) along mixing stage for blends studied; and elastic modulus (E') at 20ºC and 100ºC for protein-based bioplastics (EW: egg white albumen; T: tragacanth gum; G: glycerol; W: water). Different letters in the same column indicate a significant difference for each blend ($p<0.05$).

			t: 1 d		**t: 15 d**	
			T:20ºC	**T:100ºC**	**T:20ºC**	**T:100ºC**
BIOPOLYMER	**PLASTICIZER**	**SME (kJ/kg)**	**E´ (Pa) ·10^{-8}**	**E´ (Pa) ·10^{-8}**	**E´ (Pa) ·10^{-8}**	**E´ (Pa) ·10^{-8}**
EW60	**W40**	69 ± 6^{b}	-	-	-	-
	G20W20	255 ± 25^{c}	10.1± 2.0^{a}	1.1 ± 0.03^{b}	6.7 ± 3.8^{a}	0.88 ± 0.12^{b}
	G40	22 ± 9^{a}	7.9 ± 2.6^{a}	0.6 ± 0.01^{a}	2.6 ± 2.7^{a}	0.31± 0.24^{a}
EW50T10	**W40**	198 ± 9^{b}	-	-	-	-
	G20W20	251 ± 44^{b}	10.0 ± 2.3^{b}	0.7 ±0.1^{b}	-	-

	G40	85 ± 13[a]	4.0 ± 1.0[a]	0.4 ±0.08[a]	-	-
EW40T20	**W40**	214 ± 31[b]	-	-	-	-
	G20W20	308 ± 38[c]	2.7 ± 0.7[b]	0.34 ± 0.14[a]	4.0 ± 0.71[b]	0.46 ± 0.049[b]
	G40	63 ± 6[a]	1.3 ± 0.07[a]	0.21± 0.014[a]	0.94 ± 0.021[a]	0.13 ± 0.014[a]