EFFECT OF BLEND MIXING AND FORMULATION ON THERMOPHYSICAL PROPERTIES OF GLUTEN-BASED PLASTICS

Estefanía Álvarez-Castillo [1] ✉, María Ramos [1], Carlos Bengoechea [1], Inmaculada Martínez [2], Alberto Romero [3]

[1] Departamento de Ingeniería Química, Universidad de Sevilla, Escuela Politécnica Superior. Calle Virgen de África 7, 41011, Sevilla, Spain

[2] 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

[3] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, 41012, Sevilla, Spain

✉ malvarez43@us.es

Departamento de Ingeniería Química, Universidad de Sevilla

Abstract

Gluten-based plastics represent an interesting biodegradable alternative to conventional plastics, due to their greater mechanical properties when compared to other protein-based materials. In this manuscript, the effect that both blend mixing and the presence of sugars exerts on the gluten-based material properties was evaluated through rheological assays, water immersion tests, and microscopy. Thus, two different mixing procedures were performed (extrusion and internal mixing) for gluten samples plasticized by water and glycerol that were eventually injection moulded. The effect of trehalose and sucrose was studied including a 20 wt.% content in the material formulation. It was observed that, even if no great differences were noticed when using different mixing processes, extruded blends, as well as the resulting bioplastics, displayed higher viscoelastic moduli than those mixed using an internal mixer. For both mixing procedures, bioplastics displayed a much more pronounced thermoplastic behaviour when compared to blends. The plasticising effect of the sugar in the gluten-based plastics was apparent, denoted by a decrease in the viscoelastic properties, together with an increase in the water uptake capacity and their porosity. Interestingly, a superabsorbent material could be obtained when including trehalose in extruded samples, showing the feasibility of using gluten plastics in this field.

Keywords: *gluten; sucrose; trehalose; plastic*

1. Introduction

There is an increasing interest in replacing common synthetic polymers with more environmentally friendly materials, which are commonly composed by organic materials, such as by-products of the food industry (Álvarez-Castillo et al., 2020a). In this sense, polysaccharides (e.g., starch and cellulose derivatives) and proteins (e.g., soy, pea, plasma, or wheat gluten) have been widely used in the manufacture of green plastics (Álvarez-Castillo et al., 2020a, 2018; Jerez et al., 2005).

Gluten is a protein that may be obtained from wheat, a cereal that started to be grown about 10,000 years ago in Southeast Asia. Wheat is one of the most important cereals along with corn and rice, with an annual production of 750.4 Mt in 2016-2017, occupying some 221.6 million hectares worldwide, according to the Foreign Agricultural Service (USDA, 2018). This production decreased 2.7% in 2018, when around 736.1 Mt were produced, being Asia the largest producer (~ 44 %), followed by European Union (~ 20 %) and America (~ 14 %) (Food and Agricultural Organization of the United Nations, 2019) . Gluten is obtained as a by-product of the starch industry, the production of which seems to be increasing due to its allergenicity. It contains at least 75% protein by dry weight, being the rest mostly starch and lipids.

Gluten is the prolamin fraction of wheat, mainly composed of gliadins and glutenins (Sothornvit and Krochta, 2005). Gliadins are monomeric proteins, soluble in aqueous alcohols, with a molecular weight ranging from 30 to 100 kDa, and responsible for the cohesiveness of gluten. Regarding their structure, four types are distinguished, such as: α, β, γ and ω-gliadins. Instead, glutenins are partially insoluble in most solvents due to their large size, with a molecular weight around 3000 kDa. This protein fraction seems to be responsible of the elastic properties of gluten (Hoseney and Rogers, 1990), that are explained by the formation non-covalent interactions, like intra and inter hydrogen bonds of the individual glutenin chains. On the other hand, gliadins would hinder interactions between glutenin chains, acting as plasticizers that decrease the elasticity of the dough. Therefore, the ratio of monomeric gliadin to polymeric glutenin would determine the rheological behaviour of the gluten dough and therefore affects the performance of gluten protein (Hernandez-Izquierdo and Krochta, 2008).

Gluten is widely applied in the food industry, but it should also be highlighted its applications in the field of plastic materials (Gómez-Martínez et al., 2011; Jerez et al., 2005; Jiménez-Rosado et al., 2019; Ullsten et al., 2009; Zárate-Ramírez et al., 2011). For the latter purpose, the use of a plasticizer, such as glycerol or water, is frequently needed to obtain a cohesive and homogeneous blend that would give rise to plastic materials through common processing techniques. Additionally, some studies have reported the use of monosaccharides, disaccharides or oligosaccharides, polyols or even lipids as plasticizers for the manufacture of protein-based plastics. Sugars have also been reported to generally protect proteins from denaturation, being trehalose exceptionally superior to most of them (Kaushik and Bhat, 2003). Sucrose and trehalose are non-reducing disaccharides formed by the union of two monosaccharides by means of an *O*-glycosidic bond, not possessing any free anomeric carbon. Sucrose is obtained from sugar cane or sugar beet, while trehalose is mainly obtained from mushrooms, possessing a much lower sweetening power.

Injection moulding is one of the most widely used techniques to obtain protein-based plastic (Álvarez-Castillo et al., 2018). However, gluten based plastic materials have been mostly processed by other techniques (e.g. compression, extrusion, casting), not existing many studies focused on injection-moulded materials (Cho et al., 2011). Considering that a homogeneous blend of the ingredients is a prerequisite for an optimal injection process, blends used to feed the injection moulding unit were obtained after mixing the raw materials in a prior stage. For this purpose, two different mixing procedures were compared in the present study: an internal mixing rheometer system where both temperature and torque can be monitored throughout the mixing stage (Gómez-Heincke et al., 2016; Zárate-Ramírez et al., 2014a); and a lab-scale twin extruder, which allowed setting a temperature profile from the hopper to the exit die (Jiménez-Rosado et al., 2019). Both techniques apply high shear stresses to the blend due to the action of the corresponding dispersing element (i.e. counter-rotating shafts and endless screw, respectively).

As the properties of protein-based plastics depend largely on both the processing conditions and the formulation (Álvarez-Castillo et al., 2020a, 2019, 2018), the effect of both the mixing procedure (i.e., internal mixing, extrusion) and the presence of sucrose or trehalose (20 wt. %) was considered for gluten-based bioplastics. Therefore, the goal of the present study has been to assess the consequences of different mixing stages, as well as of the presence of sugars, on the rheological properties, water absorption response and microstructure of gluten-based plastic materials.

2. Materials and Methods

2.1 Materials and processing methods

2.1.1. Materials

Wheat Gluten (WG), provided by Productos Riba S.A. (Spain), is a protein concentrate composed of 83 % protein, 3 % lipids, 1 % ash, 8 % humidity, and small amounts of carbohydrates. Sucrose was supplied by Sigma Aldrich (USA) and trehalose anhydrous by Guangxi Biochemical Science and Technology (China). Deionized water (W) and glycerol (Gly) supplied by PANREAC.S.A. (Spain) were used as plasticizers.

2.1.2. Processing methods

Blends

In order to obtain homogeneous blends which could be correctly injection moulded, two different blending strategies were studied.

In the first one, a premix was made initially through stirring by hand every fraction (solid and fluid). Then, solid (gluten, disaccharides) and fluid (glycerol, water) ingredients were introduced separately in a two-blade counter-rotating batch mixer Haake Polylab QC (ThermoHaake, Karlsruhe, Germany) at room temperature (around 25 ºC) under adiabatic conditions. Different rates were tested (50, 100 and 200 rpm) for a certain mixing time (5 and 10 min).

In the second method, two feeding units for a twin-screw extruder EuroLab 16 (ThermoScientific, USA) were used: a peristaltic pump for the fluid fraction; and a powdery solid dispenser, for the addition of wheat gluten. As disaccharides were added in an aqueous solution onto the extruder, the amount of disaccharide added was limited by the maximum solubility of trehalose in water at 20 ºC (68.9 / 100 g), as the water solubility of sucrose is exceedingly higher (200 / 100 g). The procedure was as follows: 1) A 60 wt. % disaccharide solution was prepared at 20 ºC; 2) then, it was mixed with the proper volume of glycerol; and 3) finally, the plasticizer fraction was pumped onto the barrel using a peristaltic pump when the extrusion process started. An adequate selection of the pump rates allowed to achieve the specified formulation (Table 1). The extruder internal diameter was 19 mm with a length ratio/diameter (L/D) of 40. Temperature was controlled along the extruder cylinder through ten electrical resistances (50, 55, 50, 50, 50, 50, 50, 50, 50, 60, 60 ºC), being the temperature profile defined based on previous work in order to avoid excessively high temperatures that could promote protein degradation (Fang et al., 2014).

The extruder torque per shaft was controlled in order to always remain under the maximum allowed value (12 N·m). The chosen outlet head was circular, obtaining extruded polymeric filaments that were later cut into pellets suitable for feeding the injection unit.

For both procedures, the protein / (water + glycerol) ratio was always kept at 2/1, containing the same amount of water and glycerol. This ratio was kept constant when sugar was added. Table 1 displays the details of each material composition.

Table 1. Composition of the systems studied

System	Sucrose (%)	Trehalose (%)	Wheat Gluten (%)	Glycerol / Water (%)
Reference	-	-	67.00	16.50/16.50
20% sucrose	20.00	-	53.30	13.35/13.35
20% trehalose	-	20.00	53.30	13.35/13.35

Plastic

Blends, obtained in the previous mixing stage either as doughs or extrudates, were further processed into rectangular plastic probes (1x10x60 mm) through injection moulding using a Minijet II Piston Injection Moulding System (ThermoHaake, Karlsruhe, Germany). Blends were first introduced in a cylinder at 80 ºC, applying a pressure of 500 bar for 20 s in order to inject the samples into a mould at 100 ºC, where the samples remained for 180 s under the same pressure.

2.2. Characterization methods

2.2.1. Rheological properties

Small amplitude oscillatory measurements were carried out within the linear viscoelastic range in compressional and torsional mode to characterize the blends and plastics, respectively. For blends, temperature ramp tests were performed from 25 ºC to 140 ºC in a RSA3 rheometer (TA Instruments, USA), using an 8 mm cylindrical geometry at a heating rate of 5 ºC/min and constant frequency (1 Hz). For plastics, temperature ramp tests were carried out from -30 ºC to 140 ºC in a DHR-3 rheometer (TA Instruments, USA) employing a rectangular geometry in torsion mode, at a heating rate of 5 ºC/min and 1 Hz.

2.2.2. Water uptake and soluble matter loss

Water uptake capacity (WUC) for gluten-based plastics was determined as in previous studies (Álvarez-Castillo et al., 2018): initially, samples were submitted to a dehydrothermal treatment in an oven at 50 ºC for 24 h (w_1); then, they were submitted to an immersion stage in deionised water during 24, 48 and 72 h (w_2); and, finally, swollen samples were again dried at 50 ºC for 24 h (w_3). WUC and soluble matter loss (SML) values were obtained as indicated in equation 1 and equation 2:

$$WUC\ (\%) = \frac{w_2 - w_3}{w_3} \cdot 100 \qquad (Eq. 1)$$

$$SML\ (\%) = \frac{w_1 - w_3}{w_1} \cdot 100 \qquad (Eq. 2)$$

2.2.3. Scanning Electron Microscopy

Some swollen samples were transformed onto dried matrices through freeze-drying (-80ºC and 0.01 mbar). Thus, small pieces of them (~ 2.5 mm) were gold coated and examined by Scanning Electron Microscopy (SEM), in a ZEISS EVO (USA). The analysis was carried out in the microscope with a working distance of 6 mm, a beam current of 11-12 pA, and 10 kV as acceleration voltage. The magnification used was 200x. The obtained micrographs were analysed with the public domain software ImageJ, in order to estimate porosity values, considering 10 measurements at least.

2.2.3. Statistical analysis

Data is presented as mean ± standard deviation of three technical repeats using Statsgraphics software, considering significant probability value of $p < 0.05$.

3. Results and Discussion

3.1. Effect of the mixing stage

When observing how temperatures evolved along the extruder for the different formulations studied, it could be observed that temperatures initially matched the set value, but as mixtures went forward onto the extruder die, a slight deviation towards higher temperatures was detected. This could be attributed to the dissipation of thermal energy due to the existing friction forces in the interior of the extruder. Therefore, in the initial section, no deviation was observed, but in the central section (50 ºC as set value), a greater deviation was observed in the reference system (59.8 ºC ± 2.9 ºC) than in those containing 20 % of sucrose or trehalose (54.7 ºC ± 1.6 ºC, or 56.8 ºC ± 3.6 ºC, respectively). Lower relative deviations were found at the exit section (60 ºC) either for the reference or for the samples containing 20 % of sucrose or trehalose (63.5 ºC ± 0.7 ºC, 60.5 ºC ± 3.6 ºC or 60.7 ºC ± 3.5 ºC, respectively). The slightly greater increase of temperature taking place in the absence of sugar would support the idea of those disaccharides acting as plasticizers, as they may diminish shear stresses applied to the sample in the extruder due to the relaxation of the interactions between the polymeric chains exerted by the plasticizers (Ghanbarzadeh et al., 2006).

On the other hand, the evolution of torque and temperature with mixing time recorded in the mixer rheometer for the reference sample systems was illustrated in Figures 1A and 1B for different mixing rates (25, 100, 200 rpm) and in Figures 1C and 1D for the different formulations studied (0 % sugar, 20 % sucrose or trehalose) at 100 rpm. When observing the torque evolution, similar profiles were observed for the reference sample at all mixing rates: at the start of mixing, the torque suffered a sudden increase up to a maximum value (M_{max}), after which it dropped continuously until a steady value was achieved. For the reference blend, M_{max} values were lower at higher mixing rates, being located also at shorter times. Thus, when the reference blend was obtained at 25 rpm, M_{max} was achieved after 3 min, being around 1.5 times higher than the maximum value obtained at 200 rpm after 0.5 min, which could indicate than a more intimate mixing was previously achieved at higher rates. Mixing conditions have already been reported to influence largely the properties of blends, finding also similar events when different mixing rates were used to homogenize WG/Gly blends (Jerez et al., 2005; Redl et al., 1999).

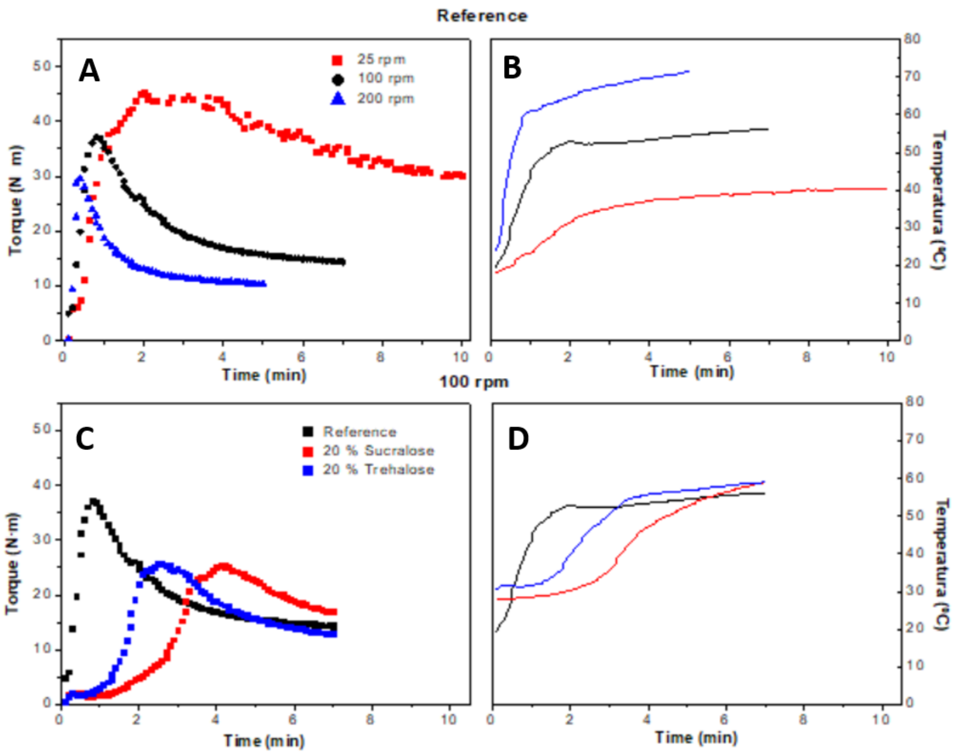

Figure 1. Evolution of torque (A, C) and temperature (B, D) during the mixing process for blends at 25, 100 and 200 rpm (A, B); and for blends containing 20 % disaccharide (sucrose, trehalose) at 100 rpm (C, D)

Regarding the temperature, an upward evolution was observed during the whole mixing process, reaching higher temperatures at the torque peak (T_{peak}) when mixing rates were higher. Moreover, this temperature profile also reflected the two regions commented for torque, as displayed by the modification of the dT/dt slope after M_{max} was achieved. T_{peak} achieved a value of 40 ºC when the reference was mixed at 25 rpm, while it raised up to 60 ºC at 200 rpm. This temperature increase may be caused by a higher heat dissipation due to the existence of numerous friction forces, which are higher when the mixing rate increased, as have been observed in previous studies for WG systems (Jerez et al., 2005; Andréas Redl et al., 1999; Zárate-Ramírez et al., 2011).

When sucrose or trehalose was included at 20 wt. % (Figure 1C), M_{max} was displaced onto longer mixing times, as a lag phase was detected, where no significant increase in the torque occurred. Moreover, the presence of disaccharide resulted in a significant drop of the M_{max} values, which may be attributed to the already commented plasticizing effect of sugars, just like observed in the temperature profile along the extruder. The torque profile was seemingly independent of the nature of the disaccharide, as they reached similar values. On the other hand, the temperature evolution along mixing (Figure 1D) revealed the previously commented correspondence with the mixing torque, observing, in this case, retardation in the temperature increase when the sugar is included in the formulation. Anyway, all samples, independently of the formulation, reached similar T_{max} values (~ 60 ºC) at the same mixing rate.

3.2. Viscoelastic properties

Blends

The viscoelastic properties of the reference and disaccharide-containing blends obtained either after extrusion (Figure 2A) or thermoplastic mixing at different rates (Figure 2B, figure 2C and figure 2D) were assessed carrying out temperature sweep tests in compression mode at a temperature range from 20 to 120 ºC within the LVR. Temperature ramp tests were performed in order to obtain valuable information that could help to determine the optimal injection conditions that would facilitate the flow of the blend from the cylinder into the mould. This fact would permit a complete and homogeneous filling of the mould cavity, which would finally result in a higher reproducibility in the production of probes (Álvarez-Castillo et al., 2020a; Felix et al., 2015). Furthermore, from an industrial perspective, a proper study of rheology would imply lower energy consumption during the injection process. In Figure 2, similar qualitative behaviours were presented by all samples, independently of the mixing device or mixing rate employed, being the elastic modulus always higher than the viscous modulus. Thus, a predominantly elastic response was detected for all blends, typical of solid materials for which both modules show a low dependence with frequency (Álvarez-Castillo et al., 2018; Jerez et al., 2005) (data not shown). When observing the effect of the mixing device, extruded blends showed slightly higher values for both elastic and viscous moduli (E' and E'', respectively) at room temperature. As identical raw materials were fed onto both mixing devices, the greater viscoelasticity displayed by extruded blends could be related to the efficiency of the mixing process. In a continuous twin-screw extruder, like the one used in the present study, high shear zones are produced at the clearances either between the screws and the barrel or between the two screws inside the barrel, at which the flow is ruled by simple shear flow (Emin and Schuchmann, 2013). On the other hand, the discontinuous mixing device used was a type of internal mixer, consisting of two rotors enclosed in a mixing chamber, where also high shear forces are generated, dispersing the added ingredients (Drobny, 2014). In both mixing devices, dispersive and distributive mechanisms must have taken place. The evaluation of the properties of the blend is helpful to determine the performance of the mixing process that led to its formation (Kajiwara and Nakayama, 2011). Thus, under the processing conditions employed, it seems that extrusion resulted in a higher mixing efficiency than the internal mixer. However, it should be highlighted that the mixing mechanism should not be inferred from the results obtained, requiring an special assessment of the existing flow patterns within the mixer, which is a complex issue, beyond the scope of the present manuscript.

The addition of a disaccharide, either sucrose or trehalose, in the formulation of these gluten-based blends prompted a reduction in both viscoelastic moduli during the whole temperature range studied for both mixing devices. This could be related to the plasticizing effect that the saccharides seem to possess that relaxes the interactions of the protein chain, allowing greater mobility and thus lowering the viscoelastic values (Ghanbarzadeh et al., 2006). However, it should be noticed that the addition of sugar implied a decrease in the gluten, glycerol and water content in the formulation, which may also help to explain the results. However, the gluten/(glycerol+water) ratio was kept constant in every case (2/1), which may highlight the commented role of plasticizer exerted by the disaccharide. When observing the effect of temperature, a moderate decrease in E’ occurred initially, generally reaching a rubbery plateau at higher temperatures. On the other hand, E’’ displayed a continuous decrease during the whole thermal treatment. Although, as commented, greater viscoelastic moduli could be found at lower temperatures for the extruded blend, finally, all blends displayed similar moduli at higher temperatures, independently of the mixing procedure and speed rate. Those quite alike results when varying rotation speed in the internal mixer was also observed in similar WG/Gly systems by Redl et al. (Redl et al., 1999). It is interesting how no significant effect of the sugar (sucrose or trehalose) was observed on the viscoelastic properties, although slightly superior viscoelastic moduli values could be determined for sucrose-containing blends.

Loss tangent (tan δ) decreased during the whole temperature test (30 - 120 ºC), displaying a steeper decay at the higher temperature region. This event can be explained by the milder decrease presented by E’ (about an order of magnitude) when compared to E" (about two orders of magnitude), enhancing the solid-like behaviour of the samples as they were heated. As no distinctive maximum can be easily distinguished in the obtained results, no clear information regarding the glass transition temperature (T_g) of these plastic materials could be withdrawn from the tan δ profiles. When comparing extruded samples to the rest, general lower tan δ values could be distinguished, being related to the slightly greater viscoelastic properties already observed. Regarding the effect of the presence of disaccharides on this profile for samples obtained through internal mixing, the presence of sugars in samples generally resulted in higher tan δ values compared to the reference sample at all mixing rates studied. This would be an agreement with the plasticizer role already commented that sugars may possess (Ghanbarzadeh et al., 2006), which would promote the viscous character of the blend. However, even if the plasticizer effect of the sugars could be implied by the observed lowering of the viscoelastic moduli, no great differences in tan δ among the formulations were perceived in extruded samples, being virtually independently of the nature of the disaccharide.

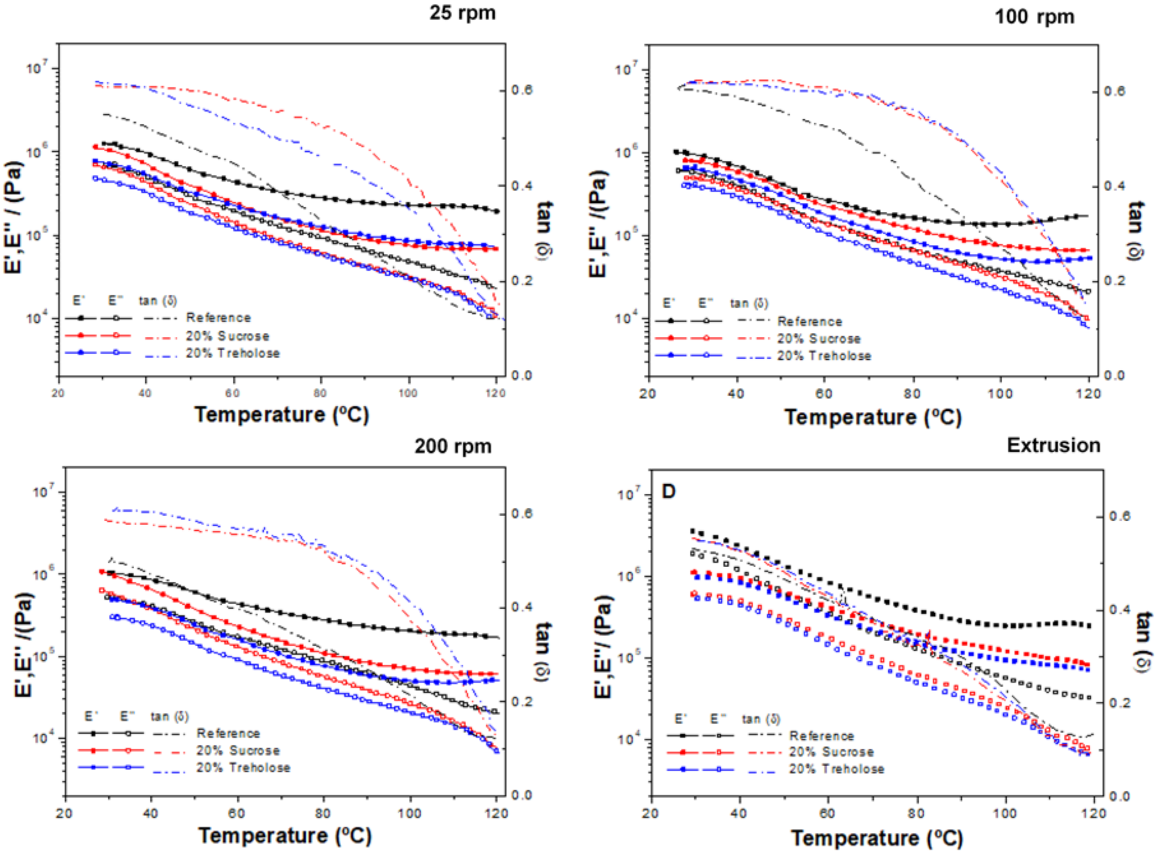

Figure 2. Values from dynamic mechanical analysis (DMA) temperature ramp measurements performed at constant frequency (1Hz) and heating rate (5 ºC min^{-1}) for the systems: A) 25 rpm for 10 min., (B) 100 rpm for 10 min., (C) 200 rpm for 5 min. and y (D) extrusion.

Plastics

The viscoelastic properties of the injection moulded gluten-based plastics (reference and sucrose o trehalose containing samples) were evaluated through temperature sweep tests in torsional mode from -30 to 120 ºC within the LVR. As happened with blends, a continuous decrease was observed for all samples, not finding any plateau at higher temperatures this time, as may be observed in Figure 3A for plastics from extruded blends. This denoted the greater thermoplasticity of plastic samples when compared to the original blends. Lower tan δ values were again detected for the disaccharide free sample being related to the plasticizing effect of the sugars (Ghanbarzadeh et al., 2006). In this case, a tendency onto tan δ maximum around 120 ºC was suggested, with a smaller shoulder al lower temperatures.

When observing how the elastic moduli, G’, is affected by the presence of a disaccharide in the formulation of gluten-based plastics (Figure 3B), an apparent decrease may be distinguished by either sucrose or trehalose, corroborating the already mentioned plasticizer effect of the disaccharide. Although no significant differences may be found between sucrose or trehalose, it seems as lower G’ values were obtained for trehalose systems. Although no significant effect of the mixing rate could be implied from these results, a slight increase in G’ may be appreciated for extruded samples, which could be explained by a higher structuration degree when applying a greater amount of mixing energy.

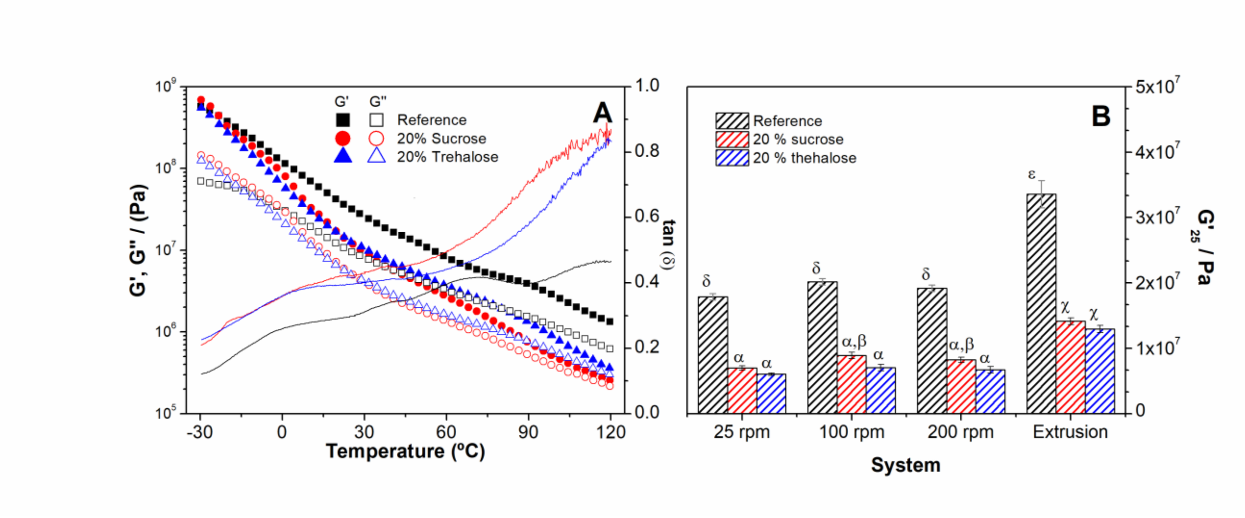

Figure 3. Linear viscoelastic properties in torsion mode for gluten based injection moulded plastics from extruded blends (A); and storage modulus (*G*') at 1 Hz and 25 ºC (G'_{25}) for gluten based injection moulded plastics obtained from internally mixed blends at 25, 100 and 200 rpm, and from extruded blends (B). Standard deviations are presented as error bars (3 technical repeats).

3.3. Water uptake and effect of the immersion time

The water content of all samples was 13.4 ± 1.3 %, which approximately matches the water content stated in Table 1. Therefore, no major loss of water has taken place during the sample processing, as it is now stated in the revised manuscript.

The water uptake capacity (WUC) and soluble mater loss (SML) values for plastic materials obtained from blends processed though different mixing devices (internal mixing at 25, 100 and 200 rpm (Figure 4A, 4B and 4C) or extrusion (Figure 4D)) are shown in Figure 4 and Table 2 for different immersion times (24, 48 and 72 h).

A slight trend towards higher WUC values can be observed when one of the two disaccharides was introduced in the composition of the plastics, independently of the immersion time. This increase may be related to two different processes happening within the matrix: first, the solubilization of protein onto the aqueous media which would promote an increase in the porosity in the structure, facilitating the penetration of water (Álvarez-Castillo et al., 2018); and secondly, the plasticizer effect exerted by the saccharides may diminish the interactions between polymeric chains, finally reducing the crosslinking during the processing and promoting the swelling of the matrix. Previous studies have highlighted that moulding gluten samples at temperatures of 130 ºC favoured crosslinking dominated by disulphide bonds (Jansens et al., 2013). Thus, the crosslinking degree is a parameter that has been closely related to the water uptake capacity of matrices, existing an equilibrium between the strength of the protein matrix and the swelling resulting from water molecules penetrating into it (Buonocore et al., 2003). Consequently, a greater crosslinking hampers the formation of a porous structure, inhibiting the swelling and water absorption of samples (Álvarez-Castillo et al., 2018). This might be the reason why most of the samples studied in the present manuscript showed low water uptake capacities. However, it is worth pointing out that the WUC was noticeably high when the added disaccharide was trehalose. Thus, samples with 20 wt. % of trehalose could even duplicate the WUC value of the reference plastic sample (e.g. 72 h as immersion time for extruded samples, or for samples internal mixed at 100 or 200 rpm). The fact that no effect of trehalose addition on WUC was observed at lower rates (i.e., 25 rpm) when using the internal mixer could be related to a poor mixing. On the other hand, the enhancement of the water absorption by the presence of trehalose at the rest of mixing conditions may be related to the protecting properties reported to trehalose, which has also been found to be very effective in the stabilization of proteins during exposure to high temperatures (Kaushik and Bhat, 2003), which might hinder crosslinking and eventually be the reason behind the higher WUC values found.

When observing the effect of immersion time, WUC values always displayed a remarkable increase with time when samples contained a disaccharide, while the WUC values remained unaffected with the immersion time for those samples taken as reference, keeping a nearly constant value around 115 % in any case. Furthermore, the mixing rate used in internal mixing did directly influence the absorption capacity of plastics, as higher WUC values were obtained when 200 rpm was employed. Thus, for those samples obtained from blends mixed at different rates, an increase of WUC for longer immersion times was generally found, being slighter for lower rates (25 rpm,) and more apparent for samples mixed at higher rates (100, 200 rpm). Thus, in general terms, it seems that a lower specific mechanical energy applied through blend mixing promoted a greater WUC, especially in the presence of disaccharide. On the other hand, the greatest WUC was achieved for the gluten-based sample obtained by extrusion, which could absorb more than 10 times its original dry weight after 72 h of immersion when trehalose was included. For this system, a different absorption kinetic was detected depending on the formulation: the sugarless reference system kept virtually the same WUC value for all immersion times studied, which would imply that equilibrium was reached before than for the sugar-containing samples; when sucrose was used, the equilibrium needed longer times to be achieved, as WUC first increased up to 350 % after 24 h of immersion, eventually decreasing to 200 % for longer times; on the other hand, trehalose-containing systems displayed upwards evolution of WUC with immersion time, eventually reaching a WUC around 1200 %, which surpasses the threshold defined for superabsorbent materials (Figure 4D). Other proteins, such as soy or porcine plasma protein, have been used in the development of superabsorbent materials through different strategies (Álvarez-Castillo et al., 2020b, 2020a, 2018), mostly at relatively low moulding temperatures (lower than 100 ºC). Most of commercial superabsorbent materials are not renewable, nor sustainable, nor biodegradable, as they are acrylic based. This causes a negative environmental impact which may be avoided if natural biodegradable components are used (Álvarez-Castillo et al., 2018). Furthermore, gluten is a by-product of the starch industry, which would enhance the sustainability of the process. those protein sources are rich in hydrophilic polar residues, such as aspartic or glutamic acid, which are not predominant in gluten (Stauffer, 2007). Moreover, gluten-based plastics must be processed at higher temperatures, not observing superabsorbent character for gluten plastics as crosslinking is promoted. Notwithstanding, the presence of trehalose enhanced water absorption in the conditions mentioned.

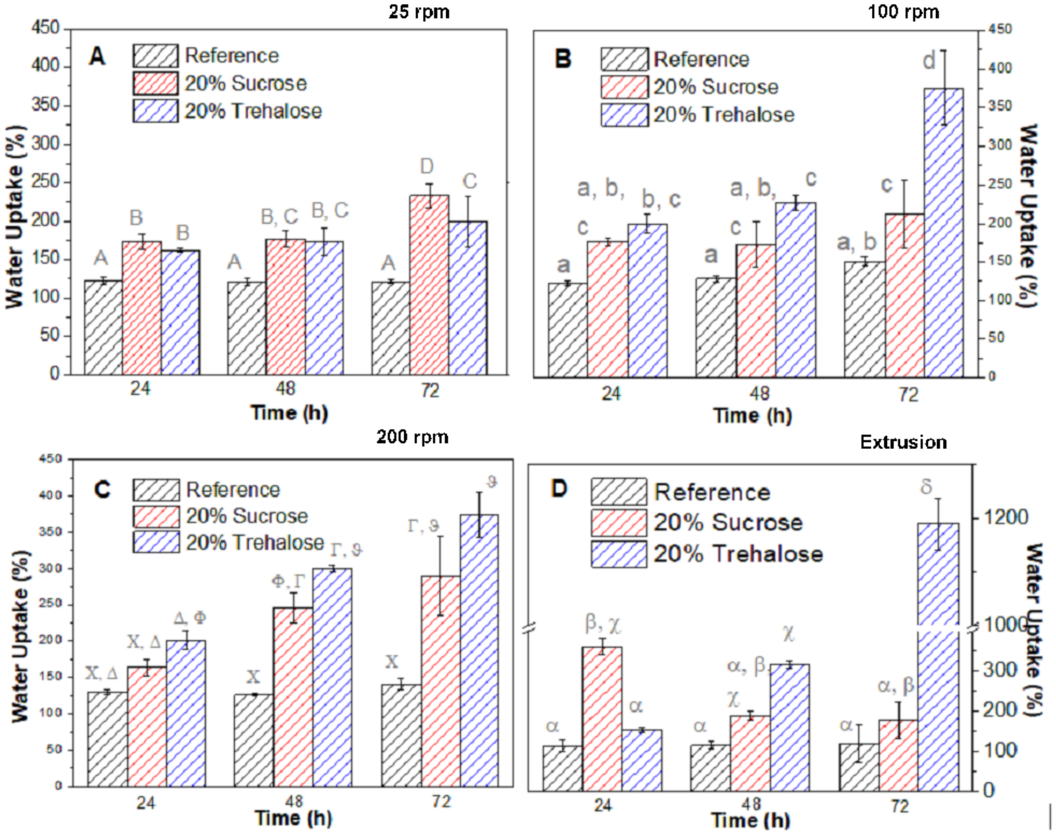

Figure 4. Evolution of water absorption capacity (%) after immersion for 24, 48 and 72 h for the systems: (A) 25 rpm for10 min., (B) 100 rpm for 10 min., (C) 200 rpm for 5 min. and (D) extrusion. Standard deviations are presented as error bars (3 technical repeats).

The values of Soluble matter loss (SML) of the plastic materials (reference, 20 % sucrose and 20 % trehalose) obtained through different mixing devices and subjected to different immersion times (24, 48 and 72 hours) are presented in Table 2. In most cases, SML was not greater than the amount of plasticizer originally contained in the formulation, neither considering only glycerol and water (33 %), nor when also including the sugars (46.7 %). Only when immersion time was 72 h for samples containing sugars, a small portion of the protein fraction would be solubilized. This fact would point out that the gluten structure formed after immersion moulding was basically insoluble, obtaining a gluten matrix, devoid of plasticizers (Zárate-Ramírez et al., 2014b) after immersion. In the case of the reference sample, the SML was kept at approximately 22-29 %, while for the systems that contained sucrose or trehalose, a small amount of protein was lost onto the immersion media, being greater when immersion time was longer. This increase was associated to the fact that the immersion media, in addition to extracting glycerol, facilitated the sugar loss.

		Soluble matter loss (%)			
Systems	**Immersion Time (h)**	**25 rpm**	**100 rpm**	**200 rpm**	**Extrusion**
Reference	24	22.7 ± 2.6	21.4 ± 1.6	20.7 ± 0.2	21.1 ± 0.4
20% Sucrose		41.7 ± 8.5	22.3 ± 0.9	43.7 ± 0.2	42.8 ± 1.2
20% Trehalose		36.8 ± 4.8	22.0 ± 0.9	40.6 ± 0.5	32.5 ± 0.1
Reference	48	20.0 ± 0.3	44.9 ± 2.7	20.5 ± 0.2	23.4 ± 0.3
20% Sucrose		42.7 ± 8.0	45.6 ± 1.6	46.9 ± 1.1	40.7 ± 0.6
20% Trehalose		44.9 ± 2.0	49.4 ± 2.0	43.9 ± 2.8	40.9 ± 5.9

Reference	72	22.2 ± 0.5	40.5 ± 1.3	23.6 ± 4.2	24.9 ± 0.3
20% Sucrose		44.3 ± 4.9	42.5 ± 2.5	49.8 ± 2.3	33.9 ± 5.5
20% Trehalose		48.3 ± 5.7	45.8 ± 1.5	47.9 ± 3.1	52.9 ± 0.6

Table 2. Soluble matter loss (%) after immersion for 24, 48 and 72 h for the systems: 25 rpm for 10 min., 100 rpm for 10 min., 200 rpm for 5 min. and extrusion. Standard deviations are presented preceded by ± (3 technical repeats).

3.4. Scanning Electron Microscopy

This methodology can provide some information about the morphology and microstructure of the plasticizer-free matrices resulting from the lyophilisation of the swollen gluten-based plastic after immersion. Thus, in Figure 5 the SEM images of the matrices obtained after 72 h immersion of gluten-based reference sample (A), and in the presence of a disaccharide in their composition (20 wt. % sucrose or trehalose (B and C, respectively)) are shown. It might be observed how both the presence of either sucrose or trehalose in the matrix enhanced the formation of a more porous structure which could have been promoted by the solubilization of sugars in the immersion media. Hence, a porous structure could help with the penetration and holding of water, which is coherent with the higher WUC values resulted when sugars were included in the formulation. A greater number of pores could be observed in the matrix obtained for the plastic containing 20 wt. % of trehalose, with an estimated superficial porosity of approximately 22.5 %, compared to porosities of around 3.9 % and 9.4 % presented by reference and containing-sucrose samples, respectively. The much greater porosity estimated for the trehalose system would result from the superabsorbent character that this sample displayed, as it has been found in previous studies for analogous protein superabsorbent materials (Álvarez-Castillo et al., 2018).

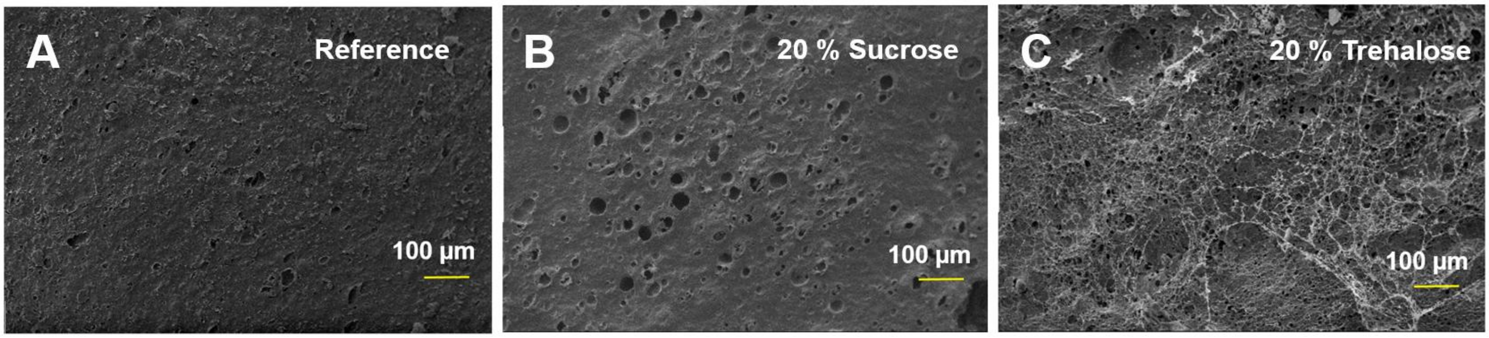

Figure 5. SEM images (x250) acquired after 72 h of immersion for plastic blended at 200 rpm during 5 minutes: (A) reference, (B) 20% of sucrose and (C) 20% of trehalose.

4. Conclusions

The presence of sugars in the formulation of gluten-based plastics did influence the temperature profile during the mixing stage prior injection moulding. Thus, a greater shear-induced heating of the sample during processing was generally observed when no sugar was added in the formulation of gluten-based plastics processed through extrusion. On the other hand, a lag phase was commonly detected initially in both torque and temperature when using an internal mixer, before an increase in both parameters took place. The presence of a disaccharide did not significant differ at a certain rotation speed, as all samples reached nearly the same steady values. Lower torque and higher temperatures were observed with increased rotation speed, which could be presumably due to the existence of higher friction forces inside the mixer.

When temperature sweep tests were performed on blends, all samples displayed a similar evolution for the viscoelastic properties, independently of the presence of disaccharide. Thus, a continuous decrease of both viscoelastic moduli (E’ and E’’) along with heating was perceived. Lower viscoelastic moduli values were obtained for disaccharide-containing blends, especially when trehalose was included. Furthermore, higher tan δ values were observed in the presence of a disaccharide, explained based on sugars plasticizing effect.

Gluten-based blends were furtherly processed through injection moulding, displaying quite similar performances for the viscoelastic moduli, as they continuously decreased as higher temperatures were reached. Additionally, the plasticizing effect of the disaccharide resulted again in a reduction of both viscoelastic moduli and a remarkable rise of tan δ. Water uptake capacity of injection moulded plastics followed three distinct trends: i) the presence of a disaccharide promoted the absorption capacity of gluten-based plastics, specially the trehalose; ii) longer immersion times resulted in higher values of water uptake ; iii) the mixing procedure affected the water absorption of injection moulded plastics, as blends obtained through extrusion gave rise to plastics with higher water uptakes capacities, even leading to the formation of a superabsorbent material for the sample with 20 % of trehalose after 72 h of immersion. It should be considered that a small percentage of protein was solubilized during long immersions (e.g. 72 h). SEM micrographs showed a more porous and open structure when disaccharides were included in the formulation, which promoted the formation of voids within the matrix structure, finally enhancing water holding.

Thus, the presence of disaccharides in gluten-based plastics exerted a plasticizer role, diminishing the friction inside the mixer device, and finally giving rise to plastics with lower viscoelastic moduli and higher tan δ. Thus, swelling was ultimately promoted in the presence of sucrose and, specially, trehalose, which could have enormous potential when searching for environmentally-friendly superabsorbent materials.

5. Acknowledgements

The authors would like to thank Spanish Ministerio de Ciencia e Innovación – Agencia Estatal de Investigación (MICINN) and FEDER programme for the financial support provided through the funding of the RTI2018-097100-B-C21 project. They would also like to thank the full access and assistance provided to the LECO-CHNS-932 and Zeiss Evo by the Microanalysis and Microscopy Service (CITIUS-Universidad de Sevilla).

6. References

Álvarez-Castillo, E., Bengoechea, C., Guerrero, A., 2020a. Composites from by-products of the food industry for the development of superabsorbent biomaterials. Food Bioprod. Process. 119, 296–305. https://doi.org/https://doi.org/10.1016/j.fbp.2019.11.009

Álvarez-Castillo, E., Bengoechea, C., Guerrero, A., 2020b. Effect of pH on the properties of porcine plasma-based superabsorbent materials. Polym. Test. 85. https://doi.org/10.1016/j.polymertesting.2020.106453

Álvarez-Castillo, E., Bengoechea, C., Rodríguez, N., Guerrero, A., 2019. Development of green superabsorbent materials from a by-product of the meat industry. J. Clean. Prod. 223, 651–661. https://doi.org/10.1016/j.jclepro.2019.03.055

Álvarez-Castillo, E., Del Toro, A., Aguilar, J.M., Guerrero, A., Bengoechea, C., 2018. Optimization of a thermal process for the production of superabsorbent materials based on a soy protein isolate. Ind. Crops Prod. 125, 573–581. https://doi.org/10.1016/j.indcrop.2018.09.051

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. J. Control. Release 90, 97–107. https://doi.org/https://doi.org/10.1016/S0168-3659(03)00154-8

Cho, S.-W., Gällstedt, M., Johansson, E., Hedenqvist, M.S., 2011. Injection-molded nanocomposites and materials based on wheat gluten. Int. J. Biol. Macromol.

Drobny, J.G., 2014. 4 - Processing Methods Applicable to Thermoplastic Elastomers, in: Drobny, J.G.B.T.-H. of T.E. (Second E. (Ed.), Plastics Design Library. William Andrew Publishing, Oxford, pp. 33–173. https://doi.org/https://doi.org/10.1016/B978-0-323-22136-8.00004-1

Emin, M.A., Schuchmann, H.P., 2013. Analysis of the dispersive mixing efficiency in a twin-screw extrusion processing of starch based matrix. J. Food Eng. 115, 132–143. https://doi.org/https://doi.org/10.1016/j.jfoodeng.2012.10.008

Fang, Y., Zhang, B., Wei, Y., 2014. Effects of the specific mechanical energy on the physicochemical properties of texturized soy protein during high-moisture extrusion cooking. J Food Eng 121, 8–32.

Felix, M., Romero, A., Cordobes, F., Guerrero, A., 2015. Development of crayfish bio-based plastic materials processed by small-scale injection moulding. J. Sci. Food Agric. 95, 679–687. https://doi.org/10.1002/jsfa.6747

Food and Agricultural Organization of the United Nations, 2019. Biannual Report of Global Food Markets.

Ghanbarzadeh, B., Oromiehie, A.R., Musavi, M., D-Jomeh, Z.E., Rad, E.R., Milani, J., 2006. Effect of plasticizing sugars on rheological and thermal properties of zein resins and mechanical properties of zein films. Food Res. Int. 39, 882–890. https://doi.org/10.1016/j.foodres.2006.05.011

Gómez-Heincke, D., Martínez, I., Partal, P., Guerrero, A., Gallegos, C., 2016. Development of antimicrobial active packaging materials based on gluten proteins. J. Sci. Food Agric. https://doi.org/10.1002/jsfa.7525

Gómez-Martínez, D., Barneto, A.G., Martínez, I., Partal, P., 2011. Modelling of pyrolysis and combustion of gluten–glycerol-based bioplastics. Bioresour. Technol. 102, 6246–6253. https://doi.org/10.1016/j.biortech.2011.02.074

Hernandez-Izquierdo, V.M., Krochta, J.M., 2008. Thermoplastic Processing of Proteins for Film Formation—A Review. J. Food Sci. 73, R30–R39. https://doi.org/10.1111/j.1750-3841.2007.00636.x

Hoseney, R.C., Rogers, D.E., 1990. The formation and properties of wheat flour doughs. Crit. Rev. Food Sci. Nutr. 29, 73–93. https://doi.org/10.1080/10408399009527517

Jansens, K.J.A., Vo Hong, N., Telen, L., Brijs, K., Lagrain, B., Van Vuure, A.W., Van Acker, K., Verpoest, I., Van Puyvelde, P., Goderis, B., Smet, M., Delcour, J.A., 2013. Effect of molding conditions and moisture content on the mechanical properties of compression molded glassy, wheat gluten bioplastics. Ind. Crops Prod. 44, 480–487. https://doi.org/10.1016/j.indcrop.2012.10.006

Jerez, A., Partal, P., Martínez, I., Gallegos, C., Guerrero, A., 2005. Rheology and processing of gluten based bioplastics. Biochem. Eng. J. 26, 131–138. https://doi.org/10.1016/j.bej.2005.04.010

Jiménez-Rosado, M., Zarate-Ramírez, L.S., Romero, A., Bengoechea, C., Partal, P., Guerrero, A., 2019. Bioplastics based on wheat gluten processed by extrusion. J. Clean. Prod.

Kajiwara, T., Nakayama, Y., 2011. Capturing the Efficiency of a Melt-Mixing Process for Polymer Processing. J. Chem. Eng. JAPAN 44, 831–839. https://doi.org/10.1252/jcej.11we081

Kaushik, J.K., Bhat, R., 2003. Why Is Trehalose an Exceptional Protein Stabilizer? J. Biol. Chem. 278, 26458–26465. https://doi.org/10.1074/jbc.M300815200

Redl, Andréas, Morel, M.H., Bonicel, J., Guilbert, S., Vergnes, B., 1999. Rheological properties of gluten plasticized with glycerol: Dependence on temperature, glycerol content and mixing conditions. Rheol. Acta. https://doi.org/10.1007/s003970050183

Redl, Andreas, Morel, M.H., Bonicel, J., Vergnes, B., Guilbert, S., 1999. Extrusion of wheat gluten plasticized with glycerol: Influence of process conditions on flow behavior, rheological properties, and molecular size distribution. Cereal Chem. 76, 361–370. https://doi.org/10.1094/CCHEM.1999.76.3.361

Sothornvit, R., Krochta, J.M., 2005. Plasticizers in edible films and coatings, in: Innovations in Food Packaging. Elsevier, pp. 403–433. https://doi.org/10.1016/B978-012311632-1/50055-3

Stauffer, C.E., 2007. Principles of Dough Formation BT - Technology of Breadmaking, in: Cauvain, S.P., Young, L.S. (Eds.), . Springer US, Boston, MA, pp. 299–332. https://doi.org/10.1007/0-387-38565-7_11

Ullsten, N.H., Cho, S.-W., Spencer, G., Gällstedt, M., Johansson, E., Hedenqvist, M.S., 2009. Properties of Extruded Vital Wheat Gluten Sheets with Sodium Hydroxide and Salicylic Acid. Biomacromolecules 10, 479–488. https://doi.org/10.1021/bm800691h

Zárate-Ramírez, L.S., Martínez, I., Romero, A., Partal, P., Guerrero, A., 2011. Wheat gluten-based materials plasticised with glycerol and water by thermoplastic mixing and thermomoulding. J. Sci. Food Agric. 91, 625–633. https://doi.org/10.1002/jsfa.4224

Zárate-Ramírez, L.S., Romero, A., Bengoechea, C., Partal, P., Guerrero, A., 2014a. Thermo-mechanical and hydrophilic properties of polysaccharide/gluten-based bioplastics. Carbohydr. Polym. 112, 24–31. https://doi.org/10.1016/j.carbpol.2014.05.055

Zárate-Ramírez, L.S., Romero, A., Martínez, I., Bengoechea, C., Partal, P., Guerrero, A., 2014b. Effect of aldehydes on thermomechanical properties of gluten-based bioplastics. Food Bioprod. Process. 92, 20–29. https://doi.org/10.1016/j.fbp.2013.07.007