Evaluation of different strengthening methods in the mechanical and functional properties of soy protein-based bioplastics

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Abstract

The bioplastics manufactured are still not competitive compared to conventional plastics, due to their higher price and poor mechanical properties. For this purpose, different physical strengthening methods have been studied to evaluate the reinforcement of soy protein-based bioplastics: mold temperature increase (from 70 °C to 130 ºC), as the application of a dehydrothermal (4 and 24 h at 50 ºC) or ultrasound (for 5 and 45 min at 20 kHz) treatment. In this sense, the crosslinking degree, the mechanical properties, water uptake measurements and scanning electron micrographs of the different bioplastics were compared. The results conclude that suitable processing conditions and post-treatments could favor some characteristics of bioplastics (such as maximum stress (~5 times) or strain at break (~3 times)), although worsening others (such as water uptake capacity (~50%)). Specifically, thermal treatments improved the mechanical properties of the structures obtained, whereas the ultrasound treatment leads to the formation of a structure with smaller pores.

keywords: Soy bioplastic; pea bioplastic; mold temperature; dehydrothermal treatment; ultrasounds.

1. Introduction

Nowadays, plastics have an ever-increasing production and consumption due to the large number of domestic and industrial applications, such as packaging or hygienic products (Ahmed et al., 2018). However, this trend involves great contamination due to the low degradability of plastics according to the studies driven by Ahmed et al. (2018) or Derraik (2002). Thus, the weight of plastics in the ocean will be greater than that of fish in 2050 (Ellen MacArthur Foundation, 2016). Furthermore, most products we eat contain traces of plastics (Carrington, 2019). The substitution of these petroleum-based plastics with bioplastics (bio-based plastics) is seen as a potential alternative (Russo et al., 2019), since it will minimize the dependency of plastic on petrol fuels and the pressure on landfills from solid wastes (Álvarez-Chávez et al., 2012).

Bioplastics can be either bio-sourced (from biomass) or biodegradable, including fossil resources, but also there are bioplastics that are both bio-sourced and biodegradable (European Bioplastics, 2019). The latter, which have both characteristics, are the most interesting and the most recently studied by using different raw materials as soy protein (Jiménez-Rosado et al., 2019), cornhusk fibers (Saenghirunwattana et al., 2014) or porcine plasma protein (Álvarez-Castillo et al., 2019), among other alternatives (Flieger et al., 2003), by injection molding or extrusion processing (Verbeek and van den Berg, 2010). In this context, bioplastics can be made of polymers derived from biomass, such as proteins, which, in combination with a plasticizer, can be easily processed (Tummala et al., 2006). In addition, these proteins could come from byproducts or waste of agri-food industry, generating added value to these products and closing its life cycle (Mohammadhosseini and Tahir, 2018a), therefore encouraging the circular

economy. However, their mechanical properties are much lower (i.e. they are a young's modulus of 7 MPa in contract with 25 MPa of PE or 35 MPa of PS) (Zhuang et al., 2014), and their price is often higher than that of conventional plastics (around 10 times more expensive), thus their use is still limited (Confente et al., 2019). These mechanical properties can be improved by using chemical, enzymatic or physical treatments. Thus, formaldehyde, glutaraldehyde or glyoxal (Marquié, 2001) as well as even citric acid (Awadhiya et al., 2016) have been used such as chemical crosslinker and transglutaminase (Giosafatto et al., 2018) have been used like enzymatic ones. In addition, fillers (i.e. metalized plastic fibers) can be added, which without interacting with proteins, improve the final properties of the bioplastic (Mohammadhosseini et al., 2018). However, the use of some of these materials can cause toxicity or the transmission of harmful properties to the product they are being used for (Gerrard, 2002). In this context, the use of physical treatments is the most recommended to could prevent these disadvantages and improve the mechanical properties of bioplastics without causing toxicity problems that may affect its subsequent use. Among the different physical treatments (i.e. ultrasound, microwaves or thermal treatments), thermal treatments are the most used, both during processing (Mo et al., 1999) and post-processing (Álvarez-Castillo et al., 2018). In addition, there are also works where ultrasound (Jayasooriya et al., 2004) or microwaves (Ghasri et al., 2019) are used to increase the degree of crosslinking of bioplastics. However, these improvements normally affect, apart from the mechanical properties, other properties such as water uptake capacity, which could decrease between 1000-2500%, (Álvarez-Castillo et al., 2018) or even the morphology of the final structure obtained and the degradation

rate. Therefore, an optimization is required to reach the final desired properties (Perez et al., 2016).

Among the possible applications, protein-based bioplastics can develop a sustainable product that may act as superabsorbent materials (SABs) (Cuadri et al., 2017). SABs are materials with the ability to absorb and retain more than 10 times their own weight of water, keeping their integrity without dissolving (Cuadri et al., 2016). The forces which allow water absorption by polymers are mainly the osmotic pressure and the formation of hydrogen bonds (Barbe and Storz, 2005). There are several applications for these materials, such as construction (Mohammadhosseini and Tahir, 2018b), waste management, agriculture, electronics and horticulture (Nnadi and Brave, 2011). Among them, personal hygiene products constitute most of the market of these materials (Magnay et al., 2010).

Specifically, soy protein has a high content of glutamic and aspartic acids, more than other proteins such as rice or pea, which favor the formation of these hydrogen bonds and, therefore, making soy protein-based bioplastics a potential option as SABs (Fernández-Espada et al., 2016a). In addition, soy protein normally used in this field is a co-product with soy oil and one of the cheapest proteins in nature, which is another advantage to consider it the best alternative to plastics (Fernández-Espada et al., 2016b). However, the properties (especially the mechanical properties) shown by soy protein-based bioplastics (Gironi and Piemonte, 2011) or other compostable alternatives (Song et al., 2009) manufactured to date are not good enough to compete with conventional plastics; therefore, further research is necessary to find a methodology that can generate an adequate relationship between the properties of these bioplastics (Karana, 2012).

In this context, the main objective of this work was the use of different physical strengthening in order to enhance the mechanical properties of soy protein-based bioplastics without worsening their functional properties (i.e. water uptake capacity). To this end, the manufacturing conditions were evaluated and different post-treatments (dehydrothermal or ultrasounds treatments) were conducted to reinforce the structure of the bioplastics. In order to compare the results, mechanical and microstructural measurements were carried out, as well as water uptake capacity tests.

2. Materials and methods

2.1 Materials

Bioplastics are usually composed of three different elements: matrix, plasticizer and additives (optional). In this work, the matrix was soy protein isolate (SPI, 91 wt.% protein), which was supplied by Protein Technologies International (SUPRO 500E, Belgium). This raw material is a byproduct of a soybean oil production, so its use in this work gives it an added value. Its chemical composition was evaluated obtaining a minimum of 90 wt.% of protein, maximum of 6 wt.% of humidity, 1 wt.% lipids, maximum of 0.1 wt.% carbohydrates and 5 wt.% of ash. In addition, its pH is between 6.9 and 7.4, being negatively charged (isoelectric point 4.5). Thus, this source of soy protein have a higher protein content than others such as used by Tummala et al. (2006), 91 vs. 52 wt.%.

In addition, glycerol (Gly) was used as plasticizer, which was purchased from Panreac Química Ltd. (Spain). The use of other plasticizers has been proved (Tummala et al., 2006), but glycerol is the one that generate bioplastics with the best mechanical properties.

2.2 Preparation of soy protein-based bioplastics

The soy protein-based bioplastics were prepared in a two-step process, which was used and optimized in previous studies related to the production of soy-based bioplastics combined with albumen (Fernández-Espada et al., 2016b), lignocellulose (Gamero et al., 2019) or even functionalized soy protein (Cuadri et al., 2017). Firstly, SPI and Gly in a 1:1 mass ratio was homogenized in a mixing step. For this, both raw materials were introduced in a two-blade counter-rotating ThermoHaake mixer Polylab QC (Germany) for 10 min at 50 rpm and adiabatic conditions. In this step, the temperature and torque were controlled to prevent the plasticization of the blends in the chamber. In this case, the temperature was below 37 ºC and the maximum torque was 5 Nm, so the blends were always under their plasticization parameters (Fernández-Espada et al., 2016b).

The second step consisted in an injection molding of the dough-like blend obtained in the mixing. For this, a ThermoHaake MiniJet Piston Injection Molding System II (Germany) was used to obtain rectangular bioplastics (60x10x1 mm^3) and dumb-bell-type bioplastics (UNE-EN ISO 527-2, 2012). The parameters used, according to previous studies, were cylinder and mold temperature of 40 and 70 ºC, respectively, injected for 20 s at 500 bar and using a post- injection pressure of 200 bar for 300 s. These parameters were selected to highlight the water uptake capacity of soy protein-based bioplastics, but they have poor mechanical properties (Fernández-Espada et al., 2016a). In this work we aimed to enhance these mechanical properties without significantly affecting the water uptake capacity.

2.2.1 Strengthening modifications

To enhance the mechanical properties, three different experiments were carried out. In the first case, the mold temperature was increased. Previous studies state that protein-based bioplastics have a better crosslinking with a higher mold temperature (Thakur, 2017). This greater crosslinking could improve the mechanical properties of these bioplastics. In this context, an increment of 60 ºC in the mold temperature was applied during the injection molding step, so the final mold temperature used was 130 ºC instead of 70 ºC. It worth mentioning that higher temperatures could cause protein disintegration without achieving the desired effect.

The second experiment consisted in the addition of a post-treatment. Thus, the bioplastics were subjected to a thermal step at 50 ºC in a conventional oven (Memmert, Germany). The application of this step, called dehydrothermal treatment (DHT), for a long time (12-14 h) can favor the formation of covalent bonds in it (Álvarez-Castillo et al., 2018). In order to study the effect of this step with the time of treatment in the bioplastic properties, different times (4 and 24 hours) were evaluated.

Finally, the last experiment was an ultrasound post-treatment (US). US treatment produces localized cavitation, which facilitates the disintegration of the particles (Karki, 2009), allowing the structure to rearrange and, consequently, improving the mechanical properties of the bioplastics. To carry out this experiment, the bioplastics, after the injection molding step, were inserted into vacuum bags. Then, these bags with the bioplastics were immersed in an ultrasound bath (JP Selecta) at 50 kHz with 100 W (power) and 0,4 A (intensity), using water as medium. In this case, the treatment time was also evaluated at 5 and 45 min.

166 The selected times in both treatments (thermal and US) were chosen to achieve a
167 maximum change in the bioplastic properties (long times) and a less radical
168 change (short times) to assess if all the bioplastic properties change uniformly with
169 the application of the treatment.

170 ***2.3 Characterization of soy protein-based bioplastics***

171 *2.3.1 Degree of crosslinking*

172 The degree of crosslinking was determined in order to evaluate the modification
173 produced in the microstructure caused by each of the variations in the process. For
174 this, the same protocol used by Zárate-Ramírez et al. (2014) was following. Thus,
175 a portion of each bioplastic (15x10x1 mm^3) was immersed for 2 h in a denaturing
176 agent solution (0.086 mol/L Tris base, 0.045 mmol/L glycine, 2 mmol/L EDTA, 10
177 g/L sodium dodecyl sulfate (SDS) pH 6 buffer) to denaturalize the uncrosslinked
178 protein. Subsequently, the solutions were subjected to a centrifugation step at
179 10000*xg* for 10 min to separate the denatured protein solution. Finally, the soluble
180 protein fraction was determined using Lowry's method (Markwell et al., 1978).
181 From these data, the degree of crosslinking of the bioplastics was estimated using
182 as reference the bioplastic for which the process was not modified (0%
183 crosslinking) and denaturing agent solution without bioplastic (100% crosslinking).
184 In this way, the crosslinking produced by the different treatments added can be
185 evaluated with respect to that performed by the traditional method (without physical
186 treatment carried out).

187 *2.3.2 Mechanical properties*

188 *2.3.2.1 <u>Tensile tests</u>*

189 Breakage resistance under tensile stress is a widely measured property of
190 materials used in structural applications. Tensile tests were performed in a MTS

191 insight Electromechanical-10kN Standard Length (Eden Prairie, USA) where the
192 dumb-bell-type bioplastics were subjected to an axial force until breakage,
193 following the UNE-EN ISO 527-2 standard (2012). The tests were carried out at
194 room temperature (22 ± 1 ºC), using an extensional rate of 10 mm/min. The
195 equipment used can detect when the material breaks and provides parameters
196 such as maximum tensile stress (σ_{max}), strain at the break (ε_{max}) and Young's
197 modulus.

198 *2.3.2.2 Bending tests*

199 Bending tests were performed in order to evaluate the flexural resistance of the
200 bioplastics. In order to compare these results with the literature results (Patel et al.,
201 2016), a dynamic-mechanical analyzer RSA3 (TA Instrument, USA) with a dual
202 cantilever geometry were used to carry out these tests to rectangular bioplastics.
203 First, the linear viscoelastic range (the linear region where the elastic and viscous
204 moduli remain independent of the applied stain) were determined through strain
205 sweep tests (between 0.002 and 2% and 1 Hz). Subsequently, frequency sweep
206 tests were carried out between 0.02 and 20 Hz at a constant strain below the
207 critical strain (maximum strain where the bioplastics remain in the linear
208 viscoelastic range). All these measurements were performed at room temperature
209 (22 ± 1 ºC). Thus, the elastic modulus (E') and loss tangent (tan δ =E''/E') were
210 evaluated over the entire frequency range.

211 *2.3.4 Water uptake capacity & soluble matter loss*

212 In order to determine the superabsorbent character of the bioplastics (functional
213 property), water uptake capacity and soluble matter loss tests were performed.
214 These tests were carried out following a modification of the ASTM standard
215 ("ASTM D570-98: Standard Test Method for Water Absorption of Plastics," 2005).

Therefore, rectangular bioplastics (60x10x1 mm^3) were immersed in 300 mL of distilled water for 24 h at room temperature (22 ± 1 ºC). Later, the samples were subjected to a freeze-drying step for 24 h at -80 ºC and low pressure (< 15 Pa) using a Lyoquest freeze-dryer (Telstar, Spain) in order to remove all the bioplastic water.

The water uptake capacity was then obtained using equation 1 in order not to modify the water uptake capacity with the elimination of glycerol (plasticizer) that occurs during immersion.

$$Water\ uptake\ capacity\ (\%) = \frac{w_2 - w_3}{w_3} \cdot 100 \qquad (1)$$

Where w_2 (g) is the weight of the bioplastic after the immersion step and w_3(g) is the weight of the bioplastic after the freeze-drying stage.

Finally, soluble matter loss was determined using equation 2.

$$Soluble\ matter\ loss\ (\%) = \frac{w_1 - w_3}{w_1} \cdot 100 \qquad (2)$$

Where w_1 (g) is the weight of the bioplastic before the immersion step.

2.3.5 Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) was used in order to compare the microstructure of the bioplastics processed by different treatments before and after the water uptake capacity tests. For this, Orawan et al. (2006) protocol was used, who used it for similar systems with good results. The samples were subjected to a previous sputtering treatment with palladium/gold to improve the electrical conductivity of the bioplastics and improve the quality of the micrographs. Then, the samples were observed using a Zeiss EVO microscope (Germany) at an acceleration voltage of 10 kV and x100 magnification.

2.4 Statistical analysis

In order to confirm a variation of the measurements according to the parameters tested, at least three replicates of each measurement were carried out and the sample standard deviation was calculated with EXCEL (Microsoft public domain).

3. Results & Discussion

3.1 Degree of crosslinking

The degree of crosslinking induced by each of the strengthening actions is shown in Table 1. It is important to mention that the bioplastic processed with a molding temperature of 70 ºC and without post-treatment was used as a 0% crosslinking reference, with which the calculated degree of crosslinking of the other samples was compared.

Table 1: Degree of crosslinking (DC, %) of the different bioplastics: Reference (Ref.), mold temperature increase (130 ºC), dehydrothermal treatment (DHT) at 4 and 24 h and ultrasound treatment (US) at 5 and 45 min. Different letters show significant differences in the degree of crosslinking of the systems ($p \leq 0.05$).

Sample		DC (%)
Ref.		**-**
130 ºC		**32.2[a]**
DHT	**4 h**	**0.8[b]**
	24 h	**22.5[c]**
US	**5 min**	**1.9[b]**
	45 min	**19.2[c]**

As it can be observed, the action that caused the most significant improvement in the degree of crosslinking was the increase in the mold temperature to 130 ºC (32.2% more crosslinked than in the reference system). Therefore, it is intuited that more severe processing parameters induce a greater number of covalent bonds in the bioplastics, as anticipated in previous studies (Fernández-Espada et al., 2016a). Moreover, the use of a long-time post-treatment (24 h DHT or 45 min US) also caused a higher degree of crosslinking (22.5 and 19.2%, respectively), although this improvement was not as significant as the increase in mold temperature. On the other hand, at short times, neither of the two post-treatments induced a great degree of crosslinking (0.8% in DHT and 1.9% in US). In this regard, it should be noted that the US treatment needs much less time to cause the same crosslinking as DHT (several minutes instead of several hours). This fact could be decisive in their large-scale manufacture since a shorter processing time can reduce the costs of the product, making it more competitive in the market (Fowler et al., 2006), as long as this shorter processing time does not generate additional costs to the process. Therefore, all the alternatives seem to produce some crosslinking degree, being a greener method than the addition of other chemical substances (i.e. glutaraldehyde) that can produce environmental contamination. However, it is important evaluate the carbon footprint caused for the different physical strengthening methods, since an excess in their use can cause global warming problems.

3.2 Mechanical properties

3.2.1 Tensile tests

Figure 1 shows the maximum stress (σ_{max}), strain at break (ε_{max}) and Young's modulus of the different processed bioplastics. The increase in the mold

temperature (130 ºC) caused an improvement in σ_{max}, ε_{max} and Young's modulus., as in other previous studies (Fernández-Espada et al., 2016a). This also occurred when a stage of DHT was added, although without significant differences between the different treatment times. However, the US treatment did not significantly improve any of the bioplastic tensile parameters, neither at short nor long times. These results suggest that a heat treatment (i.e. higher mold temperature or DHT) improves the mechanical tensile properties of bioplastics, probably due to a greater strengthening of the bioplastic (increasing both the maximum stress and the strain at break) through covalent bonds (Álvarez-Castillo et al., 2018; Fernández-Espada et al., 2016b). However, the US treatment, although it induces a greater degree of crosslinking than the reference system, can affect the proteins by denaturing them; leading to weaker bonds (Park et al., 1993).

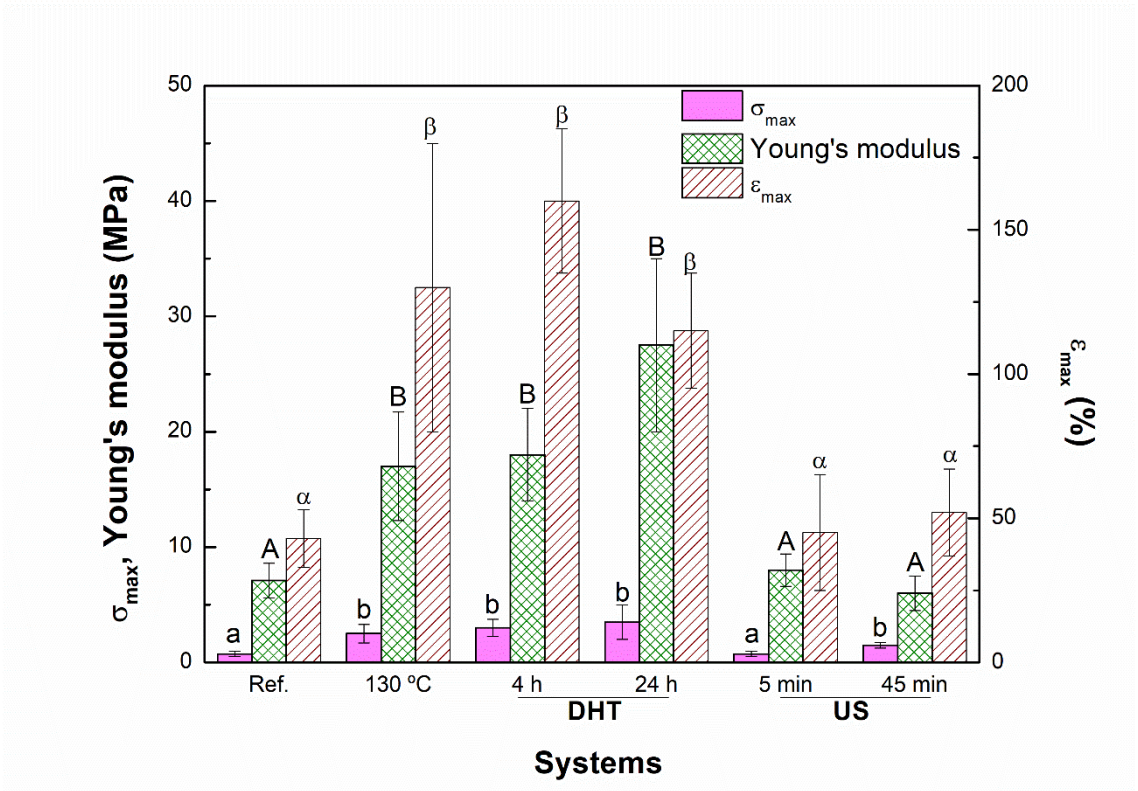

Figure 1: Maximum stress (σ_{max}), strain at break (ε_{max}) and Young's modulus obtained in tensile tests of the different bioplastics (systems): Reference (Ref.),

mold temperature increase (130 ºC), dehydrothermal treatment (DHT) at 4 and 24 h and ultrasound treatment (US) at 5 and 45 min. Different letters show significant differences ($p \leq 0.05$).

3.2.2 Bending tests

Figure 2 shows the results obtained from the bending tests. In Figure 2A, the evolution of the elastic modulus (E') with the frequency can be observed. All the bioplastics studied showed a dependency of the flexural elastic modulus (E') on the frequency studied range. This means that the properties of the bioplastics vary depending on the force applied and the application time, making it more difficult to predict their behavior. This dependence is higher in bioplastic processed thermally, since its rise is steeper than US processed bioplastics. Thus, it seems that higher tensile parameters lead to less flexural stability. These results may be because the bioplastic, although it supports higher stresses, deforms plastically when a certain force is applied, changing its structure and flexural stability. Similar results were obtained in a previous work (Jiménez-Rosado et al., 2018). On the other hand, Figure 2B shows the elastic modulus and loss tangent at 1 Hz (E'_1 and $\tan \delta_1$, respectively). As in the tensile tests, a higher mold temperature or the incorporation of a thermal stage (DHT) caused an improvement in the flexural strength of the bioplastics. However, the US treatment worsened the elastic modulus of the bioplastics, which was higher when a longer treatment time was applied. These results are consistent with those obtained in the tensile tests, showing that a thermal treatment caused a strengthening of bioplastic bonds, whereas an ultrasound treatment weakened them. As for $\tan \delta_1$, it remained invariant below 1 in all the systems analyzed. This reflects the high solid character of the systems,

320 which was not altered by the change in the processing method. This solid
321 character was also observed in previous studies (Fernández-Espada et al., 2016a;
322 Perez-Puyana et al., 2018).

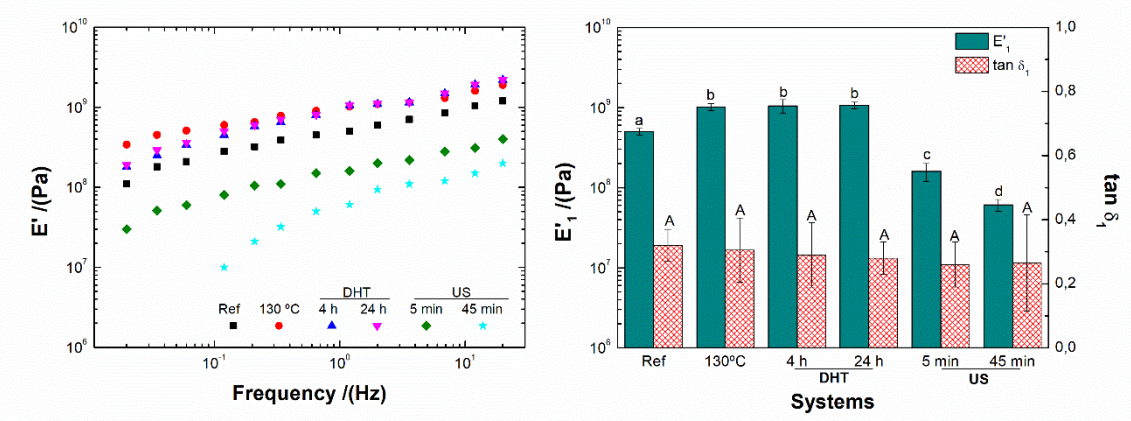

Figure 2: Bending tests of the different processed bioplastics. (A) Elastic modulus (E') in the entire frequency range and (B) elastic modulus and loss tangent at 1 Hz (E'_1 and tan δ_1, respectively). Systems: Reference (Ref.), mold temperature increase (130 ºC), dehydrothermal treatment (DHT) at 4 and 24 h and ultrasound treatment (US) at 5 and 45 min. Different letters show significant differences ($p \leq 0.05$).

If the mechanical properties of these bioplastics are compared with those of conventional plastics, it can be observed that after thermal strengthening of the bioplastics have better mechanical properties. In fact, it achieves properties similar to that of polyethylene or polyvinylchloride (Young's modulus 25 and 35 MPa, respectively), but not so high as polystyrene (3-3.4 GPa). However, the US treatment does not improve these properties.

3.3 Water uptake capacity & soluble matter loss

The measurements of water uptake capacity and soluble matter loss are shown in Figure 3. Considering the soluble matter loss, there were no significant differences between the systems, where, in all cases, all Gly is lost, due to its hydrophilic character, and the soluble part of the protein (about 10 wt.%). These results were similar to those obtained in previous works, where all Gly and part of the protein were lost (Fernández-Espada et al., 2016b).

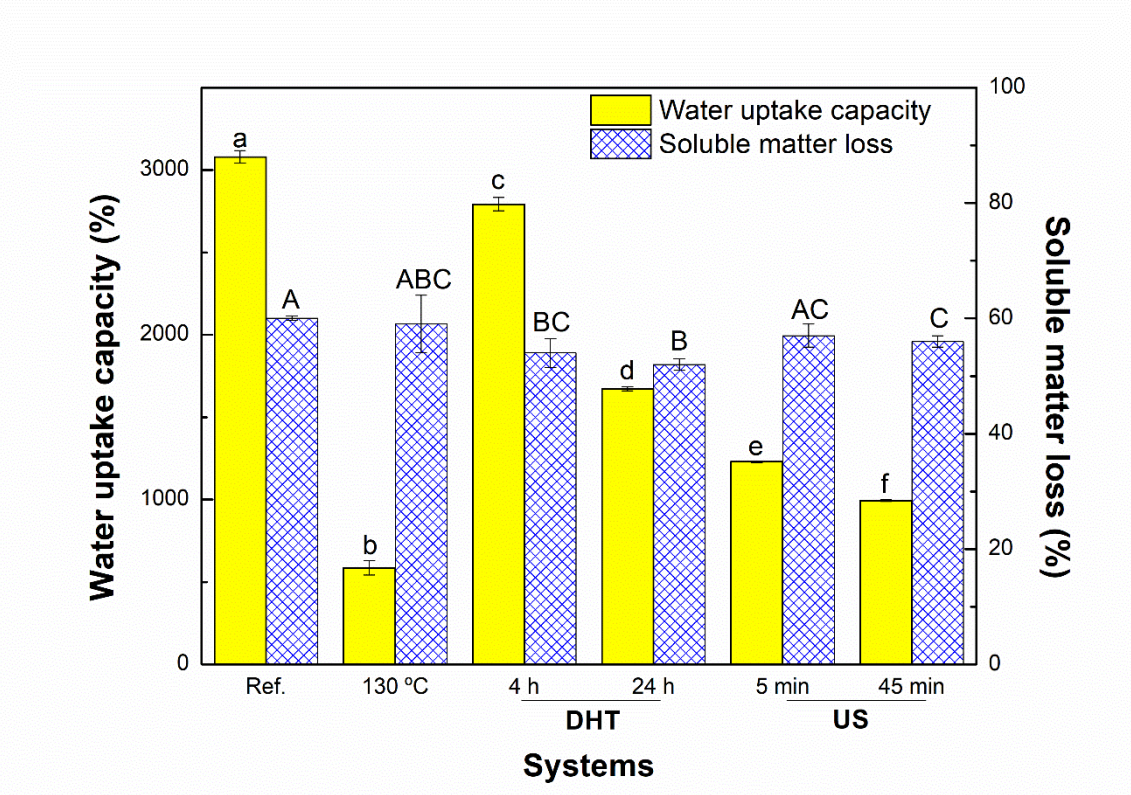

Figure 3: Water uptake capacity and soluble matter loss measurements for the different processed bioplastics (systems): Reference (Ref.), mold temperature increase (130 ºC), dehydrothermal treatment (DHT) at 4 and 24 h and ultrasound treatment (US) at 5 and 45 min. Different letters show significant differences ($p \leq 0.05$).

On the other hand, for the water uptake capacity, there were major differences between the bioplastics studied. The greatest difference was observed with the increase in mold temperature, losing the superabsorbent capacity (<1000%) of the bioplastic. This is due to the greater structuring of the bioplastic while it is conferred. It creates a more reinforced structure with more difficulty to swell, and therefore, with less capacity to retain water inside (Fernandez-Espada et al., 2016a).

As for the application of DHT, it also caused a decrease of the bioplastic's water uptake capacity, which was more significant when longer DHT time was needed.

However, the functionality of the bioplastic was not lost (they were superabsorbent). These results show that, although there is a strengthening of the system, improving its mechanical properties, it was not sufficiently consolidated. Consequently, the bioplastic could swell to retain a greater amount of water than in the case of mold temperature change.

The US treatment also worsened the water uptake capacity of the bioplastics, which remained at the limit of being considered superabsorbent. These results are not consistent with those obtained in previous studies (Álvarez-Castillo et al., 2018), since these predicted that the mechanical properties of bioplastics were closely linked to their water uptake capacity, which worsens when the better mechanical properties are presented by the bioplastics. In this case, this prediction is not fulfilled, which may be due to the protein denaturation that was previously intuited. Thus, the protein loses the ability to create hydrogen bonds with water, which worsens its retention capacity.

3.4 Scanning electron microscopy (SEM)

SEM imaging was performed in the different systems studied before and after the water uptake measurements. The micrographs of the bioplastics before (A, B, C and D) and after (A', B', C' and D') the water immersion can be seen in Figure 4.

Before water absorption, the different processes performed on the bioplastics had an impact on the microstructure in each case. The reference system (A) was the one with the greatest number of pores, which were also larger than in the rest of the systems. This is due to the lower structuring of the bioplastic when softer processing conditions were used. This lower structuring induced a greater water uptake capacity, with the subsequent swelling and opening of the structure

reflected by the increase in the number and size of pores in the microstructure (A'). Previous work also shows these results (Fernandez-Espada et al., 2016a).

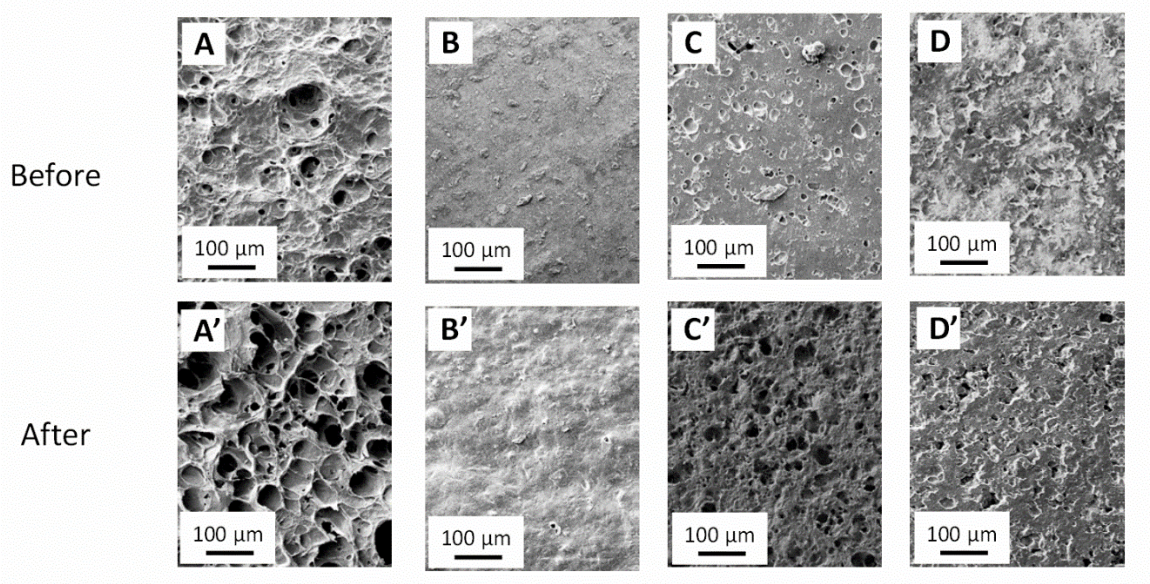

Figure 4: Microstructure images of the different processed bioplastics obtained by scanning electronic microscopy (reference without modification (A, A'), processed with 130 ºC in mold temperature (B, B'), with 24 h dehydrothermal treatment (C, C') and with 45 min ultrasounds treatment (D, D')) before (A, B, C and D) and after (A', B', C' and D') water immersion.

On the other hand, any change in the processing method led to a less porous structure (B, C and D), probably due to the reinforcement produced by the crosslinking achieved in these bioplastics. The most solid and compact structure was achieved with the highest mold temperature (B). The tightness obtained implies a lower water absorption (as seen in Section 3.3) and, therefore, no variation in the structure after the immersion (B'). A similar effect took place after performing the DHT post-treatment (C), but with a slight modification of the microstructure with the formation of some pores on it (C'). These results are similar to these obtained by Álvarez-Castillo et al. (2018). As for the bioplastic submitted to US (D), it can be seen that the ultrasound caused a modification of the

microstructure respect to the reference system, presenting a large number of small pores that did not vary after the water immersion (D'). This was surely due to the denaturation discussed above.

4. Conclusions

Soy protein-based bioplastics processed by injection molding have demonstrated their potential to replace conventional plastics. However, suitable processing conditions are extremely important to reach the required properties for these bioplastics.

Thus, thermal treatments (both higher mold temperature and dehydrothermal treatment) improve the mechanical properties of the bioplastics (specially the Young's modulus and strain at break). Nevertheless, higher mold temperature leads to bioplastics more compact and without superabsorbent capacity, while dehydroythermal treatment decreases slowly this capacity, allowing bioplastics to still have superabsorbent capacity. Thus, the first ones could be used in applications as packaging, and the second ones could be used in hygienic applications.

On the other hand, ultrasound treatment leads to a change in the structure of bioplastic with a decrease in the water uptake capacity and worse mechanical properties. In this way, however a greater crosslinking normally results in an improvement of the mechanical properties and a loss of the water uptake capacity, this is only true if the microstructure of the materials is not altered too much.

Finally, this work opens the possibility of creating sustainable bioplastics competitive with commercial ones, making the consumption of plastics cleaner without creating pollution problems. However, further study is necessary where

combinations between the different strengthening used (i.e. mold temperature in addition to post-treatment), as well as the use of other intermediate temperatures and times, would be evaluated.

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References

AENOR, 2012. UNE-EN ISO 527-2:2012. Plásticos. Determinación de las propiedades en tracción. Parte 2: Condiciones de ensayo de plásticos para moldeo y extrusión.

Ahmed, T., Shahid, M., Azeem, F., Rasul, I., Shah, A.A., Noman, M., Hameed, A., Manzoor, N., Manzoor, I., Muhammad, S., 2018. Biodegradation of plastics: current scenario and future prospects for environmental safety. Environ. Sci. Pollut. Res. 25, 7287–7298. https://doi.org/10.1007/s11356-018-1234-9

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/https://doi.org/10.1016/j.indcrop.2018.09.051

Álvarez-Chávez, C.R., Edwards, S., Moure-Eraso, R., Geiser, K., 2012. Sustainability of bio-based plastics: general comparative analysis and recommendations for improvement. J. Clean. Prod. 23, 47–56. https://doi.org/10.1016/j.jclepro.2011.10.003

Carrington, D., 2019. People eat at least 50,000 plastic particles a year, study finds [WWW Document]. Guard. URL https://www.theguardian.com/environment/2019/jun/05/people-eat-at-least-50000-plastic-particles-a-year-study-finds

Confente, I., Scarpi, D., Russo, I., 2019. Marketing a new generation of bio-plastics products for a circular economy: The role of green self-identity, self-congruity, and perceived value. J. Bus. Res. https://doi.org/10.1016/j.jbusres.2019.10.030

Cuadri, A.A., Bengoechea, C., Romero, A., Guerrero, A., 2016. A natural-based polymeric hydrogel based on functionalized soy protein. Eur. Polym. J. 85, 164–174. https://doi.org/10.1016/j.eurpolymj.2016.10.026

Cuadri, A.A., Romero, A., Bengoechea, C., Guerrero, A., 2017. Natural superabsorbent plastic materials based on a functionalized soy protein. Polym. Test. 58, 126–134. https://doi.org/10.1016/j.polymertesting.2016.12.024

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–52.

Ellen MacArthur Foundation, 2016. The new plastics economy: Rethinking the future of plastics. Word Economic Forum, New York.

European Bioplastics, 2019. Commission's Circular Economy Proposal leaves room for more ambitious actions on bio-industries such as bioplastics [WWW Document]. URL https://www.european-bioplastics.org/commissions-circular-economy-proposal-leaves-room-for-more-ambitious-actions-on-bio-industries-such-as-bioplastics/

Fernández-Espada, L., Bengoechea, C., Cordobés, F., Guerrero, A., 2016a. Thermomechanical properties and water uptake capacity of soy protein-based bioplastics processed by injection molding. J. Appl. Polym. Sci. 133, 43524. https://doi.org/10.1002/app.43524

Fernández-Espada, L., Bengoechea, C., Cordobés, F., Guerrero, A., 2016b. Protein/glycerol blends and injection-molded bioplastic matrices: Soybean versus egg albumen. J. Appl. Polym. Sci. 133, 42980. https://doi.org/10.1002/app.42980

Flieger, M., Kantorová, M., Prell, A., \vRezanka, T., Votruba, J., 2003. Biodegradable plastics from renewable sources. Folia Microbiol. (Praha). 48, 27. https://doi.org/10.1007/BF02931273

Fowler, P.A., Hughes, J.M., Elias, R.M., 2006. Biocomposites: technology, environmental credentials and market forces. J. Mech. Behav. Biomed. Mater. 86, 1781–1789. https://doi.org/10.1002/jsfa.2558

Gamero, S., Jiménez-Rosado, M., Romero, A., Bengoechea, C., Guerrero, A., 2019. Reinforcement of Soy Protein-Based Bioplastics Through Addition of Lignocellulose and Injection Molding Processing Conditions. J. Polym.

Environ. 27, 1285–1293. https://doi.org/10.1007/s10924-019-01430-1

Gerrard, J.A., 2002. Protein–protein crosslinking in food: methods, consequences, applications. Trends Food Sci. Technol. 13, 391–399. https://doi.org/https://doi.org/10.1016/S0924-2244(02)00257-1

Ghasri, M., Bouhendi, H., Kabiri, K., Zohuriaan-Mehr, M.J., Karami, Z., Omidian, H., 2019. Superabsorbent polymers achieved by surface cross linking of poly(sodium acrylate) using microwave method. Iran. Polym. J. 28, 539–548. https://doi.org/10.1007/s13726-019-00722-6

Giosafatto, C., Al-Asmar, A., D'Angelo, A., Roviello, V., Esposito, M., Mariniello, L., 2018. Preparation and Characterization of Bioplastics from Grass Pea Flour Cast in the Presence of Microbial Transglutaminase. Coatings 8, 435. https://doi.org/10.3390/coatings8120435

Gironi, F., Piemonte, V., 2011. Bioplastics and Petroleum-based Plastics: Strengths and Weaknesses. Energy Sources Part A: Re, 1949–1959. https://doi.org/10.1080/15567030903436830

Jayasooriya, S.D., Bhandari, B.R., Torley, P., D'Arcy, B.R., 2004. Effect of High Power Ultrasound Waves on Properties of Meat: A Review. Int. J. Food Prop. 7, 301–319. https://doi.org/10.1081/JFP-120030039

Jiménez-Rosado, M., Pérez-Puyana, V., Cordobés, F., Romero, A., Guerrero, A., 2018. Development of soy protein-based matrices containing zinc as micronutrient for horticulture. Ind. Crops Prod. 121, 345–351. https://doi.org/10.1016/j.indcrop.2018.05.039

Jiménez-Rosado, M., Zarate-Ramírez, L.S., Romero, A., Bengoechea, C., Partal,

519 P., Guerrero, A., 2019. Bioplastics based on wheat gluten processed by extrusion.
520 J. Clean. Prod. 239, 117994. https://doi.org/10.1016/j.jclepro.2019.117994

521 Julavittayanukul, O., Benjakul, S., Visessanguan, W., 2006. Effect of phosphate
522 compounds on gel-forming ability of surimi from bigeye snapper (Priacanthus
523 tayenus). Food Hydrocoll. 20, 1153–1163.
524 https://doi.org/10.1016/j.foodhyd.2005.12.007

525 Karana, E., 2012. Characterization of 'natural' and 'high-quality' materials to
526 improve perception of bio-plastics. J. Clean. Prod. 37, 316–325.
527 https://doi.org/10.1016/j.jclepro.2012.07.034

528 Karki, B., 2009. Use of high-power ultrasound during soy protein production and
529 study of its effect on functional properties of soy protein isolate. Iowa State
530 University, Digital Repository, Ames. https://doi.org/10.31274/etd-180810-
531 3395

532 Magnay, J.L., Nevatte, T.M., Dhingra, V., O'Brien, S., 2010. Menstrual blood loss
533 measurement: validation of the alkaline hematin technique for feminine
534 hygiene products containing superabsorbent polymers. Fertil. Steril. 94, 2742–
535 2746. https://doi.org/10.1016/j.fertnstert.2010.03.061

536 Markwell, M.A.K., Haas, S.M., Bieber, L.L., Tolbert, N.E., 1978. A modification of
537 the Lowry procedure to simplify protein determination in membrane and
538 lipoprotein samples. Anal. Biochem. 87, 206–210.
539 https://doi.org/https://doi.org/10.1016/0003-2697(78)90586-9

540 Marquié, C., 2001. Chemical Reactions in Cottonseed Protein Cross-Linking by
541 Formaldehyde, Glutaraldehyde, and Glyoxal for the Formation of Protein Films
542 with Enhanced Mechanical Properties. J. Agric. Food Chem. 49, 4676–4681.

https://doi.org/10.1021/jf0101152

Mo, X., Sun, X.S., Wang, Y., 1999. Effects of molding temperature and pressure on properties of soy protein polymers. J. Appl. Polym. Sci. 73, 2595–2602. https://doi.org/10.1002/(SICI)1097-4628(19990923)73:13<2595::AID-APP6>3.0.CO;2-I

Mohammadhosseini, H., Tahir, M.M., 2018a. Durability performance of concrete incorporating waste metalized plastic fibres and palm oil fuel ash. Constr. Build. Mater. 180, 92–102. https://doi.org/10.1016/j.conbuildmat.2018.05.282

Mohammadhosseini, H., Tahir, M.M., 2018b. Production of sustainable fibre-reinforced concrete incorporating waste chopped metallic film fibres and palm oil fuel ash. Sādhanā 43, 156. https://doi.org/10.1007/s12046-018-0924-9

Mohammadhosseini, H., Tahir, M.M., Sam, A.R.M., 2018. The feasibility of improving impact resistance and strength properties of sustainable concrete composites by adding waste metalized plastic fibres. Constr. Build. Mater. 169, 223–236. https://doi.org/10.1016/j.conbuildmat.2018.02.210

Nnadi, F., Brave, C., 2011. Environmentally friendly superabsorbent polymers for water conservation in agricultural lands. J. Soil Sci. Environ. Manag. 2, 206–211.

Park, K., Shalaby, W.S.W., Park, H., 1993. Biodegradable hydrogels for drug delivery. CRC Press, Boca Raton.

Patel, A. V, Panchal, T.M., Rudakiya, D., Gupte, A., Patel, J. V, 2016. Fabrication of bio-plastics from protein isolates and its biodegradation studies. Int. J. Chem. Sci. Technol. 1, 1–13. https://doi.org/18.04.001/20160104

566 Perez-Puyana, V., Felix, M., Romero, A., Guerrero, A., 2018. Development of eco-
567 friendly biodegradable superabsorbent materials obtained by injection
568 moulding. J. Clean. Prod. 198, 312–319.
569 https://doi.org/https://doi.org/10.1016/j.jclepro.2018.07.044

570 Perez, V., Felix, M., Romero, A., Guerrero, A., 2016. Characterization of pea
571 protein-based bioplastics processed by injection moulding. Food Bioprod.
572 Process. 97, 100–108. https://doi.org/10.1016/j.fbp.2015.12.004

573 Russo, I., Confente, I., Scarpi, D., Hazen, B.T., 2019. From trash to treasure: The
574 impact of consumer perception of bio-waste products in closed-loop supply
575 chains. J. Clean. Prod. 218, 966–974.
576 https://doi.org/10.1016/j.jclepro.2019.02.044

577 Saenghirunwattana, P., Noomhorm, A., Rungsardthong, V., 2014. Mechanical
578 properties of soy protein based “green” composites reinforced with surface
579 modified cornhusk fiber. Ind. Crops Prod. 60, 144–150.
580 https://doi.org/10.1016/j.indcrop.2014.06.010

581 Song, J.H., Murphy, R.J., Narayan, R., Davies, G.B.H., 2009. Biodegradable and
582 compostable alternatives to conventional plastics. Philos. Trans. R. Soc. Lond.
583 B. Biol. Sci. 364, 2127–2139. https://doi.org/10.1098/rstb.2008.0289

584 Thakur, V.K., 2017. Soy-Based Bioplastics. Smithers Information Limited,
585 Shawbury.

586 Tummala, P., Liu, W., Drzal, L.T., Mohanty, A.K., Misra, M., 2006. Influence of
587 Plasticizers on Thermal and Mechanical Properties and Morphology of Soy-
588 Based Bioplastics. Ind. Eng. Chem. Res. 45, 7491–7496.
589 https://doi.org/10.1021/ie060439l

590 Verbeek, R.C.J., van den Berg, L.E., 2010. Extrusion Processing and Properties of
591 Protein-Based Thermoplastics. Macromol. Mater. Eng. 295, 10–21.
592 https://doi.org/doi:10.1002/mame.200900167

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

597 Zhuang, H., Barth, M.M., Cisneros-Zevallos, L., 2014. Modified Atmosphere
598 Packaging for Fresh Fruits and Vegetables, in: Innovations in Food Packaging.
599 Elsevier, pp. 445–473. https://doi.org/10.1016/B978-0-12-394601-0.00018-7