Rice bran-based bioplastics: effects of the mixing temperature on starch plastification and final properties.

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ABSTRACT

The agro-food industry produces huge amounts of wastes and by-products with high levels of carbohydrates and proteins, basic food groups that, properly treated, can be employed for the development of bioplastics. These high added-value products represent an alternative to traditional polymers. In this research work, rice bran was mixed with glycerol and water obtaining homogeneous blends which then are processed into bioplastics via injection moulding. The mixing temperature aids starch plastification and thus, affects the properties of the final specimens. In this way, the mechanical characterization revealed improvements for the highest temperature (110 ºC) used which, at the same time, exhibited poor physical integrity during water immersion. Although the mechanical properties of the dried system obtained at 80 ºC are slightly inferior to those obtained for the non-dried 110 ºC system, these specimens are considered more adequate since they exhibited higher physical integrity and, consequently, better operating conditions.

Keywords: bioplastics; starches; injection-moulding.

1. INTRODUCTION

The annual production of plastics exceeded 368 million metric tons in 2019 [1]. One of the main environmental impacts of plastic production is pollution, caused by its difficult decomposition by microorganisms present in the soil [2]. One possible solution to solve this problem is the production of eco-friendly plastics, that is, bioplastics exhibiting biodegradable capacity. Bioplastics can be generated from two different sources: biodegradable polyesters (petroleum-based), and biopolymers from biodegradable resources [3].

Bioplastics formulated with plant proteins are becoming an increasingly popular source of raw materials for bioplastic products, since they are not only biodegradable but are also made from renewable resources, such as polysaccharides (i.e., starch), lipids and proteins [4]. Bioplastics from plant proteins have been already developed based on the protein content of plant protein concentrates commercially available, such as rice [5], pea [6], wheat gluten [7], and soy [8] among others. In addition, a large number of studies have been carried out using proteins, coming either from plant sources such as zein, wheat gluten or soybean [9–11] or from animal sources such as milk proteins, collagen or gelatin [12,13]. For this purpose, they are often mixed with certain plasticizers such as glycerol, sorbitol, water or ethylene glycol [14], to reduce the protein-protein interactions, replacing them with protein-plasticizer ones. Thus, the resulting material is suitable for processing by thermomechanical techniques [15]. However, the scientific literature related to the development of bioplastics based on their starch content is scarce except for the research related to thermoplastic starches processed by extrusion [16,17]

Starch can be found in different plants, such as wheat, maize, potato and rice [18]. This material has a granular structure, which consists of two main carbohydrate polymers: the linear amylose and the highly branched amylopectin. Although starch is in abundance all

over the world, there are not many applications in material science, since the granular structure must be totally destroyed in order to produce thermoplastic starch (TPS), a process called gelatinization. For this purpose, starch is processed under the action of temperature (between 70 and 90 ºC), shearing action and water excess. Although water is required, when used on its own it produces brittle products so it is often included with the aid of another plasticizer [19].

Proteins and carbohydrates are two basic food groups that can be found in wastes from the rice industry as is the case of rice bran (RB). RB is a residue from brown rice obtained during the rice milling process [20]. Nowadays, this by-product is mostly used for animal feeding due to its low price and nutritional richness [21]. Rice is the staple food for more than half of the world´s population, and yet by 2030, global rice production must doble to meet the demand [22] thus, emerging technologies that allow the valorization of its by-products are highly convenient. The European Commission has granted projects for a "near zero-waste" society such as the NoAW (No Agro-Waste) proposal [23]. In this sense, the development of innovative approaches that allow the conversion of growing agricultural wastes and by-products into eco-efficient bio-based products has been in the spotlight in recent years. In this way, RB could be transformed into high-added-value materials after suitable processing, meeting the requirements of circular economy, which aims at enhancing the continuous flow of technical and biological materials while keeping products, components and materials at their highest utility and value and reducing waste to a minimum [24].

This research work aimed to analyse the effect of the mixing temperature during the processing of RB-based bioplastics *via* injection moulding. The temperature employed during the mixing of the raw materials is believed to be a key factor in the properties of the final bioplastics specimens. In order to evaluate the effects of this parameter, different

mixing temperatures will be used characterizing the properties obtained by these means. The different temperatures were selected with respect to the gelatinization of starch, which is estimated to happen between 70 and 90 ºC. In this way, the authors aim to verify that temperatures around 80 ºC will allow starch gelatinization, producing materials suitable for injection mounding with proper mechanical and functional properties. In this process, the RB/plasticizer ratio was kept constant, whereas the mixing temperature varied from 50 to 110 ºC. To evaluate the effects of the different mixing temperatures on the final specimens, the mechanical properties were measured through frequency and temperature sweep tests (rheological characterisation) and tensile tests. Finally, water absorption tests were carried out to evaluate the water uptake capacity and soluble matter loss, whereas SEM microscopy was employed to study the surface morphology of the different specimens.

2. EXPERIMENTAL

2.1 Materials

The rice bran (RB) was provided by Herba Ingredients (San José de la Rinconada, Seville, Spain). The by-product supplied by this company is obtained from the polishing process of the rice variety "vaporized indica white rice". This variety contains for 7.06 ± 0.09% moisture, 10.50 ± 0.16% ashes, 13.22 ± 0.52% proteins, 22.77 ± 1.33% lipids and approximately 19 and 22 % starch and fibre, respectively (results not published yet). The remaining percentage, i.e., 46.45% of the sample, can be attributed to carbohydrates, according to previous works [25,26]. Water (W) and glycerol (Gly) were employed as plasticizers; the former was deionized-grade water, and the latter was supplied by PANREAC S.A. (Spain). All other reagents were supplied by Sigma Aldrich (USA).

2.2. Processing and characterization of blends

110 2.2.1. *Mixing of blends*

111 To obtain homogeneous blends, sieved RB (< 500 μm) along with water and glycerol
112 were introduced into a HAAKE POLYLAB QC (ThermoScientific, Germany) mixer
113 equipped with counter-rotating rotors. Water is needed in order to break the hydrogen
114 bonds present in starch, which allows its gelatinization, obtaining TPS [15]. In addition,
115 it is well established that, when used on its own, water (W) produces brittle products, thus
116 it is often used along with some other plasticizers such as glycerol (Gly) or sorbitol [27],
117 which are also compatible with proteins [8,28,29]. In this research work, different blends
118 containing 55% RB and 45% plasticizer mixture (2:1 W/Gly) were obtained by mixing
119 the raw materials for 1 h at 200 rpm, while different mixing temperatures (50, 80, 90 and
120 110 ºC) were analysed to evaluate the influence of the mixing temperature used on the
121 plasticization process of RB-based bioplastics.

122 2.2.2. *Drying of blends*

123 In general, the blends mixed at 50, 80, 90 and 110 ºC, which can be denoted as M50,
124 M80, M90 and M110, respectively, had to be subjected to drying to lose some of their
125 moisture content. This process was carried out in opened containers at room temperature,
126 achieving a final moisture content below 30%. Otherwise, the bioplastics obtained with
127 high moisture-containing blends exhibited voids and cracks that were easily observed,
128 probably due to the water excess that evaporated in the mould cavity, as can be seen in
129 Figure S1. It is worth mentioning that the boiling point of glycerol/water solutions is
130 strongly affected by concentration as reported in the literature [30]. Thus, the boiling point
131 of the solution containing 33,3% Gly in water is ca. 103ºC, which is clearly exceeded
132 inside the mould. During this drying process, they acquired a firmer appearance, which
133 was quantified by the determination of the linear viscoelastic moduli every 24 h until they
134 were ready for injection moulding. Only the blend obtained at 110 ºC was suitable for

injection right after the mixing stage (M110*) following the processing conditions mentioned above. This is because moisture was already lower than 30% after mixing at 110ºC. In this way, this system was the only one injected as soon as it was homogenised during the mixing stage.

2.2.3. Dynamic Mechanical Thermal Analysis (DMTA) of blends

A dynamic mechanical analyser (RSAIII, TA Instruments, USA) was employed in compression mode using a cylindrical geometry (8 mm in diameter) for each blend everyday during drying. Strain sweep tests were performed at constant frequency (1 Hz) and temperature to establish the linear viscoelastic region (LVR). Subsequently, the lower critical strain was selected to characterize the viscoelastic modulus at 1 Hz and room temperature. Finally, once the blends remained unchanged, a temperature sweep test was also carried out at 5 °C/min to study the rheological behaviour of the blends between 30 and 160 °C. In these measurements, the storage modulus (E'), loss modulus (E'') and loss tangent (tan δ) were determined for the whole temperature range studied. In addition, the water content was calculated during the drying process by following the A.O.A.C. methods [31]. In this way, 3 g of sample were placed in a conventional oven (Memmert B216.1126, Germany) at 105 ºC for 24 h to calculate the exact water content by mass difference.

For comparison purposes, the M110 blend, was also characterized during drying to evaluate this system processed under the same conditions.

2.2.4. *Scanning Electron Microscopy (SEM) of blends*

Scanning electron microscope (SEM) observations were carried out to determine the importance of the mixing temperature to produce the TPS material. For this purpose, two blends obtained for the same mixing velocity and time, but different mixing temperature

(room temperature, M20, and 80 ºC, M80) were compared to another blend obtained by milder conditions (M20mc, mixing for 10 min at 50 rpm and room temperature with no added water) trying to observe the starch structure with minimal modifications. The samples were first sputtered with a 10 nm thickness Pd/Au coating using an AC600 Metallizer (Leica, Germany), and then they were observed at 10 kV acceleration voltage and 500x magnification.

2.3 Processing and characterization of bioplastics

2.3.1. *Injection moulding*

Each resulting blend either mixed at 50, 80, 90 or 110 ºC after drying (M50, M80, M90 or M110) or at 110 ºC right after mixing (M110*), was introduced in the cylinder of a Haake pneumatic piston injection moulding equipment (MiniJet II ThermoScientific, Germany) at 50 ºC. The temperature of the mould was fixed at 150 ºC to favour the thermosetting of the specimens according to the results obtained from the temperature sweep tests of the blends and a previous study [32]. Regarding injection and holding pressures, they both were fixed at 500 bar, selecting 15 and 200 s for injection and holding time, respectively. By employing these conditions, 60 x 10 x 1 mm rectangular bioplastic specimens, denoted as I50, I80, I90, I110 and I110*, were obtained for subsequent characterization.

2.3.2. *Dynamic Mechanical Thermal Analysis (DMTA) of bioplastics*

DMTA tests were carried out with an RSA-III (TA Instruments, USA) on I50, I80, I90, I110 and I110* rectangular specimens using two grips, one at the bottom and one at the top, to study their rheological behaviour in tension mode. Firstly, strain sweep tests were performed to establish the LVR at 1 Hz and room temperature. Then, frequency sweep tests were carried out within the LVR at room temperature from 0.01 to 20 Hz. Finally,

183 temperature sweep tests were also carried out within the LVR at 1 Hz between 30 and
184 140 ºC using a heating rate of 5 ºC/min.

185 *2.3.3. Tensile tests of bioplastics*

186 Tensile tests were performed with the RSA III (TA Instruments, USA) equipment in
187 continuous deformation mode. Measurements were carried out according to the ISO 527-
188 2:2012 [33], using I50, I80, I90, I110 and I110* rectangular specimens and an extensional
189 rate of 1 mm/min at room temperature. Strain-stress curves were obtained for each sample
190 and three parameters were calculated: maximum tensile strength (σ_{max}), Young´s modulus
191 (E) and strain at break (ε_{max}).

192 *2.3.4. Water uptake capacity of bioplastics and soluble matter loss*

193 The absorption capacity of the bioplastic samples was evaluated by water uptake capacity
194 measurements following the ASTM D570 standard [34]. I50, I80, I90, I110 and I110*
195 rectangular specimens (20 x 10 x 1 mm) were used. According to the standard, the
196 samples were subjected to a dehydrothermal treatment in an oven at 50 ºC for 24 h to
197 determine the initial dry weight, followed by the weighing of the sample after immersion
198 in distilled water for 24 h. Finally, the specimens were subjected to freeze-drying in a
199 LyoQuest freeze-dryer with a Flask M8 head (Telstar, Spain). Water uptake capacity
200 (WUC) and soluble matter loss (SML) were determinate by Eqs. (1) and (2):

201 $$WUC\ (\%) = \frac{w_2 - w_3}{w_3} \cdot 100 \qquad (1)$$

202 $$SML\ (\%) = \frac{w_1 - w_3}{w_1} \cdot 100 \qquad (2)$$

203 Were w_1, w_2 and w_3 are the weights of the sample after the dehydrothermal treatment,
204 after the immersion step and after the freeze-drying stage, respectively. Samples obtained

after freeze-drying were denoted as F50, F80, F90, F110 and F110*, depending on the initial mixing and drying conditions of the blends used.

2.3.5. Scanning Electron Microscopy (SEM) of freeze-dried bioplastic matrices

The microstructure of each final bioplastic after the freeze-drying treatment (F50, F80, F90, F110 or F110*) was observed to determine the influence of the mixing temperature on the structure generated in the bioplastics after water absorption according to the protocol described by Julavittayanukul et al. [35]. The samples were first sputtered with a 10 nm thickness Pd/Au coating using an AC600 Metallizer (Leica, Germany), and then they were observed at 10 kV acceleration voltage and 500x magnification.

2.4 Statistical analyses

At least three replicates of each measurement were carried out. Statistical analyses were performed using t-test and one-way analysis of variance (ANOVA) (p <0.05) using the STATGRAPHICS 18 software (Statgraphics Technologies, Inc, NJ, USA). Standard deviations from some selected parameters were calculated. Significant differences are indicated by different letters.

3. RESULTS AND DISCUSSION

3.1. Characterization of blends

3.1.1. *Dynamic Mechanical Thermal Analysis (DMTA) of blends*

Two parameters were monitored to evaluate the drying process of the blends obtained after mixing: the elastic modulus at a constant frequency of 1 Hz (E_1') and the moisture content (%). Figure 1 shows the evolution of these two parameters over time for the four different blends obtained after mixing at 50, 80, 90 and 110 ºC (M50, M80, M90 and M110). As can be seen, the first three blends (M50, M80 and M90) underwent the same evolution, with E_1' increasing with time for the first 6-7 days and then remaining

unchanged until the end of its monitoring after 10 days of drying. The values obtained show no significant differences, with E_1' being slightly higher for the M80 and M90 blends. At the same time, the water content followed the same behaviour as the elastic moduli for the different blends, observing a stabilization after drying for six days. However, its values decreased during the firsts days, from 40 - 45% moisture right after mixing to less than 30% at the end of the experiment, for all the studied systems. At this point, the three blends were suitable for injection moulding. Moreover, the relationship between both parameters shows that the elastic modulus increased when the moisture content decreased, which reflects the plasticizer effect of the water contained in the sample [36].

On the other hand, the M110 blend did not follow such a clear tendency. Right after mixing (day 0), E_1' values were higher than the ones obtained for the other blends and, contrary to the increasing tendency observed before, the values for this system remained practically unchanged. Regarding water content, right after mixing, the moisture content was low, near 20 %, due to the high temperature employed in this process (110 ºC). However, after 1 day, the M110 blend absorbed some moisture causing the observed fluctuation. It is worth mentioning that, after several attempts, it was found that blends were only suitable for injection moulding when the water content was below 30 %, and no successful processing was achieved for higher values. Therefore, the moisture content of the blends was found to be the critical parameter to be evaluated before proceeding with injection moulding. Thus, as mentioned above, excess water (i.e. moisture higher than 30%) seems to lead to severe water/glycerol evaporation inside the mould cavity, which is maintained at 150ºC, causing the formation of voids and cracks as can be seen in Figure S1.

As can be observed in Figure 1, the only blend that can be readily injected after mixing is the one mixed at 110ºC, denoted as M110* to distinguish it from the dried blends. This blend was also injected after 10 days of drying as the other systems for comparison purposes, which also ensures that the moisture content was below 30%.

Once the blends were stabilized (i.e., 10 days after mixing), temperature sweep tests were carried out between 30 and 160 ºC using a heating rate of 5 ºC/min. Figure 2 shows the evolution of the elastic and viscous moduli (E' and E'', respectively) with temperature. Although all blends showed a predominantly elastic character, with the elastic modulus being higher than the viscous modulus for the whole range studied, different behaviours can be observed when the temperature increased. First, the M80 and M90 blends, show a decrease in E' and E'' with increasing temperature up to 100-120 ºC (from 2.6 and 1.5 MPa to 1.1 and 0.7 MPa, respectively). Then, both moduli increased again up to 2.0 and 1.3 MPa, tending to equilibrate at high temperature. For the whole temperature range, both viscoelastic moduli obtained for the M80 blend are higher than those obtained for M90. On the other hand, the M50 blend followed the same tendency for the first part of the test (between 30 and 100-120 ºC). However, above this temperature, the viscoelastic moduli underwent an abrupt decrease accounting for the lowest measured values. Finally, the blend mixed at 110 ºC was characterized after drying for 10 days (M110), just like the other systems, and right after mixing (M110*). Both blends followed a very similar tendency. At the beginning of the experiment (between 30 and 70 ºC), the viscoelastic moduli were higher for the M110* than for M110 (e.g. E' was around 1.0 and 0.6 MPa, respectively). Between 70 and 110 ºC, both blends followed the same behaviour slightly decreasing with increasing temperature (e.g. from 0.3 to 0.2 MPa). However, both systems diverge above 110 ºC up to the end of the experiment, being again M110* above M110 for the higher temperatures used (at which E' values drops from 0.09 to 0.04 MPa,

278 respectively). As can be seen, M80 and M90 were the only blends that showed certain
279 thermosetting potential, with both moduli increasing above 110 ºC up to 150ºC. For this
280 reason, and based on the results obtained in previous studies by Alonso-González et al.
281 [32], 150ºC was selected as the temperature of the mould for injection moulding. It can
282 be assumed that the higher temperatures used hinder the thermosetting potential of the
283 mixed blends leading to lower viscoelastic moduli for the whole studied range.

284 The thermosetting potential exhibited by the M80 and M90 samples has been previously
285 observed for pea protein-based blends prepared using a similar procedure at lower
286 temperatures [37]. On the other hand, Felix et al. [38] produced crayfish-based bioplastics
287 from protein/glycerol blends whose viscoelastic moduli upon temperature sweep tests
288 exhibited a decreasing tendency during the whole studied range, thus no thermosetting
289 potential was observed, showing a behaviour similar to that recorded for the two blends
290 mixed at 110 ºC.

291 3.1.2. *Scanning Electron Microscopy (SEM) of blends*

292 The SEM images revealed that after mixing with mild conditions the M20mc blend still
293 contains some granular starch (Figure 3A). It can be observed that the addition of water
294 while mixing at 200 rpm for 1 h leads to a blend (M20) where the granular structure was
295 partly destroyed (Figure 3B). This structure disappeared completely for the M80 sample
296 (Figure 3C), indicating that the mixing temperature is a key parameter to obtain TPS. Tábi
297 et al. [39] obtained similar SEM images during TPS obtention through extrusion and
298 injection moulding.

299 **3.2. Characterization of bioplastics**

300 3.2.1. *Dynamic Mechanical Thermal Analysis (DMTA) of bioplastics*

The results obtained for the frequency sweep tests between 0.01 and 20 Hz at room temperature are shown in Figure 4 for all the bioplastics injected using either the blends mixed at different temperatures and dried for 10 days (I50, I80, I90 and I110) or with no drying (I110*). The mechanical spectra obtained shows that E' was always higher than E'', which indicates that all specimens exhibit a predominantly elastic character [40]. The viscoelastic moduli show a moderate frequency dependence, following a power-law tendency to increase with increasing frequency. The power-law exponent ranges from 0.15 for I50 to 0.20 for I110*. This rheological behaviour has been previously observed for protein-based bioplastics which exhibited slightly lower power-law exponents from 0.15 to 0.18 [41] and from 0.12 to 0.14 [42] for the soy/nanoclay based composites which seems to indicate that the starch content induces stronger frequency dependence. In addition, it should be highlighted that the higher viscoelastic moduli are found for the bioplastics obtained from the blend injected right after mixing, with no drying process, being clearly superior as this system exhibits 180 and 55 MPa for the elastic and viscous moduli, respectively, which are clearly above the second highest one, which would be the I90 system, with 68 and 23 MPa for these two parameters. The other four systems showed lower viscoelastic moduli, especially the probe obtained from I110, with E'_1 being ca. 30 MPa, and the other three having higher and more similar values (ca. 46 and 60 MPa for I50 and I80, respectively). This behaviour could be related to the fact that the system obtained from the blend without drying, was injected with lower moisture content (~22%), as a result of the higher mixing temperature and no water absorption during drying. This fact involved a lower content of plasticizer which contributed a higher viscoelastic response. However, the lower proportion of water in the plasticizer also led to an increase in its boiling point, giving rise to a reduction in the evaporation inside of the mould cavity [30]. The other systems (dried ones) were injected with similar water

contents (< 30%), and thus showed more similar values. However, the higher temperatures used for the mixing stage led to slightly higher values, indicating the convenience of increasing temperature during starch gelatinization. Regarding the I110 bioplastic, the water absorbed during drying did not take place in the gelatinization process, only contributing to hindering the injection moulding stage, leading to poorer results.

The systems were also subjected to temperature sweep tests between 30 and 140 ºC (Figure 5). In this case, all specimens, except the one obtained from the dried blend mixed at 110 ºC (I110), followed the same behaviour, with E' and E'' decreasing with increasing temperature up to 90-100 ºC and reaching, at this point, a plateau value, with the viscoelastic moduli remaining almost unchanged. In addition, the elastic moduli began clearly differentiated at 30ºC, with the I110* system showing the best results (127 and 48 MPa for E' and E'' values, respectively), followed by the I80 and I90 systems, which exhibited similar results, with 71 MPa for E' and 23-25 MPa for E'', for the two different systems), with the 50 ºC system showing the worst results (E' = 36 MPa and E'' = 10 MPa). Although, once the plateau value was reached, they all showed more similar values, being the elastic modulus around 13 MPa and the viscous one approximately 3 MPa. Finally, the specimens obtained from the dried blend I110 began with the same tendency, where both moduli decrease with increasing temperature up to 90 ºC, with the elastic modulus decreasing from 16 MPa to 6 MPa although, at this point, E' and E'' underwent a more abrupt decrease (about one order of magnitude), and then reached a steady value around 120-140 ºC, with E' stabilizing at around 0.7 MPa. Again, this differentiated behaviour could be attributed to the fact that this system was the only one absorbing water after the mixing stage. Thus, this water did not ease the production of TPS but hindered the injection of the specimens resulting in higher sensitivity to temperature. Similar

tendencies can be observed for the same tests performed on defatted rice bran with similar protein/starch composition processed by extrusion [17] and also with other protein-based bioplastics [43].

3.2.2. Tensile tests of bioplastics

Figure 6 shows the stress-strain curves obtained from the tensile tests of all the samples evaluated in this work. All systems exhibited a similar behaviour. The system not submitted to drying exhibited the highest stiffness, although it only achieved the second-lowest elongation at break. The rest of the curves presented much more similar tendencies with varying parameters, with the I80 system showing the higher slope, followed by the I90 system, and finally by the I50 and I110 specimens. Regarding toughness, although the I90 specimens presented poorer stiffness than I80 samples, the former exhibited greater elongations, leading to similar areas under the curve and therefore similar toughness. To finish with, the two systems with similar slopes (50 and 110 ºC) exhibited quite different elongations, with the system processed at 50 ºC being the one with the greatest toughness and the I110 system being the one with the worst tensile properties.

Table 1 allows a more accurate evaluation of the three parameters obtained from the stress-strain curves: Young´s modulus (E), maximum tensile strength (σ_{max}) and strain at break (ε_{max}). Beginning with the four systems obtained from the dried blends (I50, I80, I90 and I110), it can be seen that all parameters improved when the mixing temperature increased from 50 to 80 ºC, although further increases had no beneficial effects, especially for the system processed at 110 ºC, which is in agreement with the previously mentioned detrimental effect for the water absorption during drying. In this way, E increased from 10 ± 1 to 33 ± 6 MPa when the temperature of the mixing stage increased from 50 to 80 ºC, although lower values (20 ± 1 and 10 ± 1 MPa) were obtained for the I90 and I110

systems, respectively. Again, σ_{max} reached a maximum value for the I80 system, showing its minimum value for I110 specimens. Finally, ε_{max} also improved from I50 to I80 and I90 systems, being the only parameter that displayed higher values for I90 than for I80 bioplastics. The dried system mixed at 110 ºC (I110) still showed the lowest value for the final elongation. Similar values have been found for protein [44] and starch-based [17] systems obtained by similar processing techniques.

On the other hand, the system with the highest Young´s modulus and tensile strength is the only one that was not subjected to the drying process. The only property that did not improve in this case was the elongation at break, reaching the poorest values (1.0 ± 0.1%.) The obtained results indicate that higher temperatures during mixing lead to stiffer materials with lower elasticities. This last behaviour is typical of protein-based systems, where E increases with the processing temperature, whereas the elongation at break does not [8].

3.2.3. Water uptake capacity of bioplastics and soluble matter loss

Figure 7A shows the water uptake capacity values for the different bioplastics obtained. The highest values are those obtained for the systems mixed at the highest temperature, either with the dried (I110) or non-dried systems (I110*), achieving 234 ± 21%, and 252 ± 75%, respectively. The other three systems (I50, I80 and I90) exhibited lower absorption capacities, not showing any significant difference among them. These values of water uptake capacity are between those typically obtained by hydrophilic protein-based bioplastics [37,45,46] and those shown by starch-based specimens [47,48].

High water uptake capacities correspond to high soluble matter losses, as can be seen in Figure 7B. In this way, the systems which underwent the highest losses were the two obtained from the blends mixed at 110 ºC, being 37 ± 7 and 37 ± 16% for the dried (I110)

399 and non-dried (I110*) systems, respectively. In addition, these two systems did not
400 maintain their integrity during water immersion, appearing fissures in the specimens,
401 which resulted in higher specific surfaces and, thus, higher water uptake capacity values.
402 The appearance of the fissured specimens can be seen in Figure S2. On the other hand,
403 the I50, I80 and I90 systems exhibited lower values, especially the last one with 25 ± 1%.
404 The other two specimens (I50 and I80) obtained SML values of 31.6 ± 0.3% and 27 ±
405 1%, respectively. This result is also in line with the ones reported in the aforementioned
406 study [43].

407 These results reveal the importance of selecting a proper mixing temperature. When the
408 temperature was fixed to 50 ºC, the gelatinization of starch is not achieved, leaving a
409 partial granular structure with poorer processability. Although the physical integrity is
410 not compromised, the inferior mechanical properties evidence this fact. However,
411 temperatures of 80 and 90 ºC allow complete gelatinization during mixing, providing a
412 material suitable for injection moulding that undergoes retrogradation and some
413 thermosetting phenomenon in the case of the protein fraction, resulting in specimens with
414 both good mechanical performance and physical integrity. Finally, the poorer physical
415 integrity provided by the higher temperature used could be because water is already lost
416 during mixing at 110 ºC, leading to an early starch retrogradation even before injection,
417 being the bioplastic sample entirely formed through protein-related events. Regarding the
418 lipid and fibre fractions, no significant effects are expected during the bioplastics
419 processing since rice bran does not undergo any changes before 150 ºC in thermal analysis
420 [26]. However, certain fibre concentrations might have a reinforcing effect upon final
421 properties [49].

422 *3.2.4. Scanning Electron Microscopy (SEM) of freeze-dried bioplastic matrices*

Figure 8 shows the SEM micrographs of the different bioplastics obtained after the freeze-drying stage. As can be seen, the observed morphologies match the results obtained before, especially regarding functional properties, in such a way that greater surface homogeneities can be associated with higher WUC, improving from samples F50 and F80 (micrographs A and B) to samples F110 and F110* (micrographs C and D). It can be assumed that this higher temperature led to fewer voids and fissures, and thus a more homogeneous distribution of the active matter, which translates into higher absorption capacities. The obtained micrographs are similar to those obtained by Tábi et al. [39] and Ferreira et al. [50] for starch-based samples processed using water as the plasticizer.

4. CONCLUSIONS

The selected conditions, compatible with both proteins and starches, led to the successful development of RB-based bioplastics by injection moulding. The mixing stage, the one including the parameter under study, produced different blends that showed rheological differences during and after drying. Those differences made one of them (the M110* system) suitable for injection moulding right after mixing while the rest of them had to undergo an drying stage in order to lose some water before being subjected to injection moulding. It was concluded that, under the experimental conditions selected for the injection moulding process, moisture of blends had to achieve a content lower than 30% to obtain proper specimens. This limitation may be explained in terms of a reduction in the boiling point of the Gly/W solution with increasing water that may induce excessive evaporation from inside the mould cavity.

The mechanical characterization carried out on the bioplastic samples revealed two superior specimens in terms of rheological and tensile parameters: the system processed at 110 ºC without drying (I110*) and the dried system mixed at 80 ºC (I80). When water uptake capacity was measured, the two systems mixed at the highest temperature (I110

and I110*) exhibited clearly improved values compared to the others, although they exhibited much poorer physical integrities, which could be due to their high soluble matter losses and the fact that they started to lose integral consistency upon water immersion. These two differentiated behaviours could be associated with different morphologies observed in the SEM micrographs, where the more homogeneous surfaces correspond to the systems processed at higher temperatures (110 ºC) leading to a more successful injection-moulding processed and a greater distribution of the active matter.

This work evidences the possibility of manufacturing bioplastics from RB, as well as the effect of water as a plasticizer, which, on the one hand, is required during the mixing stage, and, on the other hand, determines the final mechanical and functional properties, based on its content. A proper mixing temperature (i.e., 80 ºC) is required to produce suitable bioplastics with balanced properties. Furthermore, the measured properties seem to be convenient for those applications that require materials exhibiting high water absorption behaviour and still require suitable end-use mechanical properties, ensuring their physical integrity. This should be the case of biodegradable water absorption materials for healthcare, agriculture or horticulture applications. In addition, the study of water uptake capacity in bioplastic systems is receiving increasing attention recently because of their important applications in the fields of biomedical, pharmaceutical, environmental and agricultural engineering.

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FIGURE CAPTIONS

Figure 1: Fit of the elastic modulus at 1 Hz (E_1') and moisture content over drying time of the M50, M80, M90 and M110 blends. The injectability limit (i.e. the maximum moisture content below which the blends can be injected) is also shown.

Figure 2: Temperature sweep tests at 5ºC/min between 30 and 160 ºC for the blends obtained after mixing at different temperatures and drying for 10 days (M50, M80, M90 and M110) or mixed at 110 ºC with no drying (M110*).

Figure 3. SEM micrographs of the blends obtained by mixing (A) at 50 rpm for 10 min, room temperature and no added water (M20mc) (B) at 200 rpm for 1h and room temperature (M20) and (C) at 200 rpm for 1h and 80 ºC (M80).

Figure 4. Frequency sweep tests between 0.01 and 20 Hz for the bioplastics obtained from the different blends after drying for 10 days (I50, I80, I90 and I110) and with no drying (I110*).

Figure 5. Temperature sweep tests at 5ºC/min between 30 and 140 ºC for the bioplastics obtained from the different blends after drying for 10 days (I50, I80, I90 and I110) and with no drying (I110*).

Figure 6. Stress-strain curves from the tensile tests performed on the bioplastic samples obtained from the different blends (I50, I80, I90 and I110) and with no drying (I110*).

Figure 7. (A) Water uptake capacity (WUC) and (B) soluble matter loss (SML) of the bioplastic samples obtained from the different blends: mixed at 50, 80, 90 and 110 ºC after drying and mixed at 110 ºC with no drying (110 ºC*).

Figure 8. SEM micrographs of the bioplastic samples obtained after freeze-drying of bioplastics processed from different blends (A) F50, (B) F80, (C) F110 and (D) F110*.

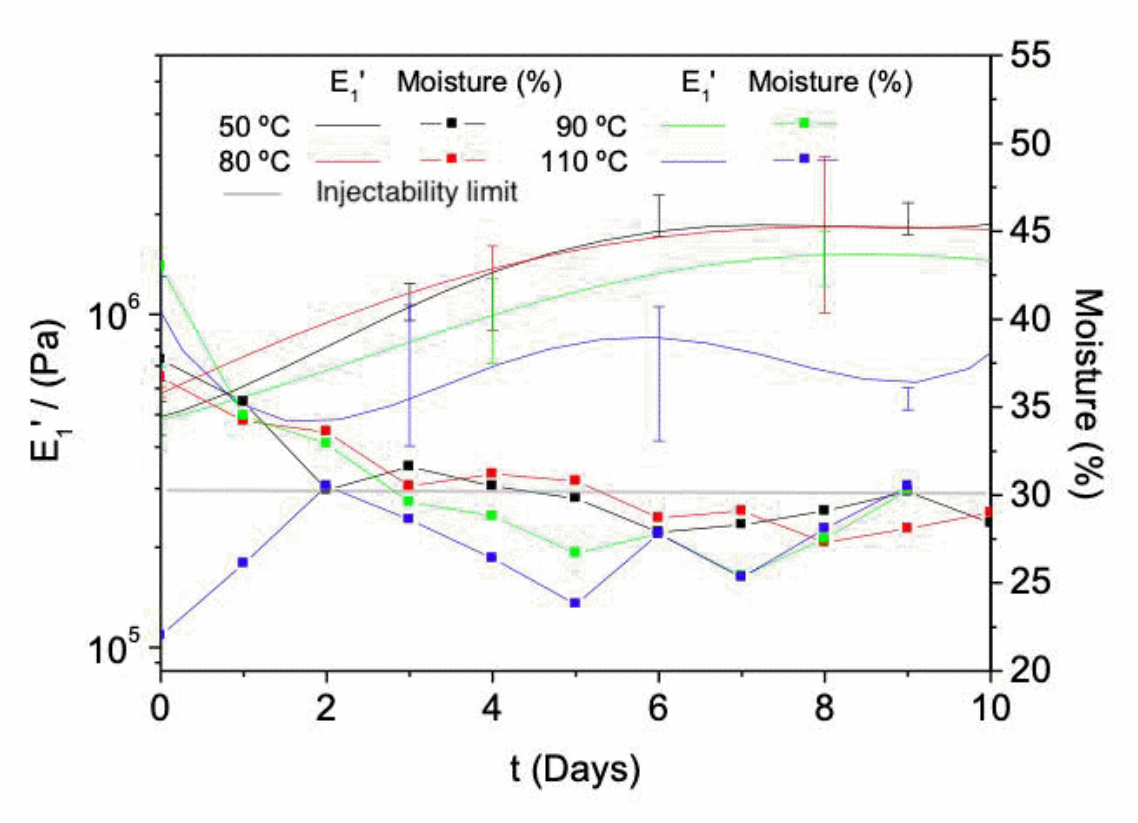

Figure 1.

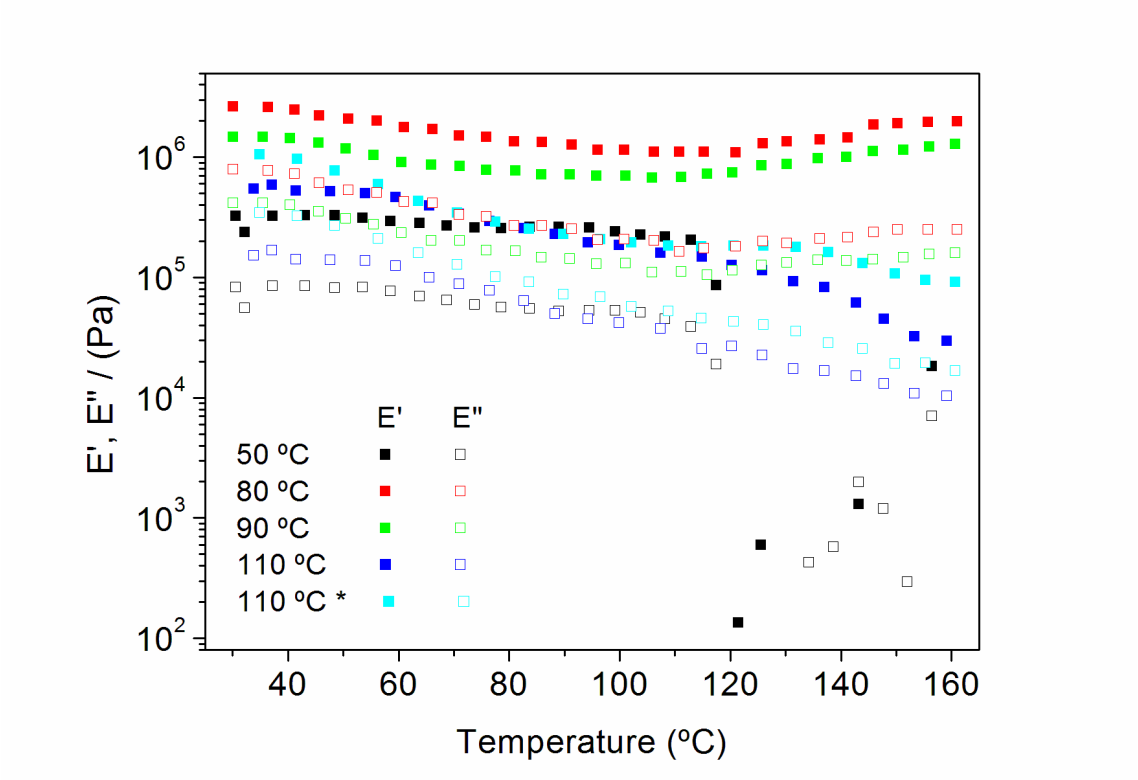

Figure 2

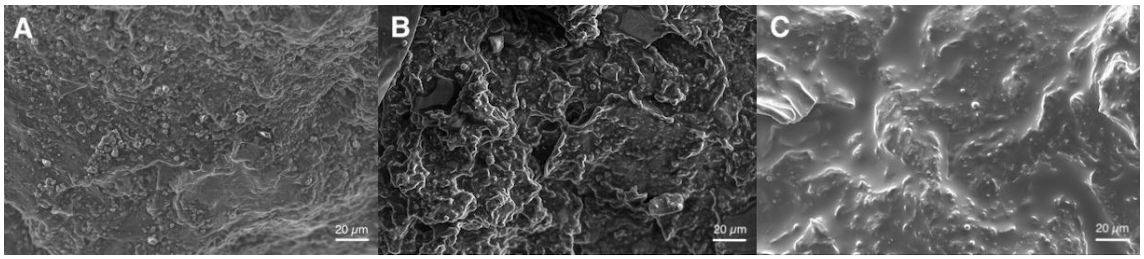

Figure 3

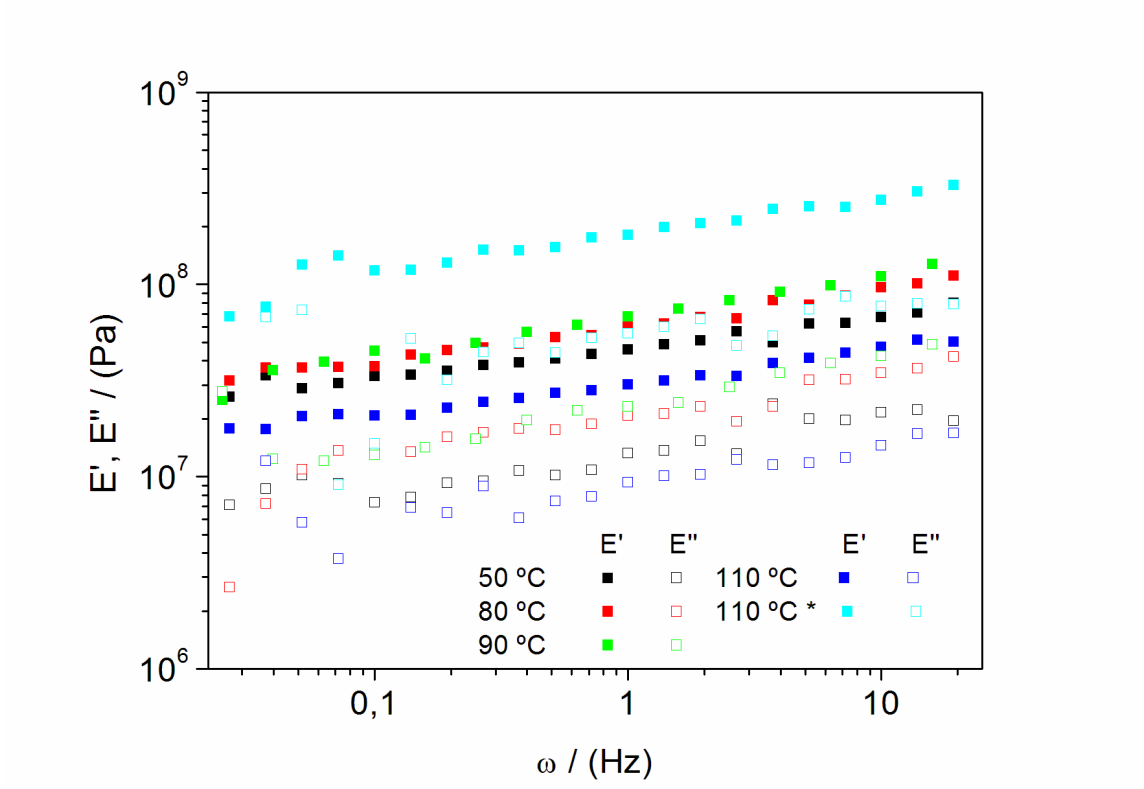

Figure 4

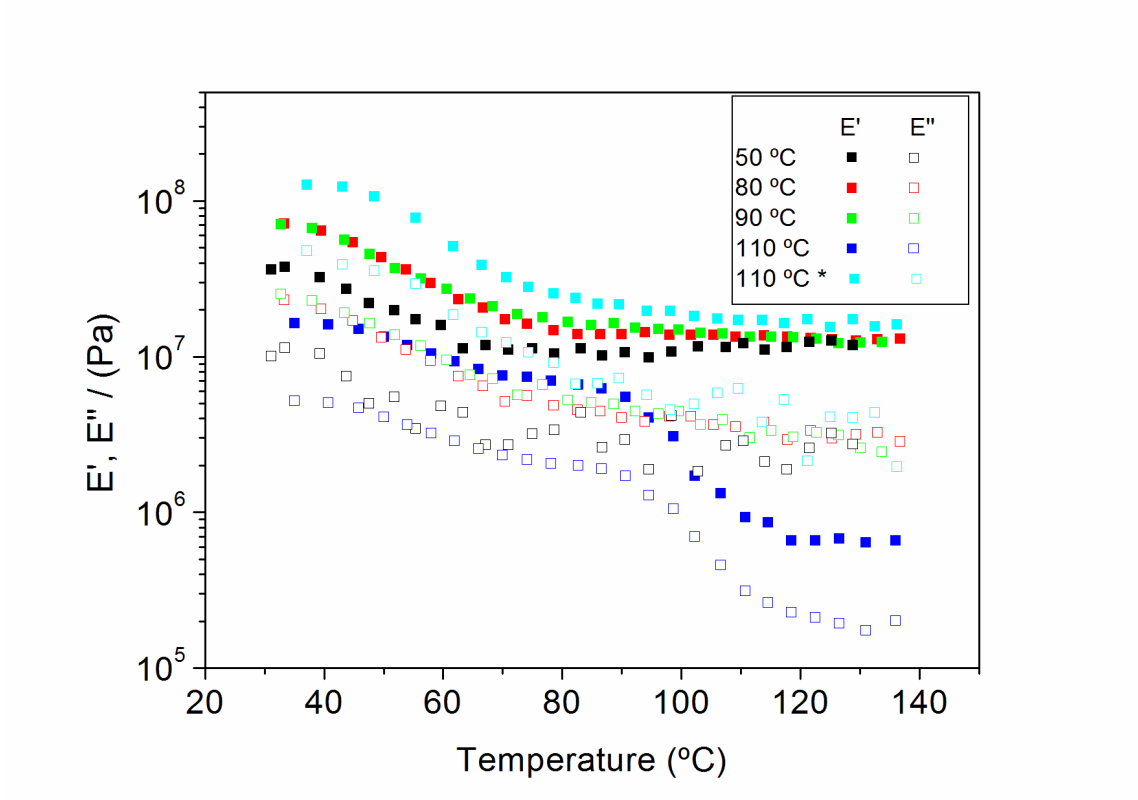

Figure 5

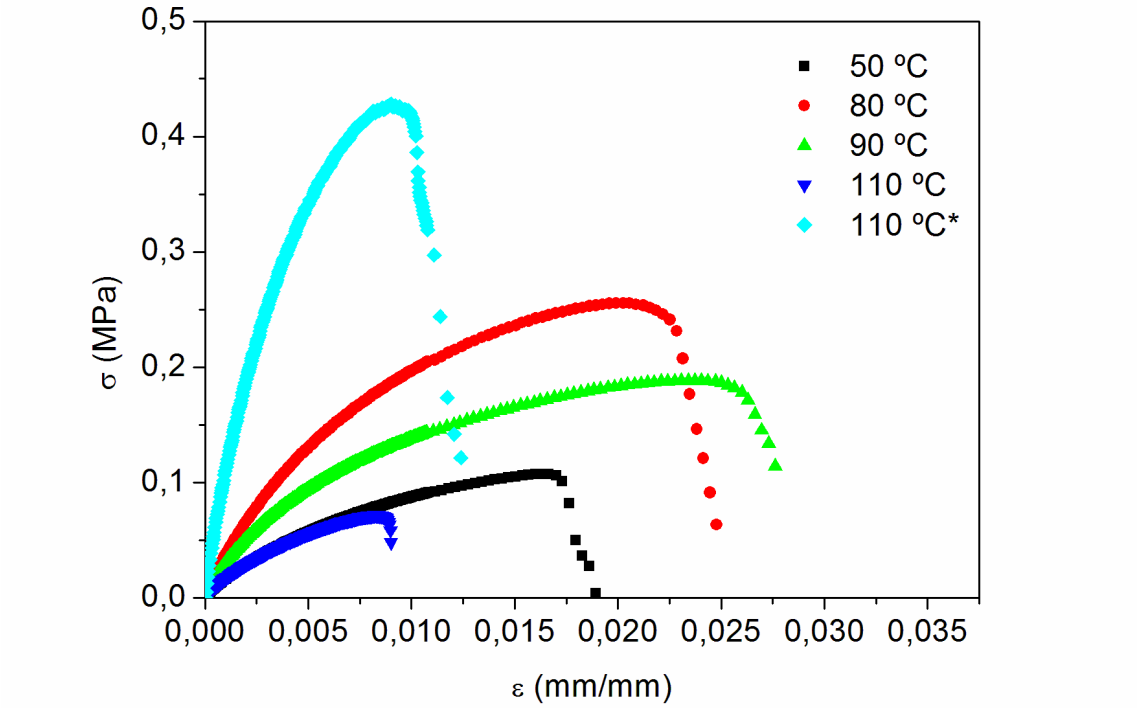

Figure 6

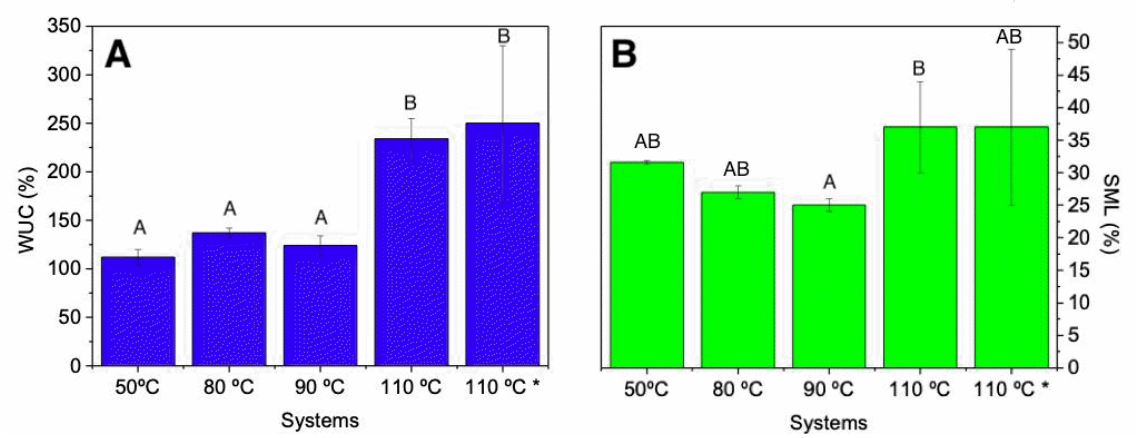

Figure 7

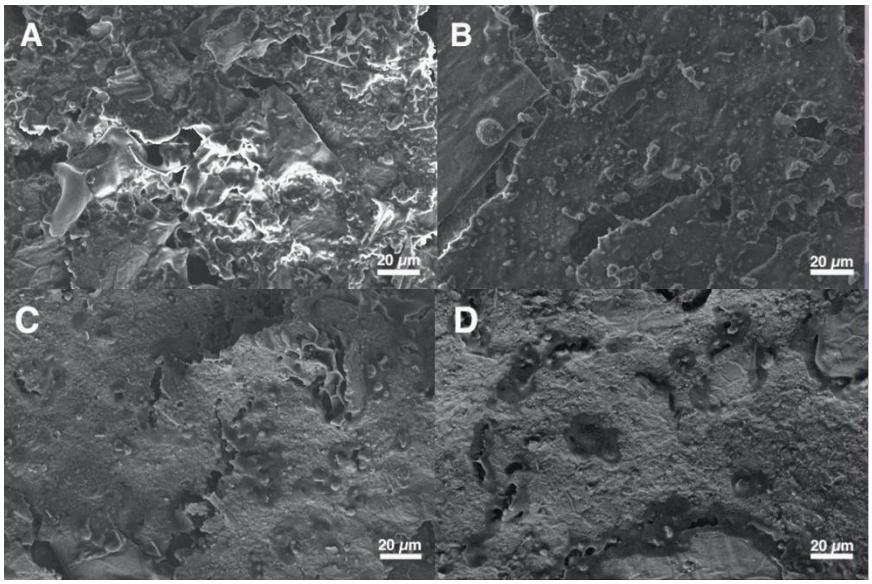

Figure 8

TABLE CAPTIONS

Table 1: Young´s modulus, maximum tensile strength and Elongation at break of the different processed systems. Different letters within a column indicate significant differences (p<0.05).

System	*E (MPa)*	σ_{max} (MPa)	ε_{max} (%)
I50	10 ± 1^{A}	0.12 ± 0.01^{A}	1.9 ± 0.2^{BC}
I80	33 ± 6^{C}	0.27 ± 0.01^{B}	2.4 ± 0.3^{C}
I90	20 ± 1^{B}	0.19 ± 0.01^{C}	2.5 ± 0.5^{C}
I110	10 ± 1^{A}	0.073 ± 0.006^{D}	1.4 ± 0.5^{B}
*I110**	80 ± 1^{D}	0.43 ± 0.04^{E}	1.0 ± 0.1^{A}