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Influence of the plasticizer on rice bran-based eco-friendly bioplastics obtained by injection moulding

María Alonso-González [a],[*],[1], Manuel Felix [b], Alberto Romero [a]

[a] *Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, 41012 Sevilla, Spain*
[b] *Departamento de Ingeniería Química, Escuela Politécnica Superior, Universidad de Sevilla, 41011 Sevilla, Spain*

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ABSTRACT

The manufacture of eco-friendly bioplastic materials from renewable resources to replace petroleum-based plastics has attracted increasing attention. For many years, proteins, lipids and polysaccharides have been proposed as natural biopolymers sources to obtain biodegradable plastic materials. As by-product from the rice industry, rice bran, is an available and non-expensive resource of both proteins and starches, food groups that, properly processed, can be employed in the development of bioplastics. Plasticizers are essential for the manufacture of bioplastics and, when carbohydrate/protein mixtures are used, an adequate selection of plasticizers must be addressed. By these means, a material suitable for thermo-mechanical processing methods is obtained if starches are subjected to shear forces under high temperatures and water excess (gelatinisation). Moreover, additional polyol-based plasticizers, such as glycerol and sorbitol, allow obtaining reinforced products with improved elasticity when protein-based bioplastics are processed. The aim of the present study was to analyse the plasticizing effect of water combined with different proportions of glycerol or sorbitol, as well as their influence on the final bioplastic properties. Results indicate that higher water ratios produce stiffer bioplastics with improved viscoelastic moduli, maximum stress and Young's modulus, while increasing the glycerol and sorbitol content leads to higher elasticities and water uptake capacities in general. Moreover, sorbitol seems to provide more suitable bioplastics with better tensile (up to 500% in Young's modulus) and functional properties compared to glycerol.

1. Introduction

Nowadays, the world shifts towards a circular, sustainable economy while the production and use of energy, the management of resources and the production and consumption patterns are being revised, especially those concerning the excessive consumption of single-use plastics, due to their negative impact on the environment (Chong et al., 2021b; Huneault and Li, 2007; Zhang et al., 2019). Bioplastics have become very attractive to many materials researchers as substitutes for conventional petroleum-based plastics, as society needs to rely on renewable raw materials, stop depending on fossil resources and reduce carbon emissions. Bioplastics are either biobased, biodegradable or both. In this way, biopolymer-based plastics avoid the use of fossil resources by using renewable biomass and being also biodegradable (Chong et al., 2021a; Pavlovskay et al., 2020).

Numerous plant proteins (i.e. corn zein, wheat gluten, soy proteins…) and animal proteins (i.e. milk proteins, collagen, gelatin, keratin…) can be used for the development of bioplastics (Chong et al., 2021a; Cuq et al., 1998). Proteins are specifically characterised by their potential reactivity to cross-linking (Félix et al., 2016). Thus, protein-based materials can be defined as stable three-dimensional macromolecular networks stabilised by low-energy interactions and strengthened by covalent bonds. The thermoplastic behaviour of proteins has been studied and used to manufacture bioplastics by thermal or thermo-mechanical processing methods. It is well known that the glass transition, characterised by the change from a glassy state to a rubbery one, is affected by the presence of plasticizers, with T_g decreasing with increasing plasticizer content improving, by these means, the processability of proteins. The plasticizer nature and content greatly influence the properties of the materials (Cuq et al., 1998; López-Castejón et al., 2015; Orliac et al., 2003). Regarding polysaccharides, starch is considered an attractive and cheap source to be used as a biodegradable

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material suitable to replace, to some extent, synthetic polymers. It is found in nature in the form of granules comprised of a biopolymer mixture of a linear homopolymer, amylose, and a highly branched one named amylopectin. The granules they form are water-insoluble at ambient temperature, whereas when heated in excess of water at temperatures around 70 °C, they undergo a gelatinisation process. During this process, they swell by absorbing water, losing the granular structure (Cooke and Gidley, 1992), and thus they can be processed by thermo-mechanical techniques (Srinivasa Rao et al., 2022). Although it is required for gelanitization, water on its own produces brittle products. Therefore, in order to produce successful bioplastics, other plasticizers are usually added, such as glycerol or sorbitol, plasticizers that, which at the same time, are also compatible with proteins.

Some studies have reported that some natural biomasses such as algae can contribute to reducing CO_2 emission (Abdul-Latif et al., 2020). However, these two basic biopolymers (proteins and polysaccharides) can be also found in wastes and by-products from the food industry (Alsafadi et al., 2020; Tsang et al., 2019), which would reduce the dependence of future bioplastics on a single specific source of raw material. For example, rice is the major cereal crop cultivated in the world. It is generally processed by shelling and polishing to remove the bran, which constitutes approximately 10% of the rice weight, being still an underutilized by-product (Luh and Mickus, 1991). It is used for the extraction of edible oil (Kim et al., 2001) and, after oil extraction, defatted rice bran is used as animal feed or boilers fuel, despite being a rich source of carbohydrates and proteins (Saunders, 1990). Due to its abundance and low cost, rice bran constitutes a good source of biopolymers for bioplastic production, which would benefit from its valorisation, becoming a by-product with high added value. Starch- and protein-based biopolymers have already been used for manufacturing bioplastics using thermomechanical techniques with the aid of certain plasticizers (Alonso-González et al., 2021c; Mutmainna et al., 2019); however, the plasticizing effect of glycerol, sorbitol and water has not been widely investigated with a combination of protein and, as it is the case of rice bran.

Bio-based, biodegradable plastics could overcome the environmental and sustainability issues posed by the production and disposal of synthetic plastics. However, their large scale commercial use to replace conventional plastics is still challenged due to different reasons, such as their relatively poor performance. For these reasons, it is important that the scientific community continue their research to improve the properties of bioplastics. In this way, the aim of the present study was to evaluate the potential of glycerol and sorbitol as plasticizers for the production of rice bran-based bioplastics. As was previously mentioned, water is required for starch plasticisation. Thus water was added along with glycerol or sorbitol in different ratios, 4:1, 2:1, 1:1, 1:2, 1:4 and 0:1 (water/glycerol and water/sorbitol), to study the effect of the plasticizers and their combination on the properties of the final bioplastics. To this end, the obtained specimens will be characterised by rheological, tensile, water uptake and scanning electron microscopy measurements.

2. Experimental

2.1. Materials

The rice bran (RB) employed in this work was a by-product obtained from the polishing process of the vaporised indica rice variety (supplied by Herba Ingredients, Seville, Spain). This variety accounts 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% content of starch and fibre, respectively (Alonso-González et al., 2021a). The composition was calculated following the A.O.A.C. methods (AOAC, 2005). Water, glycerol and sorbitol were the plasticizers used in this research for the RB-based bioplastics generated. The former was deionized-grade water, whereas the glycerol and sorbitol were purchased from PANREAC S.A. (Barcelona, Spain). All other reagents were supplied by Sigma Aldrich

(Missouri, USA).

2.2. Bioplastics processing

2.2.1. Mixing stage

RB was sieved using a 500 μm mesh. Once sieved, the RB < 500 μm was introduced along with the plasticizers into a HAAKE POLYLAB QC (ThermoScientific, MA, USA) mixer-rheometer, equipped with counter-rotating rotors, obtaining homogeneous blends. Two different plasticizers (glycerol or sorbitol) in combination with water were evaluated in this work. All the blends contained 55% RB and 45% total plasticizer and were mixed for 1 h at 200 rpm and 80 °C inside the mixing chamber of the HAAKE POLYLAB QC. The conditions were stablished based on previous researchs (Alonso-González et al., 2022, 2021b, 2021a). All the formulations analysed are gathered in Table 1.

2.2.2. Drying of blends

Although water is needed during the mixing stage to aid starch gelatinisation, once the blends are plasticized, water excess can hinder injection moulding, since it can evaporate inside the mould cavity, causing voids and cracks when the mould temperature is above 100 °C (Alonso-González et al., 2021a). For this reason, the moisture of the obtained blends must be readjusted prior to injection moulding (Alonso-González et al., 2021a). Thus, right after mixing, the blends were kept inside a desiccator until the moisture content was low enough for them to be properly processed. This final content varies depending on the plasticizer combination used. Therefore, when glycerol/water mixtures were used as plasticizer, the moisture was adjusted to approximately 30% for the 3 systems with lower glycerol content, that is, the 2:1 G, 1:1 G and 1:2 G and it had to be decreased down to 20% and 15% for the 1:4 G and 0:1 G systems., However, the blends prepared with sorbitol/water as plasticizer required 10% moisture. The water content was calculated during the drying process following the A.O.A.C. methods (AOAC, 2005). In this way, 3 g of sample were placed in a conventional oven (Memmert B216.1126, Schwabach, Germany) at 105 °C for 24 h to calculate the water content by mass difference.

2.2.3. Injection moulding

The blends were processed by injection moulding using the MiniJet II (Haake, ThermoScientific, MA, USA) as pneumatic piston injection moulding equipment to obtain the bioplastic specimens. The temperature for the injection cylinder was 50 °C, whereas the temperature of the mould was fixed to 150 °C to favour the thermosetting of the probes according to previous studies (Alonso-González et al., 2021b). The injection time was 15 s at 500 bar and the post-injection time was 200 s at 500 bar. By employing these conditions, rectangular bioplastic matrices measuring 60 × 10×1 mm were obtained for further characterisation.

2.3. Bioplastics characterisation

2.3.1. Dynamic mechanical thermal analysis (DMTA)

DMTA tests were carried out in tension mode using an RSA-III rheometer (TA Instruments, MA, USA) on rectangular probes, to study their rheological behaviour. Firsty, strain sweep tests were performed to

Table 1
Combinations of the evaluated plasticizers (water, glycerol and sorbitol). RB proportion remained constant (55%).

System denomination	Water (%)	Glycerol (%)	System denomination	Water (%)	Sorbitol (%)
			4:1 S	36	9
2:1 G	30	15	2:1 S	30	15
1:1 G	22.5	22.5	1:1 S	22.5	22.5
1:2 G	15	30	1:2 S	15	30
1:4 G	9	36	1:4 S	9	36
0:1 G	–	45	0:1 S	–	45

establish the linear viscoelastic region (LVR) at 1 Hz and room temperature. Then, frequency sweep tests were carried out within the LVR at room temperature from 0.04 to 20 Hz. Moreover, temperature ramp tests were also carried out within the LVR at 1 Hz between 30 and 140 °C using a heating rate of 5 °C/min. The elastic modulus (E'), the viscous modulus (E'') and the loss tangent (*tan δ*) were measured for the whole range evaluated. The dependence of viscoelatic moduli on frequency has been fitted to Eq. (1):

$$E^{'}(\omega) = A \cdot \omega^n \qquad (1)$$

2.3.2. Tensile tests

Tensile tests were performed using the RSA III (TA Instruments, MA, USA) equipment in continuous deformation mode. The strain-stress curves were obtained according to a modification of the ISO 527–2:2012 standard method (ISO, 2019) using rectangular probes (60 ×10×1 mm) and an extensional rate of 1 mm/min at room temperature. The strain-stress curves obtained were used to calculate the following parameters: maximum tensile strength (σ_{max}), Young's modulus (E) and strain at break (ε_{max}).

2.3.3. Water uptake capacity (WUC) and soluble matter loss (SML)

The water absorption capacity of the bioplastic samples was evaluated by WUC measurements following the ASTM D570 standard (D570, 1985) with some modifications. Rectangular probes measuring 20 × 10×1 mm were used for these analyses. The initial weight of samles was determined after drying in a conventional oven at 50 °C for 24 h (w_1). Sugsequently, the samples were immersed in distilled water over 24 h, and their wet weight was determined (w_2). Finally, the specimens were subjected to freeze-drying in a LyoQuest − 85 plus freeze-dryer (Telstar, Barcelona, Spain) equipped with a Flask M8 head, and the dry-weight of samples after water inmersion was determined (w_3). Water uptake capacities (WUC) and soluble matter losses (SML) were determined as shown in Eqs. (2) and (3):

$$WUC \quad (\%) = \frac{w_2 - w_3}{w_3} \cdot 100 \qquad (2)$$

$$SML \quad (\%) = \frac{w_1 - w_3}{w_1} \cdot 100 \qquad (3)$$

Where w_1, w_2 and w_3 are the weights of the sample after the dehydrothermal treatment, after the immersion step and after the freeze-drying stage, respectively.

2.3.4. Scanning electron microscopy (SEM)

The microstructure of the final bioplastics after the water uptake and further freeze-drying was obtained in a in an EVO SEM microscope (Zeiss, Jena, Germany). The influence of the plasticizer type and proportion on the structure generated was determined in the bioplastics after water absorption according to the protocol described by Julavittayanukul et al. (Julavittayanukul et al., 2006). The samples were first sputtered with a 10 nm thickness Pd/Au coating using an AC600 Metallizer (Leica, Wetzlar, 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. Dynamic mechanical thermal analysis (DMTA)

Fig. 1 shows the viscoelastic moduli as a function of frequency (between 0.04 and 20 Hz, at room temperature) for all the studied systems processed with different proportions of water and glycerol (A) or sorbitol (B). The mechanical spectra obtained shows that E' was higher than E'' in all cases, confirming that all specimens exhibited a predominantly elastic character. All elastic moduli follow a power-law tendency, increasing with frequency, which fit to Eq. (1).

This dependence is similar to previous ones observed for protein-based bioplastics (Bourny et al., 2017; Felix et al., 2018). However, the slope of the linear form of the equation ($log(E^{'}(\omega)) = log(A) + n \cdot log(\omega)$) differs for the different evaluated systems. The n values obtained for the different evaluated systems are gathered in Table 2.

Regarding the glycerol systems, the power-law exponents for all specimens containing water (2:1 G, 1:1 G, 1:2 G and 1:4 G) were between 0.16 and 0.18. In contrast, the system without water (0:1 G) exhibited a slighter lower dependence with a power-law exponent of 0.14. On the other hand, the systems containing sorbitol ranged from 0.10 (system 4:1 S) to 0.19 exhibited by the system with the lower water ratio (system 0:1 S). These results indicate that, in general, the glycerol containing systems show a stronger dependence with frequency, and that higher proportions of sorbitol lead to stronger dependence with frequency. Assuming that the lower dependence of the viscoelastic moduli on frequency was previously related with a more structured material (i.e., solid-like behaviour) (Ferry and Myers, 1961), these results indicate that the sorbitol-based materials showed a more rigid structure. In this sense, glycerol seems to have a stronger plasticizer effect than sorbitol since smaller amounts of glycerol led to higher dependence of the elastic modulus on frequency. However, higher amounts of glycerol do not increase significantly the mobility of biopolymer chains.

Moreover, apart from the frequency dependence, the values recorded for E' and E'' for the whole study range also varied depending on the plasticizer used and its proportion. In this way, the viscoelastic moduli increased when the water content increased regardless of the plasticizer used (glycerol (A) or sorbitol (B)), which indicates that the presence of water led to stiffer bioplastics. Similar results have been found for albumen/tragacanth based bioplastics plasticized with water and glycerol (López-Castejón et al., 2015). Although there is a similar tendency, the same water/glycerol and water/sorbitol proportions provided different rheological properties, being the viscoelastic moduli of the sorbitol-based systems always above the glycerol-based ones. Thus, the values recorded for E' at 1 Hz (E'$_1$) for the 2:1 G and 1:1 G systems were $6.0 \cdot 10^7$ and $8.1 \cdot 10^7$ Pa when they were plasticized using glycerol, while sorbitol led to systems exhibiting values of $4.7 \cdot 10^8$ and $3.8 \cdot 10^8$ Pa for E'$_1$ for the 2:1 S and 1:1 S systems, respectively. These results could be attributed to the greater plasticizer efficiency of glycerol compred to sorbitol chich is related to its lower molecular weight. By these means, lower amounts of glycerol are required to achieve similar viscoelastic moduli. By increasing the glycerol and sorbitol ratios, the detrimental effect on the viscoelastic moduli became more significant, especially for the former plasticizer. In this way, the values for E'$_1$ decreased from $1.9 \cdot 10^7$ and $2.3 \cdot 10^7$ Pa exhibited by the 1:2 G and 1:4 G systems to $2.3 \cdot 10^6$ Pa corresponding to the 0:1 G system, with the latter value being the lowest one, while the sorbitol systems presented a slighter decrease of E'$_1$, which was $2.3 \cdot 10^8$ Pa for the 1:2 S system and $9.0 \cdot 10^7$ and $5.7 \cdot 10^7$ Pa for the 1:4 S and 0:1 S systems, respectively. In addition, despite the water excess of the 4:1 S blend, which exhibited limited processability, some specimens were injected and the results obtained for the frequency sweep test (Fig. 1B) confirmed the effect of water, leading to the highest E'$_1$ value ($5.9 \cdot 10^8$ Pa). Finally, it is worth mentioning that the
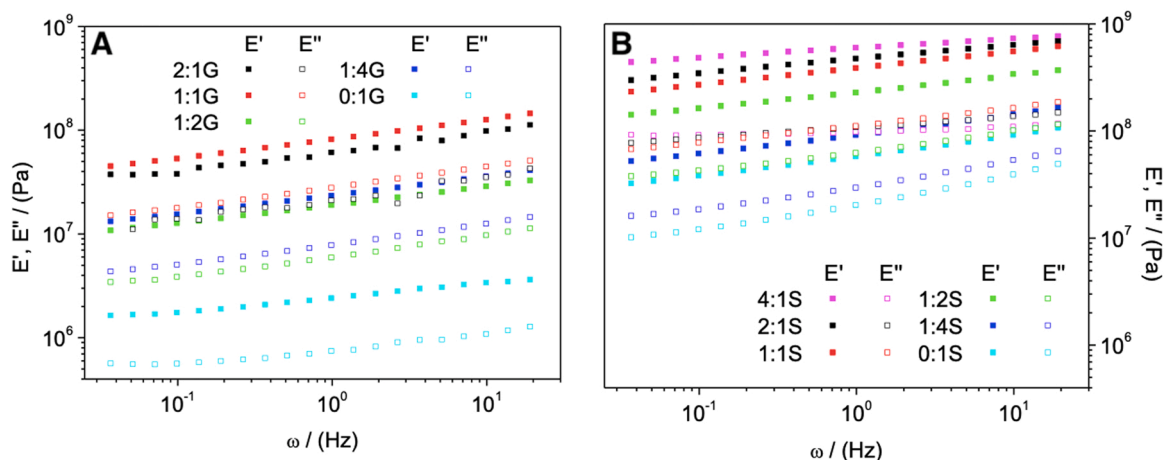
Fig. 1. Frequency sweep tests between 0.04 and 20 Hz for the systems obtained with (A) glycerol (2:1 G, 1:1 G, 1:2 G, 1:4 G and 0:1 G) and (B) sorbitol (4:1 S, 2:1 S, 1:1 S, 1:2 S, 1:4 S and 0:1 S).

Table 2
Values for parameter n calculated after fitting the frequency sweep tests to the Eq. 3 for the different processed systems. Different letters within a column indicate significant differences (p < 0.05).

System	n value	System	n value
4:1 G	–	4:1 S	0.10 ± 0.01[A]
2:1 G	0.16 ± 0.01[DEF]	2:1 S	0.14 ± 0.01[B]
1:1 G	0.18 ± 0.01[HI]	1:1 S	0.15 ± 0.01[CD]
1:2 G	0.16 ± 0.01[EFG]	1:2 S	0.15 ± 0.01[CDE]
1:4 G	0.17 ± 0.01[FGH]	1:4 S	0.18 ± 0.01[GHI]
0:1 G	0.14 ± 0.01[BC]	0:1 S	0.19 ± 0.01[I]

viscoelastic moduli of the sorbitol systems decreased progressively while the glycerol-based ones seemed to appear in pairs. Thus, for the 2:1 G and 1:1 G systems, as well as the 1:2 G and 1:4 G systems, the E'_1 values exhibited no significant differences (p < 0.05).

Fig. 2 shows the viscoelastic moduli of the processed systems as a function of temperature (from 30 to 140 °C) for the glycerol/water plasticized samples (A) and the sorbitol/water plasticized ones (B).

In this case, the same effect was observed, that is, increasing water content led to higher viscoelastic moduli. Moreover, regarding the evolution for each plasticizer, the sorbitol systems (Fig. 2B) presented a more continuous decrease for each system evaluated than the glycerol-based samples (Fig. 2A), since the lowest value was observed for glycerol-based samples above 80 °C, whereas the nearly constant value

could be deduced for the probes containing sorbitol above 130 ⁻C. A similar continuous decrease was observed by Felix et al. (Felix et al., 2016) for protein-based bioplastics processed by injection moulding. As for the evolution with temperature, two different behaviours were observed in Fig. 2A. On the one hand, at the beginning of the test, the viscoelastic moduli of the 2:1 G and 1:1 G systems decreased with temperature up to approximately 80 °C, where E' and E'' reached a stable value that remained constant for the higher temperatures. On the other hand, the other three systems obtained for higher glycerol ratios (1:2 G, 1:4 G and 0:1 G) followed the same decreasing tendency up to 80 °C, although, instead of remaining at the achieved value, the viscoelastic moduli started to increase again, showing nearly constant values at the end of the test. This thermosetting potential seems to be higher for increasing glycerol ratios; in this way, the greater recovery was that of the 0:1 G system, which shows similar values to those achieved by the systems 1:2 G and 1:4 G systems, although the latter two were clearly higher at the beginning of the test. Crayfish-based bioplastics processed by injection moulding also exhibited this thermosetting potential (Felix et al., 2015), the same exhibited by the more similar samples based on both polysaccharides and gluten studied by Zárate-Ramírez et al. (Zárate-Ramírez et al., 2014). These authors also stated that higher processing temperatures were not advisable due to protein degradation. The fact that the thermosetting potential was not observed in the 2:1 G and 1:1 G systems could be due to the higher presence of water which allows to establish more hydrogen bonds with a stronger plasticizer effect, lowering the glass transition temperature. On the other hand, the
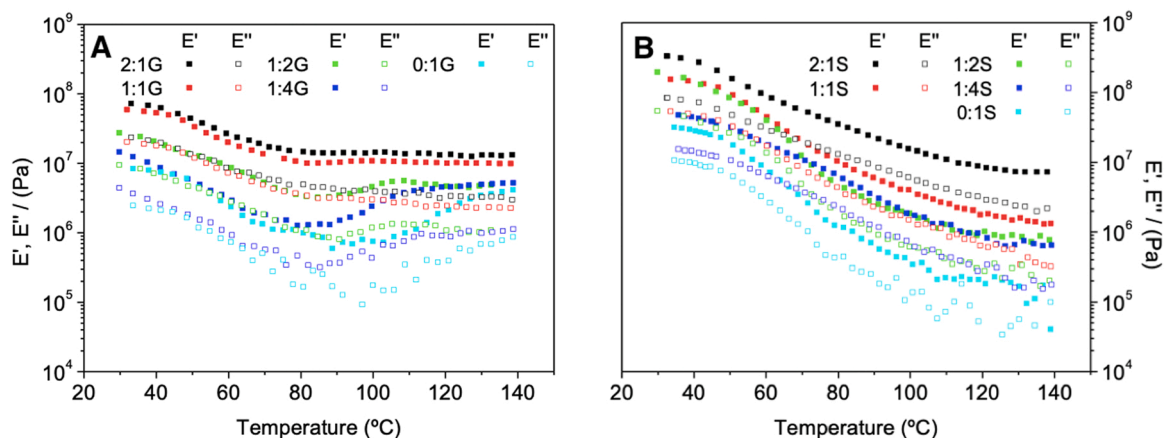

Fig. 2. Viscoelastic moduli (E′ and E'') for the temperature ramp tests between 30 and 140 °C for the systems obtained with (A) glycerol (2:1 G, 1:1 G, 1:2 G, 1:4 G and 0:1 G) and (B) sorbitol (2:1 S, 1:1 S, 1:2 S, 1:4 S and 0:1 S).

sorbitol-based systems exhibited a different behaviour (Fig. 2B). In this case, none of them exhibited thermosetting potential, and as it was previously stated, the viscoelastic moduli decreased for almost the whole study range, reaching an almost constant value at the end of the experiment, which reveals that the bioplastics thermosetting mainly occurs during the previous injection moulding process at 150 °C. The same behaviour was exhibited by albumen/tragacanth-based bioplastics when submitted to similar experiments (López-Castejón et al., 2015). Furthermore, the main difference exhibited by the different plasticizer proportions is that E′ and E′′ underwent a more pronounced decline for higher sorbitol ratios. Thus, E′ ranged from $3.3 \cdot 10^8$ Pa to $7.2 \cdot 10^7$ Pa in the 2:1 S system, while the elastic modulus of the 0:1 S system ranged from $3.1 \cdot 10^7$ Pa to $1.4 \cdot 10^5$ Pa. The viscoelastic moduli at ambient temperature (25 °C) is similar to those obtained for different protein systems obtained by injection moulding which have been proposed for food packaging applications (Felix et al., 2017).

Fig. 3 shows the tendency of the loss tangent (tan δ) obtained from the temperature ramp tests performed on the bioplastic samples for four of the systems generated (1:1 G, 1:4 G, 1:1 S and 1:4 S).

For comparison purposes, the same plasticizer proportions, i.e., 1:1 and 1:4, were selected from the glycerol and sorbitol systems (1:1 G, 1:4 G, 1:1 S and 1:4 S). Fig. 3 shows that all the samples tested presented a maximum peak associated with the glass transition of the plasticized rice bran bioplastics. The peak is relatively well defined for the glycerol systems (around 60 °C), although it became wider and less pronounced in the sorbitol samples confirming the differentiated behaviour observed before in Fig. 2. This difference in the glass transition temperature suggests that the samples plasticized with sorbitol were more stable at higher temperatures while glycerol had a greater plasticizer efficiency, as explained before, leading to a lower transition temperature. Similar glass transition temperatures are found for rice protein-based bioplastics plasticized with glycerol (Félix et al., 2016). Finally, the two used plasticizers exhibited the desired effect of lowering the glass transition temperature of rice bran since the characterisation of the material performed by Fabian et al. (Fabian et al., 2011) revealed that the glass transition temperature of rice bran is higher than the decomposition temperature above 210 °C.

3.2. Tensile tests

The stress-strain curves obtained from the tensile tests are shown in Fig. 4 for all glycerol (A) and sorbitol (B) systems. Firstly, the different scales employed in Fig. 4A and Fig. 4B are noticeable. While the stress
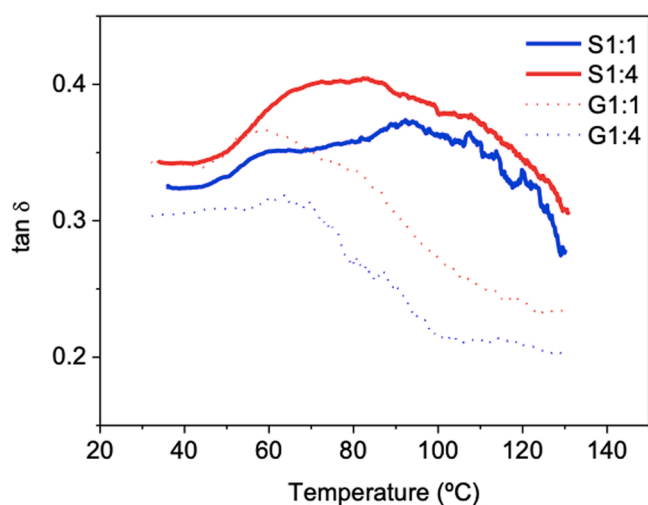

Fig. 3. Evolution of the loss tangent (tan δ) for the temperature ramp tests between 30 and 140 °C for some of the systems obtained with glycerol (1:1 G and 1:4 G) and sorbitol (1:1 S and 1:4 S).

values of the systems containing glycerol ranged from 0 to 0.3 MPa, those of the systems plasticized with sorbitol reached 1.4 MPa. Therefore, the latter specimens exhibited higher tensile strength values (one order of magnitude). The effects of glycerol and sorbitol were previously studied on soy-based bioplastics by Tummala et al. (2006), obtaining similar results, that is, the sorbitol containing systems achieving higher tensile stresses and modulus. Regarding the elongation at break, Fig. 4B shows that, when sorbitol was combined with water, the 1:4 S sample reached the highest elongation at break value (0.05 mm/mm). However, in this case, the rest of the sorbitol-based systems ranged from 0.01 to 0.03 mm/mm, which were the same values shown by the glycerol-based specimens. In addition to the effect of employing glycerol or sorbitol in the plasticisation process, the plasticizer proportion played a vital role in the tensile properties of the final bioplastics in both cases. Similar values and tendencies upon plasticizer proportions have been found for pea-based bioplastics (Perez et al., 2016).

Young′s modulus (E), maximum tensile strength (σ_{max}) and strain at break (ε_{max}) obtained from the stress-strain curves are gathered in Table 3, allowing a more accurate evaluation of the tensile properties. With respect to the effect of increasing water content on Young′s modulus, Table 3 shows the same behaviour for the systems containing glycerol and sorbitol, increasing for higher water proportions. Regarding the glycerol containing systems, there were no significant differences between the three systems with lower water content, reaching 4.33 ± 1.52, 10.3 ± 1.2 and 8.00 ± 1.00 MPa for the 0:1 G, 1:4 G and 1:2 G systems, respectively. Nevertheless, the higher water content in the 1:1 G and 2:1 G systems led to stiffer bioplastics, which exhibited 29.7 ± 7.4 and 31.3 ± 3.5 MPa for E values, respectively. Although the evolution of this parameter with increasing water content was similar in the sorbitol systems, the values achieved were significantly higher for the same plasticizer proportion. For example, the highest E value recorded for the glycerol systems was that of the 2:1 G system (31.3 ± 3.5), which had no significant differences with the value of 30.0 ± 2.6 MPa exhibited by the 0:1 S system (i.e., the one with the poorest results of all the sorbitol-based samples). From this value, the rest of the combinations with increasing water content led to significant stiffer bioplastics and, thus, greater E values, increasing from 106 ± 4 and 140 ± 7 MPa recorded for 1:4 S and 1:2 S, to 176 ± 13 and 199 ± 18 MPa exhibited by 1:1 S and 2:1 S. The values obtained for the tensile properties are similar to those obtained by Felix et al. (2017) for different protein systems. In addition the σ_{max} values match those obtained by Félix et al. (2014) for albumen/soy biobased plastic materials processed also by injection moulding. In this way, the samples obtained could be proposed for food packaging applications as those analysed in the article referred.

The second parameter evaluated was the tensile strength, which also presented an increasing tendency for greater water proportions. As was previously observed, the three glycerol systems containing lower water proportions (0:1 G, 1:4 G and 1:2 G systems) exhibited the poorest results. On the other hand, the higher water/glycerol ratios present in the 1:1 G and 2:1 G systems led to more resistant bioplastics, with tensile strength values of 0.213 ± 0.025 and 0.270 ± 0.010 MPa. As for Young′s modulus, the highest σ_{max} value of the glycerol-based systems (obtained for 2:1 G) was similar to the lowest value recorded for the sorbitol-based systems (i.e., 0:1 S), exhibiting a value of 0.263 ± 0.005 MPa. In this way, increasing the water/sorbitol ratios involved more resistant bioplastics, with tensile strengths of 0.373 ± 0.031, 0.863 ± 0.023, 1.25 ± 0.15 and 1.27 ± 0.23 MPa, corresponding to the 1:4 S, 1:2 S, 1:1 S and 2:1 S systems, respectively (the last two ratios did not present significant differences). On the other hand, although the 4:1 S system was very stiff, its high water/sorbitol ratio produced brittle specimens, which withstood lower stresses, particularly 0.880 ± 0.057 for maximum stress.

Finally, the elongation at break followed the opposite tendency, indicating that higher water proportions produced stiffer but less elastic products. This behaviour was also observed in the sorbitol systems, since
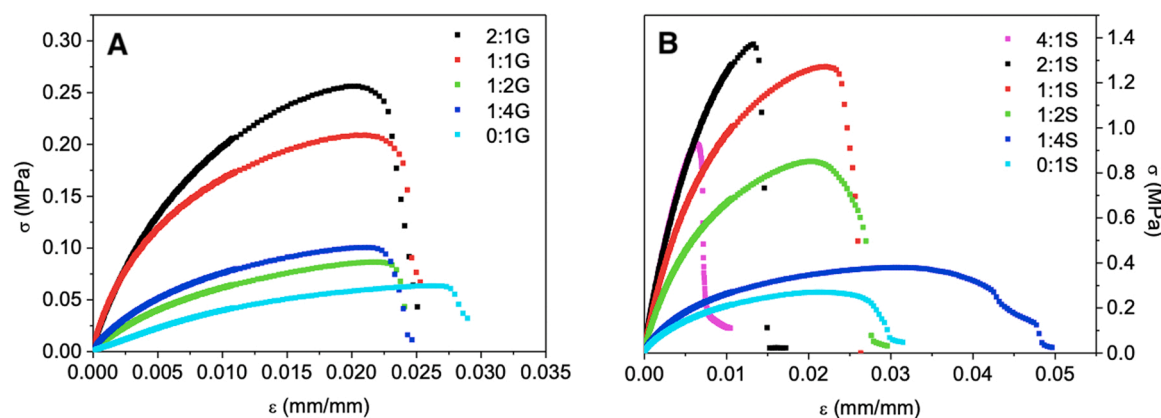
Fig. 4. Stress-strain curves from the tensile tests performed on the bioplastic samples obtained for the different plasticizers: (A) glycerol (2:1 G, 1:1 G, 1:2 G, 1:4 G and 0:1 G) and (B) sorbitol systems (4:1 S, 2:1 S, 1:1 S, 1:2 S, 1:4 S and 0:1 S).

Table 3
Young's modulus (E), Tensile strength (σ_{max}) and Elongation at break (ε_{max}) of the different processed systems. Different letters within a column indicate significant differences ($p < 0.05$).

| System	E (MPa)	σ_{max} (MPa)	ε_{max} (%)
2:1 G	31.3 ± 3.5^{BC}	0.270 ± 0.010^{JK}	2.36 ± 0.32^{PQ}
1:1 G	29.7 ± 7.4^{B}	0.213 ± 0.025^{IJ}	2.80 ± 0.27^{QR}
1:2 G	8.00 ± 1.00^{A}	0.0640 ± 0.0187^{HI}	2.10 ± 0.30^{OP}
1:4 G	10.3 ± 1.2^{A}	0.100 ± 0.010^{HI}	2.66 ± 0.67^{PQR}
0:1 G	4.33 ± 1.52^{A}	0.0573 ± 0.0081^{H}	2.60 ± 0.43^{PQR}
4:1 S	199 ± 18^{G}	0.880 ± 0.057^{L}	0.650 ± 0.071^{N}
2:1 S	176 ± 13^{F}	1.27 ± 0.23^{M}	1.47 ± 0.06^{O}
1:1 S	140 ± 7^{E}	1.25 ± 0.15^{M}	2.77 ± 0.21^{PQR}
1:2 S	106 ± 4^{D}	0.863 ± 0.023^{L}	2.70 ± 0.10^{PQR}
1:4 S	41.3 ± 4.2^{C}	0.373 ± 0.031^{K}	3.93 ± 0.90^{S}
0:1 S	30.0 ± 2.6^{BC}	0.263 ± 0.005^{JK}	3.16 ± 0.38^{R}

ε_{max} increased from $0.650 \pm 0.071\%$ registered for 4:1 S and $1.47 \pm 0.06\%$ registered for 2:1 S to 2.77 ± 0.21 and $2.70 \pm 0.10\%$ for the intermediate 1:1 S and 1:2 S systems and $3.93 \pm 0.90\%$ for the 1:4 S sample. However, further sorbitol/water ratios did not lead to improved elasticities, obtaining a ε_{max} of $3.16 \pm 0.38\%$ for the 0:1 S sample. This improvement in the elasticity of the samples as the sorbitol content increased was also observed during the frequency sweep tests where the dependence with frequency increased as the sorbitol content did. Regarding the glycerol systems, it is more difficult to establish a relationship between elasticity and water content, since the lowest value is that of the 1:2 G system, with $2.10 \pm 0.30\%$. This is followed by 2.36

$\pm 0.32\%$ of the 2:1 G system and $2.80 \pm 0.27\%$ of the 1:1 G system, with the 1:4 G and 0:1 G systems having similar values, i.e., 2.66 ± 0.67 and $2.60 \pm 0.43\%$, respectively. The rice bran-based bioplastics developed by Klanwan et al. (Klanwan et al., 2016) exhibited similar results in terms of tensile parameters.

As concluded in the previous section, higher water contents led to stiffer (higher E modulus and maximum stress) but less elastic bioplastics. However, the effect upon the elongation at break was less remarkable. These results indicate the feasibility of modulating bioplastics where the mechanical properties obtained by varying the plasticizer used and its proportion are suitable for different applications.

3.3. Water uptake capacity and soluble matter loss

Fig. 5 shows the WUC values of the different bioplastics obtained. This figure shows that the differences between the different systems were not as remarkable as in the case of tensile parameters, which makes it difficult to establish a relationship between the plasticizer proportion and the WUC. However, the absorption ability seemed to increase for higher glycerol/sorbitol proportions (lower water content) with a few exceptions. Thus, the lower WUC value was observed for the 1:1 G system, with $127 \pm 7\%$, followed by the 1:2 G systems, with an increase of 7% points, that is, $134 \pm 4\%$. These two systems were exceeded by systems 2:1 G and 1:4 G, which exhibited WUCs of 137 ± 5 and $147 \pm 7\%$, although 2:1 G contained the higher water proportion in its formulation. The least glycerol containing system (0:1 G) confirmed the tendency, exhibiting the greatest value of this group, with $148 \pm 7\%$.
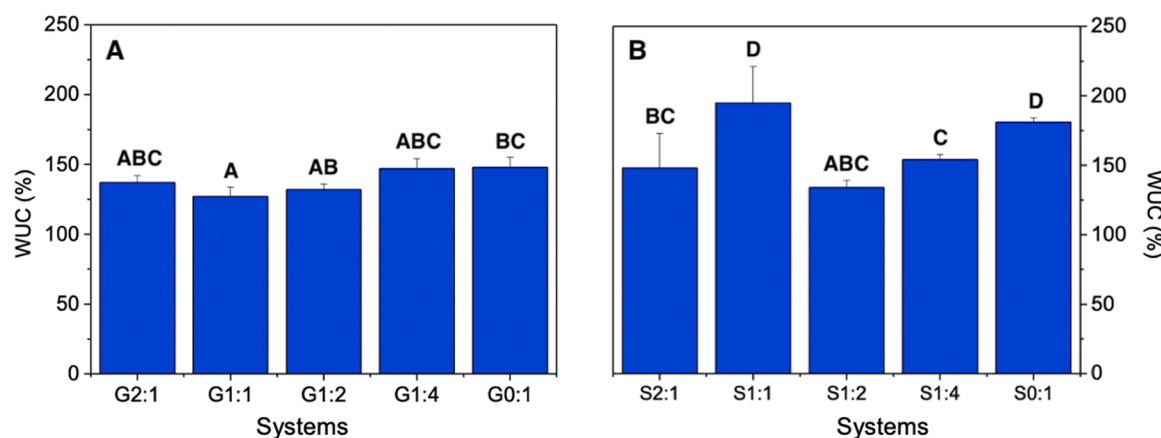
Fig. 5. Water uptake capacity (WUC) of the bioplastic samples obtained for the different plasticizers: (A) glycerol (2:1 G, 1:1 G, 1:2 G, 1:4 G and 0:1 G) and (B) sorbitol systems (2:1 S, 1:1 S, 1:2 S, 1:4 S and 0:1 S). Different letters indicate significant differences ($p < 0.05$).

The same value was registered for the 2:1 S system, which was expected to exhibit the lowest value (concerning the sorbitol samples), however, contrary to this behaviour, the poorer results were those of the 1:2 S system, accounting for $134 \pm 5\%$. The rest of the specimens showed greater values, beginning with the 1:4 S system, with $154 \pm 4\%$, and followed by the 0:1 S and 1:1 S systems, with 181 ± 3 and $195 \pm 26\%$, respectively. Although the 1:1 S system was the one with the highest value this measure had high dispersion, with the different replicates greatly differing from each other. The water absorption capacity exhibited by these specimens is significantly above those obtained for the albumen/soy based plastic materials and protein based plastics used to compare the mechanical properties before (Felix et al., 2017; Félix et al., 2014). For this reason, these samples could outstand in food packaging applications specially when water absorption is required.

The soluble matter losses (SML) measured for each system were gathered in Fig. 6. Although the SML values seemed to increase for decreasing water content following a clear tendency, it is important to consider the plasticizer losses during these tests: the glycerol and sorbitol added during plasticisation (note that water was not taken into account, since it is always lost during the final probe drying). Although SML is essentially attributed to the plasticizer content (Jiménez-Rosado et al., 2019), it is important to clarify that it can not be assumed that all the plasticizer content is lost and that some of these losses can be due to different soluble elements (i.e. soluble salts). In this way, the SMLs varied from $9.5 \pm 2\%$ attributed to 1:1 G to $14 \pm 2\%$ corresponding to the 1:2 G system, and between $9 \pm 2\%$ and $17.5 \pm 1\%$ in the case of the sorbitol systems. As can be seen, the SMLs (excluding the plasticizer loss) are relatively small, thus it is possible to conclude that the bioplastics obtained exhibited good physical integrity during the water uptake process. Similar behaviours are observed in pea protein-based bioplastics processed by following a similar method (Perez et al., 2016).

3.4. Scanning electron microscopy (SEM)

Fig. 7 shows the SEM micrographs of some of the different bioplastics obtained after the freeze-drying stage (2:1 G, 2:1 S, 0:1 G and 0:1 S). The four selected systems represent the limit plasticizer proportions, that is, the highest and lowest water content for the glycerol and sorbitol systems (except for the 4:1 S system which, as stated before, was difficult to process and only a few samples could be obtained). As can be seen, the observed morphologies match the results obtained before. Regarding the glycerol systems, it can be observed that the greater glycerol proportion present in the 0:1 G system (Fig. 7C) led to increased porosity compared to the 2:1 G system (Fig. 7A). This fact can be related to the poorer mechanical properties and higher water uptake capacity. The same explanation applies to the sorbitol systems, which seemed to exhibit

greater porosity upon lower water/sorbitol ratio (Fig. 7D), with the 2:1 S system (Fig. 7B) showing better mechanical properties but lower water uptake capacity. Finally, the porosity also increased as a consequence of the effect of sorbitol instead of glycerol, which can be observed when comparing the 2:1 G and 0:1 G systems with the 2:1 S and 0:1 S systems. This change of plasticizer is related to the improved water uptake capacity observed in the previous section. The micrographs obtained were similar to those obtained by Yue et al. (Yue et al., 2012) for bioplastics based on cottonseed protein.

4. Conclusions

Rice-based bioplastics were successfully obtained after processing the raw material with water and different proportions of glycerol or sorbitol. However, the election of a suitable plasticizer is essential for the final properties of bioplastics, which,. Although water was required to produce stiffer specimens, an excess of water hindered the injection moulding process and led to brittle bioplastics. In this way, the highest water/glycerol ratio that could be processed was 2:1 G, while some specimens with 4:1 S proportion could be obtained when sorbitol was used together with water as plasticizer.

Both rheological and tensile properties indicated that higher water proportions led to stiffer bioplastics with higher viscoelastic moduli, especially for the sorbitol-based systems As can be expected, the higher water content produced specimens with greater Young's modulus and tensile strength, although the elongation at break generally increased for higher glycerol and sorbitol ratios. Regarding WUC, results seem to indicate that lower water content (higher glycerol/sorbitol) in the formulation produced bioplastics with higher WUC. In addition, specimens containing sorbitol improved this capacity when compared to the water/glycerol-based systems. However, the tendency was less remarkable when analysing the corresponding SML. Finally, the SEM micrographs helped to establish a relationship between the observed microstructure and the measured properties, agreeing in all cases, samples containing greater glycerol proportion increased porosity, leading to poorer mechanical properties and higher water uptake capacity.

CRediT authorship contribution statement

María Alonso-González: Conceptualization, Methodology, Software, Validation, Investigation, Data curation, Writing – original draft, Visualization. **Manuel Felix:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Project administration. **Alberto Romero:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.
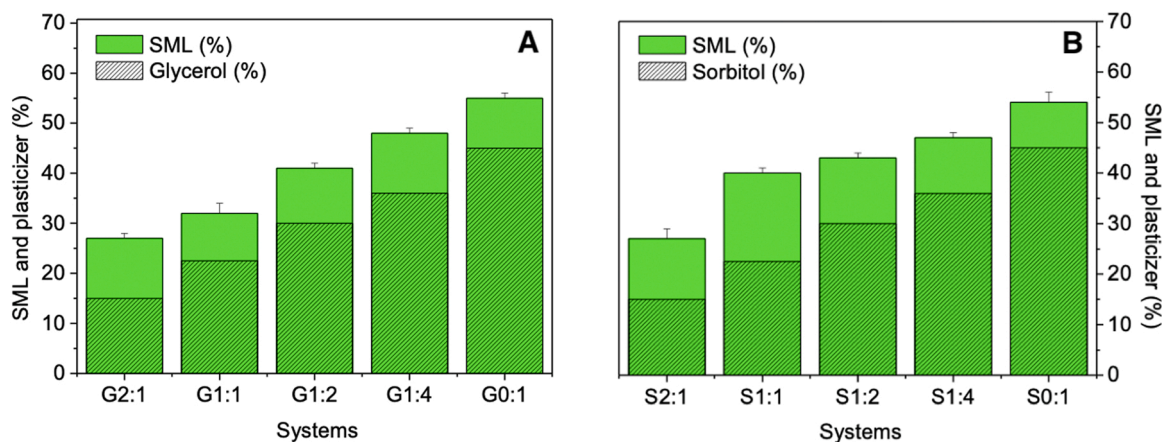

Fig. 6. Soluble matter loss (SML) of the bioplastic samples obtained for the different plasticizers: (A) glycerol (2:1 G, 1:1 G, 1:2 G, 1:4 G and 0:1 G) and (B) sorbitol systems (2:1 S, 1:1 S, 1:2 S, 1:4 S and 0:1 S). Different letters indicate significant differences ($p < 0.05$).
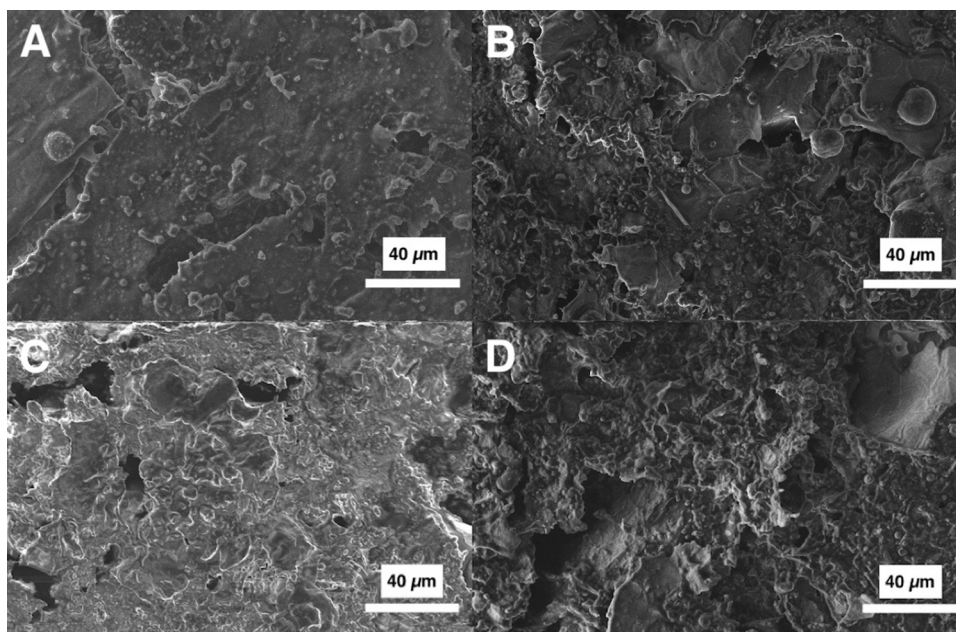
Fig. 7. SEM micrographs (500x magnification) of the bioplastic samples after the freeze-drying treatment obtained for four of the different blends: (A) 2:1 G, (B) 2:1 S, (C) 0:1 G and (D) 0:1 S.

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