Development of soy protein-based matrices containing zinc as micronutrient for horticulture

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ABSTRACT

Protein-based bioplastics may be regarded as novel biopolymer matrices based on renewable natural components. Due to technological, economic and environmental benefits, these biopolymer matrices are highly attractive for the incorporation and subsequent release of micronutrients that are essential for the development and health of plants, avoiding the typical excesses of conventional fertilizers. In addition, soy protein isolate (SPI) seems to be an adequate resource for the manufacture of natural-based superabsorbent materials due to its hydrophilic character and excellent processability when combined with a plasticizer. The objective of this work is to develop soy protein-based bioplastic matrices loaded zinc sulphate monohydrate with potential applications in horticulture. With this aim, the micronutrient loading level and water absorption are the most important properties to assess. The effect of the presence of a selected micronutrient (zinc) at different concentrations in the soy protein-based matrix was assessed, evaluating the mechanical properties, water uptake capacity, microstructure and loading level of zinc. The results confirm that important amounts of an essential micronutrient for a plant (Zn) can be incorporated into bioplastic matrices, modifying water absorption, mechanical and microstructural properties. In any case, the results obtained in this work open up a great potential for the use of this matrices as a supplying source of micronutrients for horticultural crop applications.

Keywords: Bioplastics; Soy protein; Zinc; Micronutrients; Horticulture

1. INTRODUCTION

Plastics are one of the most important and most used consumer products in human life. Almost everything used in society is made of plastic or contains plastic, so plastics are used in a great variety of applications, such as, packaging, construction, electronic devices, houseware, etc. Due to this great use, the worlḑs production of plastics reached 322 million tons in 2015, when China led the market (27.8% of production), followed by Europe (18.5%) (Plastics Europe, 2016). However, this high production of plastic products has significant disadvantages derived from their low biodegradability, which leads to high contamination, and their extremely low renewability, since most of them come from fossil sources (Bledzki and Jaszkiewicz, 2010). In 2014, recycling and recovery of plastic reached 69.2%, while the rest of this percentage is still discarded in landfills, causing garbage accumulation (Plastics Europe, 2016). For these reasons, plastics are being gradually replaced by bioplastics.

The term bioplastics refers to either those polymeric materials which are obtained from renewable sources, or those which exhibit a natural degradability that helps eliminate garbage accumulations. A superabsorbent matrices due to its hydrophilic character derived from its high content of glutamic and aspartic acid, which make it more hydrophilic than other proteins, whereas Cuadri et al. (2018, 2017, 2016) benefit from its high content in lysine to introduce some functional carboxylic groups, thereby enhancing its hydrophilic character. There are several soy products with different protein content. Among them, the soy protein isolate (SPI) contains at least 90 wt.% protein; although more expensive, it gives the best results due to the greater amount of protein and less moisture (Mo et al., 1999). Plasticizers are low molecular weight agents that have the ability to increase protein chain mobility, reducing the number of inter and intramolecular interactions and decreasing the glass transition temperature. Glycerol (Gly) is the most widely used from this group (Bourny et al., 2017; Guerrero et al., 2010; Matveev et al., 2000). A wide variety of additives are often used to improve the processability and/or the final properties of bioplastics (Bourny et al., 2017).

The properties of these soy-based matrices (bioplastic) can be controlled by the SPI/Gly ratio, the processing conditions and technologies used. Protein-based bioplastics can be processed by using existing processing technologies, from the physicochemical to thermomechanical methods. Among these thermomechanical techniques, injection moulding is one of the most important and suitable process for systems that may exhibit a mixed (thermoplastic and thermoset) character such as proteins. However, this

technique needs a previous mixing process in order to obtain a readily injectable protein-plasticizer blend (Reddy, 2015).

In the agricultural sector, the use of these materials would bring great advantages due to their null toxicity and high biodegradability (Guo et al., 2015), as well as the extra contribution of nutrients after their degradation (Saenghirunwattana et al., 2014). Besides, these materials are able to absorb and retain water from the environment, maintaining their integrity without dissolving, even though they can suffer a marked increase in volume (Fernández-Espada et al., 2016a). All of these advantages make these materials an attractive potential candidate for the incorporation of essential micronutrients for the development and health of plants in horticulture. Micronutrients are elements needed by plants in small quantities. There are seven essential micronutrients in horticulture: Boron, Copper, Iron, Zinc, Manganese, Molybdenum and Chlorine. Among them, Zinc has an important role in the growth and development of plants (Uchida, 2000). The incorporation and subsequent controlled release of micronutrients would contribute to avoid the typical excesses of conventional fertilization, while increasing the efficiency of assimilation by the plant. In addition, being able to store water and dispose it during crop growth would increase water-use efficiency of plants (Mortain et al., 2004). The present work is focused on the development of soy protein-based matrices loaded with a soluble micronutrient for their potential use in horticulture. For this purpose, the most important property to evaluate is the micronutrient loading level followed by the water uptake capacity. Zinc cation was selected as the micronutrient, and it was introduced in salt form in the mixing stage, along with SPI and glycerol. Then, bioplastic systems were obtained by injection moulding, at selected conditions, evaluating the water uptake capacity, the mechanical properties and particularly the zinc loading level. It is worth pointing out that mechanical properties of zinc-loaded bioplastics only have a relative importance for their horticulture applications. However, a minimal level of mechanical properties is necessary to ensure physical stability of these bioplastics. In addition, the microstructure of the freeze-dried matrix and their loading level of zinc have also been studied.

2. EXPERIMENTAL

2.1 Materials

Soy protein isolate (SPI, with min. 91 wt.% protein and 6 wt.% moisture) was supplied by Protein Technologies International (SUPRO 500E, Belgium). Glycerol (Gly) was used as a plasticizer and zinc micronutrient was incorporated into the matrix as zinc sulphate

monohydrate ($ZnSO_4 \cdot H_2O$, MN), which were both purchased from Panreac Química Ltd. (Spain).

2.2. Preparation of bioplastic matrices

The procedure for the preparation of matrices consisted of several stages, as can be seen in Fig. 1.

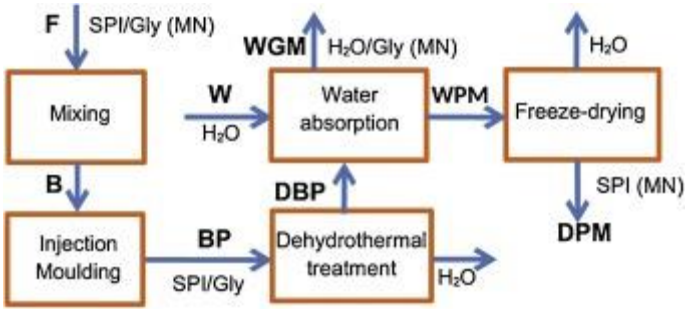

Fig. 1. Processing stages for the preparation of soy/glycerol and soy/glycerol/micronutrient matrices. F: soy, glycerol (and micronutrient) flow. B: moulding blends. BP: bioplastics after injection moulding. DBP: bioplastics after dehydrothermal treatment. W: water. WGM: water with glycerol and micronutrient which leave the matrix. WPM: matrix with water. DPM: matrix without water.

First of all, SPI and Gly (ratio 1:1) with different percentages of $ZnSO_4 \cdot H_2O$ (0.0, 2.5, 5.0, 10 and 15 wt.%), maintaining this SPI/Gly (1:1) ratio constant (Table 1), were mixed in a two-blade counter-rotating mixer Polylab QC (ThermoHaake, Germany) at room temperature and 50 rpm for 10 min, under adiabatic conditions, following the same protocol as in previous studies (Felix et al., 2016; Fernández-Espada et al., 2016a). A detailed description of this equipment and its operational conditions may be found in the literature (Dealy, 1983; Van Wazer et al., 1963). At this stage, torque (M) and temperature (T) measurements were taken during mixing time.

Table 1. Percentages and nomenclature of the different soy/glycerol/micronutrient (SPI/Gly/MN) blends in the mixing stage.

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Nomenclature	SPI (wt.%)	Gly (wt.%)	$ZnSO_4 \cdot H_2O$ (wt.%)
0.0%	50.00	50.00	0
2.5%	48.75	48.75	2.5
5.0%	47.50	47.50	5.0
10%	45.00	45.00	10
15%	42.50	42.50	15

In the second stage, SPI/Gly or SPI/Gly/MN blends (B) obtained after mixing were subsequently processed by injection moulding using a MiniJet Piston Injection Moulding System II (ThermoHaake, Germany) to obtain soy protein-based bioplastic matrices (BP). In this stage, the processing parameters were selected on the basis of a previous study (Fernández-Espada et al., 2016a), taking into account the specific requirements of this current study. As a result, the selected values were: cylinder temperature (40 °C), mould temperature (90 °C), injection pressure (600 bar, 20 s) and post-injection (holding) pressure (200 bar, 300 s). These moulding conditions will be referred to as 40/90. Subsequently, the bioplastic matrices were subjected to a dehydrothermal treatment that consisted in a heating stage in a conventional oven at 50 °C for 24 h to obtain dried bioplastic matrices (DBP). This stage involves some network strengthening and, therefore, is necessary to maintain the physical integrity of the matrices in the subsequent stage of absorption with water. The next step was an absorption process performed in a closed vessel with 300 mL of distilled water (W) for 24 h. In this stage, the glycerol, with some micronutrient, and even protein, was released into the water (WGM). Finally, the wet protein matrices after absorption (WPM) were introduced into a freeze-drying equipment (LyoQuest, Tesltar, Spain) in order to remove the water and obtain the dry protein matrix (DPM).

2.3. Characterization of bioplastic matrices

2.3.1. *Tensile strength measurements*

Tensile tests were carried out in a dynamic mechanical analyser RSA3 (TA Instruments, USA), with a modified protocol of the norm ISO 527-2:1993 ("ISO 570-2:1993, Plastics. Determination of tensile properties. Part 2: Test conditions for moulding and extrusion plastics," 1993). In this respect, bioplastic matrices (BP) were subjected to increasing axial stress until breakage. Thus, a crosshead speed of 1 mm/min was selected, and at least three replicates were tested for each sample. The stress produced (σ) against the applied strain (ε) was recorded. Besides, Young's modulus, maximum stress (σ_{max}) and maximum strain (ε_{max}) were obtained.

2.3.2. *Dynamic mechanical analysis*

Dynamic Mechanical Analyses were performed in bending mode and were carried out in a dynamic mechanical analyser RSA3 (TA Instruments, USA). In the bending tests, BPs were subjected to a small amplitude oscillatory flexural strain.

Firstly, a strain sweep test between 0.002% and 2%, at a constant frequency of 1 Hz, was performed to determine the linear viscoelastic region in which elastic moduli and viscous

moduli remained independent of the imposed strain. Subsequently, frequency sweep tests (0.02–20 Hz and room temperature) were carried out within the linear viscoelastic region. These tests are frequently performed to associate the viscoelastic properties with the unchanged matrix microstructure (Fernández-Espada et al., 2016a; Perez-Puyana et al., 2016).

The elastic (E') and the viscous (E") moduli as well as the loss tangent (tan (δ)) were measured across the frequency range. In addition, E' and tan (δ) were obtained at a constant frequency of 1 Hz (E'1 and tan (δ1)) in order to perform a suitable comparison of the systems.

2.3.3. *Water uptake capacity and soluble matter loss*

Water absorption tests were carried out according to a modification of the ASTM D570 norm (2005) (ASTM D570-98: Standard Test Method for Water Absorption Of Plastics, 2005), using rectangular DBP specimens (55 × 10 × 1 mm) after the dehydrothermal stage. Then, DBP samples were immersed into 300 mL of distilled water for 24 h. The water uptake capacity was calculated as follows:

$$\text{Water uptake capacity} = (w_{WPM}-w_{DPM})/w_{DPM}$$

Where w_{DPM} is the weight of the matrix after the freeze-drying stage (DPM) and w_{WPM} is the weight of the matrix after the absorption stage (WPM).

Finally, soluble matter loss was estimated as:

$$\text{Soluble matter loss} = (w_{DBP}-w_{DPM})/w_{DBP}$$

Where w_{DBP} is the weight of the matrix after the dehydrothermal stage (DBP).

2.3.4. *Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)*

ICP-AES measurements were performed using an ICP SpectroBlue TI (Spectro, Germany). The bioplastic matrices before (DBP) and after absorption and drying stages (DPM) were subjected to a previous treatment of microwave digestion. To do this, a known sample weight was taken and digested with the help of acids (HNO3 and H2O2 in a ratio 7:1), finally bringing the solution to a specific volume. In addition, water after absorption (WGM) was subjected to a previous gravity filtering treatment. After previous treatments, all the samples were aspirated through a nebulizer passing through a nebulization chamber where they became an aerosol. An argon stream transported the aerosol to the plasma torch, where it was subjected to a temperature around 6000 K, dissociating it into free atoms and ions with characteristic wavelengths, which were measured and recorded.

2.3.5. *Scanning electron microscopy (SEM)*

The microstructure of the selected matrices (DPM) was determined as described by Orawan et al. (2006). The samples sputter-coated with palladium-gold were mounted on a bronze stub. The specimens were observed using a Philips XL-30 Scanning electron microscope (the Netherlands) at an acceleration voltage of 8 kV and a resolution of ×50 and ×200 magnification. Then, the pore size was measured using ImageJ software, which is a public domain Java image processing program.

2.4. Statistical analysis

Al least three replicates of each measurement were carried out. The statistical analysis was performed using t-test and one-way analysis of variance (ANOVA, $p < 0.05$) by means of the statistical package SPSS 18. Standard deviations from some selected parameters were calculated.

3. RESULTS AND DISCUSSION

3.1. Preparation of bioplastic matrices

As can be seen in Fig. 1, mixing was the first stage in the thermochemical processing of the protein-based bioplastic matrices studied. SPI, Gly and $ZnSO_4 \cdot H_2O$ (MN) were introduced into the mixer in different percentages.

Fig. 2 shows torque profiles (M) and the evolution of the percentage ratio between the increase in temperature and the initial temperature ($100 \cdot (T-T_0)/T_0$) as a function of mixing time for different blends. The profiles did not show significant differences in the torque of the different blends (Fig. 2A). All the profiles displayed a fast increase in torque at the beginning, decreasing towards a constant value that was maintained during the rest of the mixing. There was a minimum torque in the system without micronutrients (0% MN), followed by a maximum of 2.5% MN. However, for higher percentages of MN the torque decreased again. On the other hand, all thermal profiles (Fig. 2B) show similar evolution with a moderate increase in temperature, ranging from 10%, for the blend without zinc, to 15% for the blends containing $ZnSO_4 \cdot H_2O$, regardless of the amount incorporated into the matrix. These results suggest that the zinc salt act as a filler, contributing a moderate increase in the mechanical energy dissipation. The increase in the filler content seems to be compensated by the reduction in protein among the different Zn-containing blends.

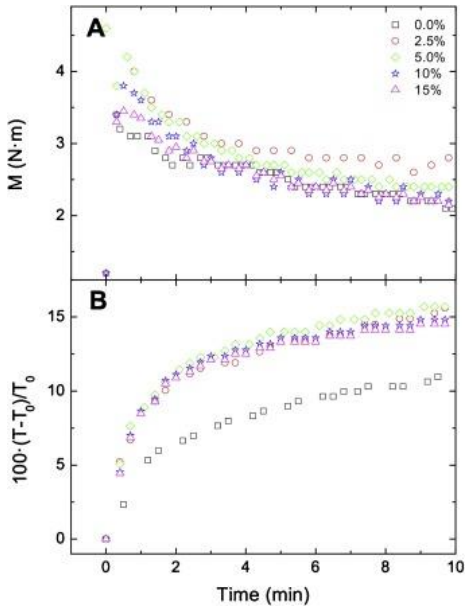

Fig. 2. Evolution of (A) mixing torque and (B) percentage ratio between the increase in temperature and the initial temperature (100·(T-T0)/T0) over the mixing time process for soy/glycerol/micronutrient (SPI/Gly/MN) blends at different zinc sulfate monohydrate content (0.0, 2.5, 5.0 and 10%).

The second stage was injection moulding. According to previous studies (Fernández-Espada et al., 2016a), the best moulding parameters to achieve the highest water absorption values in SPI/Gly bioplastics were: cylinder temperature (40 °C), mould temperature (70 °C), injection pressure (500 bar, 20 s) and post injection pressure (200 bar, 300 s). However, blends in this way were either poorly injected or led to excessively brittle bioplastic matrices. Under 40/90 processing conditions, loaded bioplastic matrices were obtained that contained up to 10 wt.% $ZnSO_4 \cdot H_2O$. In any case, the higher the percentages of $ZnSO_4 \cdot H_2O$, the more difficult were the blends to be injected, probably due to the impact of $ZnSO_4 \cdot H_2O$ as filler in matrix and its impact to the rheological properties of the blends.

3.2. Characterization of bioplastic matrices

3.2.1. *Tensile strength measurements*

The tensile properties of SPI/Gly bioplastic matrices (BP) containing different percentages of MN (0.0, 2.5, 5.0 and 10%) are shown in Fig. 3. Fig. 3A displays the results of stress-strain curves obtained from tensile strength measurements. In all cases, stress-strain curves exhibit an initial linear elastic behaviour of constant stress-strain slope yielding different values for the Young's modulus. This linear behaviour is followed by a plastic deformation stage with a continuous decrease in the strain-stress slope after the

elastic limit. All the curves for the systems containing micronutrients eventually reach a maximum value for the stress (σmax) and the strain (εmax), which is immediately followed by the rupture of the sample. This behaviour generally is similar to that of the soy-based bioplastic previously studied (Fernández-Espada et al., 2016a) although in these there is an appreciable plastic area until the rupture of the sample. However, significant differences were found between the levels of MN incorporated. The values of these parameters (Young's modulus, σmax and εmax) and their corresponding standard deviations are shown in Fig. 3B for all the systems studied as a function of the MN content. As can be observed, the incorporation of a micronutrient into the matrix produced an increase in the Young's modulus up to a maximum value (at about 5%) and progressively lower strain at breakage. These parameters indicate that the bioplastic matrices are more rigid when the MN concentration increases (i.e. toughness is progressively reduced). Finally, no significant differences were found for σmax, except for the 10% system, where σmax decreases markedly. In fact, the lower proportion of SPI and the higher proportion of $ZnSO_4 \cdot H_2O$ available during the injection moulding for the 10% system seems to contribute decisively to the decrease of the parameters in the tensile test, which reflect the lower toughness of the matrix. This may be the reason why BP loaded with a higher MN percentage cannot be obtained.

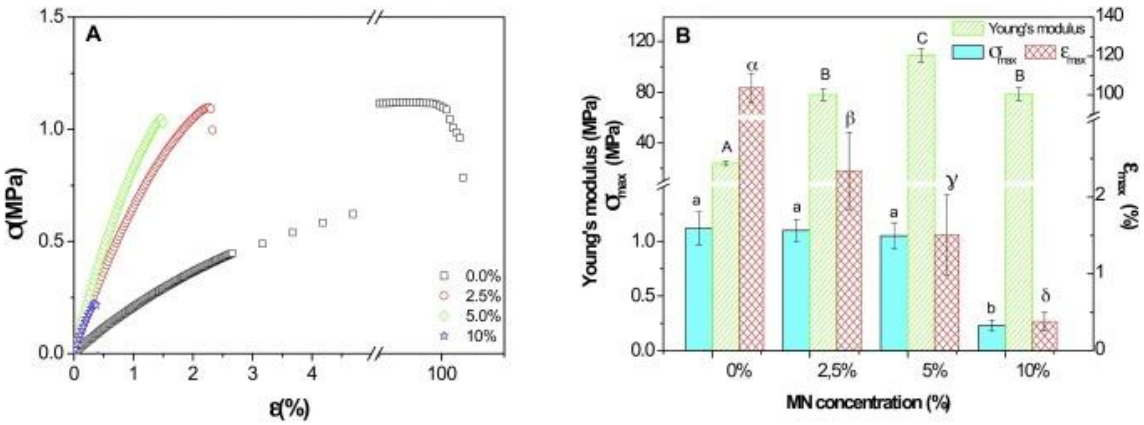

Fig. 3. Tensile strength measurements for soy/glycerol/micronutrient (SPI/Gly/MN) bioplastic matrices (BP) at different zinc sulfate monohydrate percentages (0.0, 2.5, 5.0 and 10%): (A) Stress-strain curves and (B) Parameters: Young's modulus, maximum stress (σmax) and maximum strain (εmax). Columns with different letters are significantly different ($p \leq 0.05$).

3.2.2. *Dynamic mechanical analysis*

The mechanical properties of SPI/Gly/MN bioplastic matrices (BP) containing different percentages of MN (0.0, 2.5, 5.0 and 10%) were obtained by means of small amplitude

bending frequency sweep tests and these are shown in Fig. 4. Strain sweep tests (results not shown) did not provide significant differences in critical strain (about 0.20%) of the different samples, thus delimiting their linear viscoelasticity range. A strain of 0.02% for frequency sweep tests was used.

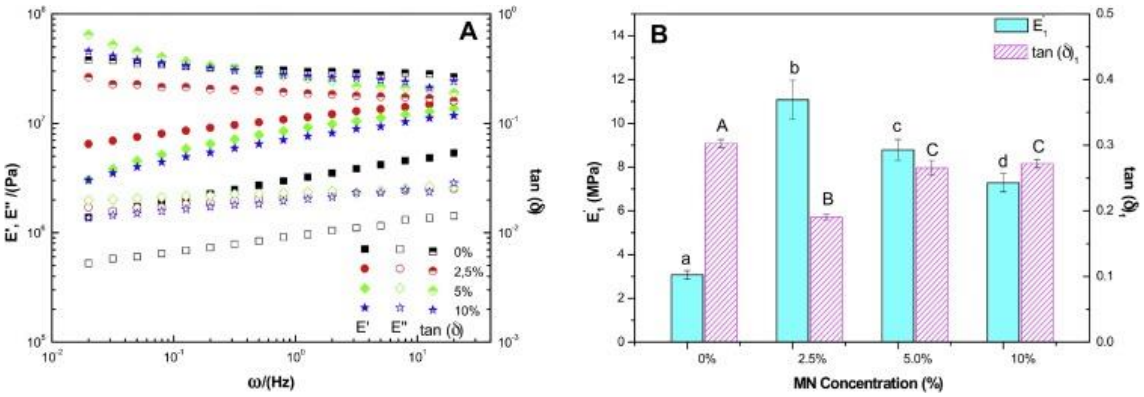

Fig. 4. Dynamic frequency sweep tests for soy/glycerol/micronutrient (SPI/Gly/MN) bioplastic matrices at different zinc sulfate monohydrate percentages (0.0, 2.5, 5.0 and 10%). A: Elastic modulus (E'), viscous modulus (E") and loss tangent (tan (δ)) versus frequency (ω). B: Linear viscoelastic parameters at 1 Hz (E'1 and tan (δ1)). Columns with different letters are significantly different ($p \leq 0.05$).

Fig. 4A shows the evolution of linear viscoelastic parameters, E', E" and tan (δ), with frequency for all SPI/Gly BP containing different concentrations of MN (0.0, 2.5, 5.0 and 10%). All the systems studied showed a predominantly elastic response (E' > E") over the whole frequency range studied, and both moduli showed a slight dependence on frequency.

Fig. 4B shows the values of E' and tan (δ) at 1 Hz (E'1 and tan (δ1)). As can be observed, the incorporation of MN led to an increase in the elastic modulus (E'1) and a decrease of the values of tan (δ1). Although the presence of MN always improved the mechanical properties of BP, it is the BP specimen which has the lowest content of MN (2.5 wt.%) that showed the highest elastic response. These results reinforce the idea of the importance of the proportion of protein available for the injection moulding of the SPI/Gly/MN blends. Thus, it seems that a decrease in the percentage of SPI and an increase in the MN content lead to a more rigid material. However, higher MN percentages (>2.5%) may interfere with the formation of the protein matrix, and may weaken its viscoelastic response.

3.2.3. *Water uptake capacity and soluble matter loss*

Fig. 5 shows the water uptake capacity and soluble matter loss for the SPI/Gly/MN specimens as a function of MN content (0.0, 2.5, 5.0 and 10%). As can be seen, the incorporation of a micronutrient produced a dramatic decrease of water uptake capacity, with this capacity being independent of the MN content (2.5–10%). These results indicate that the matrices with added $ZnSO_4 \cdot H_2O$ are not good absorbents. Several factors may contribute to this decrease in water uptake capacity. Firstly, a reduction in protein and glycerol content takes place as MN content increases, which reduces the hydrophilic character of the matrix. Secondly, a negative effect of ionic strength on water absorption has been reported by other authors (Judawisastra et al., 2017; Zayas, 1997). In addition, the above described increase in the elastic properties of the protein network impairs its swelling capability as has been previously found for different protein-based bioplastics (Felix et al., 2016; Félix et al., 2016; Fernández-Espada et al., 2016a; Perez-Puyana et al., 2016; Zárate-Ramírez et al., 2014). The lack of any significant effect of the increase in MN content (from 2.5 to 10%) on water uptake may be a consequence of the superposition of these factors, where the former two factors progressively increase with MN content, while the later decreases as MN is continuously increased.

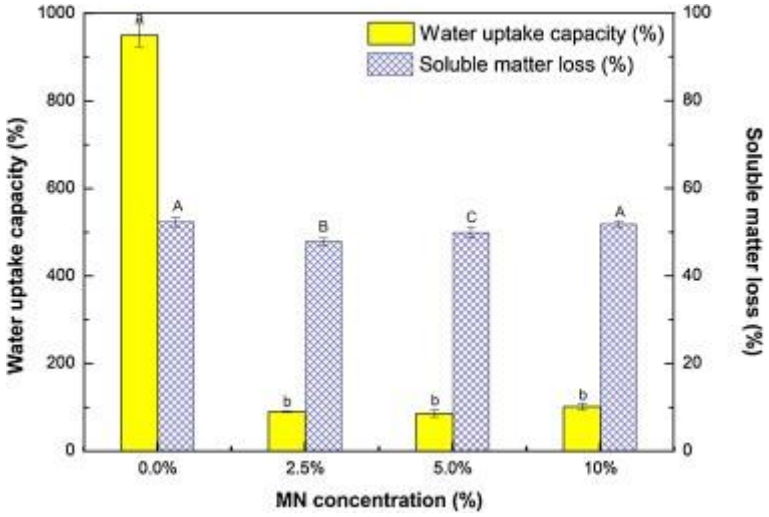

Fig. 5. Water uptake capacity and soluble matter loss for soy/glycerol/micronutrient dry (SPI/Gly/MN) matrices at different zinc sulfate monohydrate percentages (0.0, 2.5, 5.0 and 10%). Columns with different letters are significantly different ($p \leq 0.05$).

As for the results of soluble matter loss, it appears that Gly diffused completely to the medium as soluble matter. Without $ZnSO_4 \cdot H_2O$, a small proportion of SPI was also solubilized (soluble matter loss >50%). This loss of SPI was inhibited when $ZnSO_4 \cdot H_2O$ was incorporated. In fact, soluble matter loss presented a minimum for 2.5%, which

indicates that only Gly was solubilized. In fact, the percentage at 2.5% was very similar to the percentage of Gly in the sample (Table 1). Nevertheless, when the content of $ZnSO_4 \cdot H_2O$ increased from 2.5% to 5.0 or 10%, an increase in soluble matter loss was again noted. One possible explanation for this behaviour would be that this increase was due to the sum of Gly and $ZnSO_4 \cdot H_2O$ that the specimen failed to retain. According to these results, the amount of $ZnSO_4 \cdot H_2O$ that the matrix could retain would be between 2.5 and 5.0%. This explanation would also reinforce the explanation given to the little or no difference found in water uptake capacity as $ZnSO_4 \cdot H_2O$ concentration increased.

3.2.4. *Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)*

ICP-AES measurements were carried out for 2.5% and 10% bioplastic matrices before (DBP) and after water absorption and freeze-drying stages (DPM) as well as for the water outlet after absorption (WGM). These measurements are shown in Table 2. It is worth pointing out that, although the initial amount of Zn was higher at 10%, the percentage of zinc retained in the 2.5% matrix was much greater. This result is consistent with the results obtained in water uptake capacity and soluble matter loss tests (Section 3.2.3). Thus, the 10% matrix seems to show a less integrated matrix that led to a rapid release of $ZnSO_4 \cdot H_2O$ in the absorption. In fact, the retained amount of Zn was not significantly different ($p < 0.05$) for the samples containing 2.5% and 10% MN (ca. 6.00 mg). A straightforward consequence of this is that the sample at 10% released much more Zn to the water (about 80% of the initial percentage of Zn).

Table 2. Percentages (wt.%) and weight (mg) of zinc (Zn) obtained in the ICP-AES measurements for 2.5% and 10% in the studied systems before (DBP) and after absorption/drying (DPM) as well as in water after absorption (WGM). Different letters indicate statistically significant differences between weight of zinc retained ($p < 0.05$).

Table 2. Percentages (*wt.%*) and weight (*mg*) of zinc (*Zn*) obtained in the ICP-AES measurements for 2.5% and 10% in the studied systems before (DBP) and after absorption/drying (*DPM*) as well as in water after absorption (*WGM*). Different letters indicate statistically significant differences between weight of zinc retained ($p < 0.05$).

Sample	Zn in DBP (wt.%; mg)	Zn in DPM (wt.%; mg)	Zn in WGM (wt.%; mg)
2.5%	100.0; 6.8 ± 0.2^{a}	82.1 ± 2.6; 5.5 ± 1.0^{b}	17.9 ± 2.6; 1.3 ± 1.0^{c}
10%	100.0; 31.0 ± 3.2^{d}	22.4 ± 2.1; 6.5 ± 0.8^{ab}	77.6 ± 2.2; 24.5 ± 0.8^{e}

3.2.5. *Scanning electron microscopy*

Fig. 6 shows SEM images obtained for matrices (DPM) without and with 10% $ZnSO_4 \cdot H_2O$ after absorption and freeze-drying. It can be observed that the matrix without Zn presents pores larger than those in the matrix containing 10% MN. In addition, the pore size for the matrix without $ZnSO_4 \cdot H_2O$ is rather heterogeneous, roughly ranging between 10 and 100 μm, with the most prevalent size being 20 μm. On the other hand, the pore size for the 10% matrix is more homogeneous, between 5 and 10 μm, with 5 μm being the most common size. These results indicate that the incorporation of MN in the matrix entails a reduction of the pore size, with these pores being more homogeneously distributed. This is another factor contributing to explain the reduction of water uptake capacity in these matrices, as well as their greater rigidities.

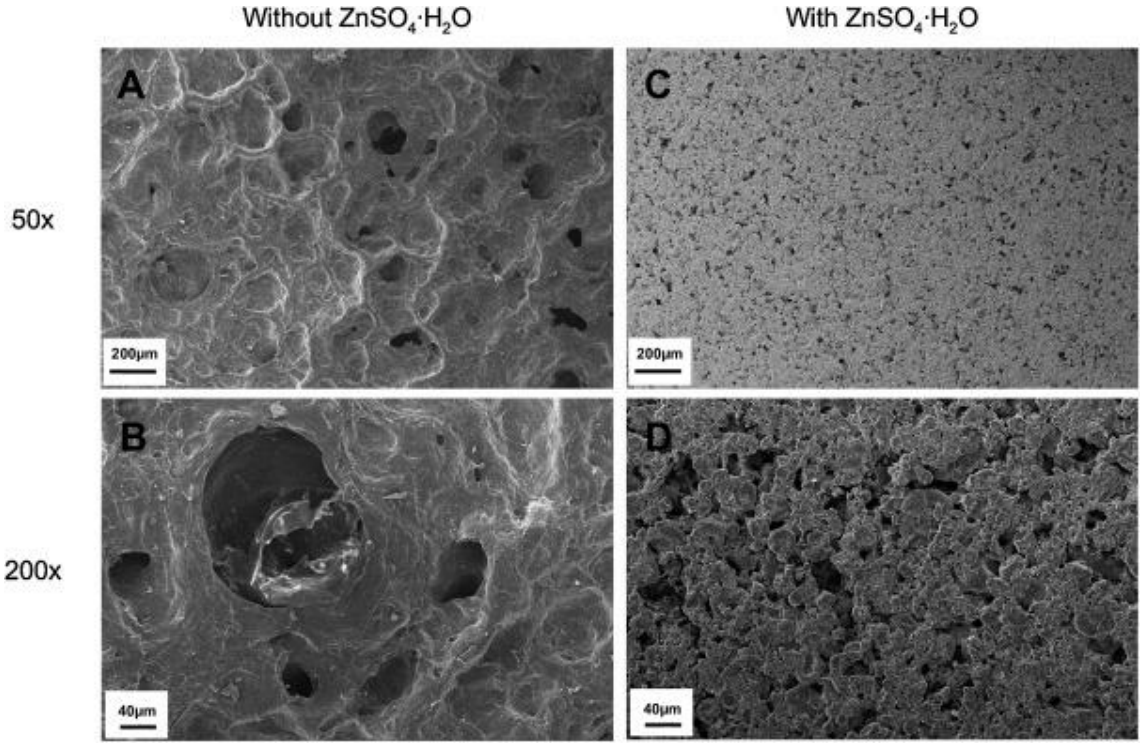

Fig. 6. SEM images of soy/glycerol/micronutrient (SPI/Gly/MN) freeze-dried matrices (DPM): Without $ZnSO_4 \cdot H_2O$ at 50 x (A) and at 200 x (B) as well as with 10% $ZnSO_4 \cdot H_2O$ at 50 x (C) and 200 x (D).

4. CONCLUSIONS

Soy-based bioplastics may be regarded as novel biopolymer matrices based on renewable natural components, highly attractive for the incorporation and subsequent release of micronutrients that are essential for the development and health of plants, avoiding the typical excesses of conventional fertilizers. Fairly promising micronutrient loading results of soy protein-based bioplastic matrices have been obtained. Thus, an important amount

of an essential micronutrient for a plant (Zn) has been incorporated to the matrix (up to 10 wt.% $ZnSO_4 \cdot H_2O$). However, the incorporation of the micronutrient Zn, in the form of $ZnSO_4 \cdot H_2O$, to bioplastic matrices during mixing leads to more rigid and less deformable bioplastic matrices, although these specimens maintain their dimensional stability. In addition, these matrices with Zn involve a significant reduction in water uptake capacity, due to some superimposed effects, including the progressive reduction of protein and glycerol, the negative effect of ionic strength on water absorption and the development of a more integrated enhanced elastic network. In fact, the pore size of these matrices is highly influenced by the presence of the micronutrient since a remarkable decrease in pore size is found when the micronutrient is present. A percentage of $ZnSO_4 \cdot H_2O$ higher than 2.5% does not lead to neither better absorption results (higher water uptake capacity, lower soluble matter loss or higher amount of Zn in the matrix after 24 h absorption,), nor better mechanical properties.

Further research is being currently carried out, including the use of different zinc carriers such as carbonate or EDTA quelling agents, as well as new strategies to incorporate the micronutrient into the protein-based matrices, in order to achieve a balance between the zinc loading level and the water uptake capacity. In any case, although a more comprehensive study will be required to optimize the incorporation and release of zinc cations from protein-based matrices, the results obtained in this work open up a great potential for the use of these matrices as a source of supplying micronutrients for horticulture applications.

ACKNOWLEDGEMENTS

This research is part of a project supported by MINECO, from the Spanish Government (Ref. CTQ2015-71164-P, MINECO/FEDER, UE). The authors gratefully acknowledge their financial support. The authors would also like to thank CITIUS (Universidad de Sevilla, Spain) for providing all access and assistance to Microanalysis and Microscopy Service.

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