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DEVELOPMENT OF SUPERABSORBENT SOY PROTEIN-BASED BIOPLASTIC MATRICES WITH INCORPORATED ZINC FOR HORTICULTURE

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The use of superabsorbent materials has recently spread in horticulture since they have great advantages such as the efficient use of water in periods of drought, being able to retain water and release it as crops need it. However, these superabsorbent materials are made of synthetic polymers, which present degradability problems and, sometimes, even toxicity. For this reason, the main objective of this work is the development of biodegradable superabsorbent bioplastic (SAB) matrices using as raw material a soy protein isolate (SPI). In addition, zinc is incorporated into these bioplastic matrices as an essential micronutrient for plants, to increase their added value. In this way, the incorporation of zinc chelated with 2,2’,2’’,2’’’-(Ethene-1,2-diyldinitrilo)tetraacetic acid (Zn AEDT) (a salt in which the micronutrient is incorporated) into soy protein-based bioplastic matrices improve their superabsorbent capacity and provided a controlled release of water and nutrients to the crops. The results obtained show the high potential of these bioplastic matrices for their use in horticulture as superabsorbent materials which can release water and nutrients in a controlled manner.

Keywords: *bioplastic matrices; superabsorbent; soy; zinc; controlled released; horticulture*

DESARROLLO DE MATRICES BIOPLÁSTICAS SUPERABSORBENTES DE PROTEÍNA DE SOJA CON ZINC INCORPORADO PARA HORTICULTURA

El uso de materiales superabsorbentes ha aumentado recientemente en horticultura ya que presentan grandes ventajas como la eficiencia en el uso del agua en periodos de sequía, siendo capaces de retener agua y suministrarla a los cultivos conforme a su necesidad. No obstante, estos materiales superabsorbentes están hechos con polímeros sintéticos, que presentan problemas de degradabilidad y, a veces, también de toxicidad. Por esta razón, el objetivo principal de este trabajo es el desarrollo de matrices bioplásticas superabsorbentes y biodegradables (SAB) usando como materia prima el aislado proteico de soja (SPI). Además, el zinc se incorpora a estas matrices bioplásticas, en forma de sal, como un micronutriente esencial en las plantas, para incrementar su valor añadido. De esta forma, la incorporación de zinc quelatado con ácido 2, 2’, 2``, 2```-(Etilen-1,2-diildinitrilo)tetraacético (Zn AEDT) a las matrices bioplásticas de soja mejora su capacidad superabsorbente y proporciona una liberación controlada de agua y nutriente en los cultivos. Estos resultados muestran el gran potencial para el uso de estas matrices bioplásticas en horticultura como materiales superabsorbentes que pueden liberar agua y nutrientes de una forma controlada.

Palabras clave: *matrices bioplásticas; superabsorbente; soja; zinc; liberación controlada; horticultura*

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1. Introduction

In recent years, the increase in carbon dioxide emissions has boosted the effect of global warming (Cox et al., 2000). One of the most significant impacts of this warming is the change in the water cycle. Lately, there are more extensive periods of drought and periods of rainfall, which, although scarce, discharge large amounts of water (Wang et al., 2006). These changes in rainfall have mainly affected horticulture, since crops need a regular supply of water, which must be controlled manually, increasing their price (Venuprasad et al., 2007).

To counteract this problem, superabsorbent polymeric materials are being used nowadays buried in the farmland. This materials can absorb and retain a large amount of water without losing their integrity (from 10 to 1,000 times their weight) (Chanda and Roy, 2007). These materials could absorb rainwater and supply it to crops during periods of drought, optimizing the water cycle (Zohuriaan-Merh and Kabiri, 2008). However, typical superabsorbent materials are not biodegradable and can be toxic to crops if they are not removed effectively. A possible alternative to these materials is the use of biodegradable superabsorbent (bio)polymeric matrices (SAB) (Mortain et al., 2004).

Among all the raw materials that can be used to make SAB, proteins have technological, economic and environmental advantages. In addition, their properties are easily modellable due to the wide range of formulations and applicable processing techniques (Bozell, 2006). On the other hand, they are the most underutilized and undervalued by-product in the agri-food industry. Their use as SAB would implie an increase in their added value (Damodaran, 1997). Finally, they can pose an extra contribution of nutrients after their degradation due to their significant nitrogen content. Another advantage of the use of these materials in horticulture is the incorporation of micronutrients in a controlled manner to the crops. In this sense, micronutrients can be incorporated into the SAB and, due to their biodegradability and water release, be captured by the crops, increasing the efficiency of the incorporation of micronutrients.

From all the different proteins studied in the literature for the elaboration of protein-based SAB, soy protein has shown the best absorbent capacity, due to its high content of aspartic and glutamic acid, which give it a good hydrophilic character (Fernández-Espada et al., 2016a).

Among the processing techniques, soy protein-based SAB allow an adequate processing by injection molding, facilitating its implementation on an industrial scale, since it is one of the most used techniques for the manufacture of synthetic plastics (Fernández-Espada et al., 2016a, 2016b; Félix et al. 2014). However, the incorporation of micronutrients generally hinders the processing of these matrices, as it results in a reduction of the strengthening potential of the matrix associated with the corresponding reduction in the protein content.

2. Objectives

The main objective of the present work was the development of sustainable, superabsorbent and biodegradable soy protein-based bioplastic matrices with zinc (micronutrient) incorporated.

3. Experimental

To achieve this objective, zinc chelated with 2,2’,2’’,2’’’-(Ethene-1,2-diyldinitrilo)tetraacetic acid (Zn AEDT) was used as a salt, which contains the essential micronutrient for plants. In addition, the mechanical and morphological properties of matrices with different concentrations of Zn AEDT incorporated were evaluated throughout the processing stages (blends and bioplastic matrices before and after the water stage), as well as their water absorption capacity.

3.1 Materials

Soy protein isolate (SPI, *Glycine max L. Merril*), supplied by Protein Technologies International (SUPRO 500E, Belgium) with min. 91 wt.% protein, and glycerol (Gly), provided by Panreac Química Ltd. (Spain), were used to obtain protein-based SAB bioplastic matrices. In addition, zinc chelated with 2,2’,2’’,2’’’-(Ethene-1,2-diyldinitrilo)tetraacetic acid (Zn AEDT), which was supplied by Trade Corporation International S.A.U. (Spain), was used as a salt, which provides the selected micronutrient.

3.2 Preparation of soy protein-based bioplastic matrices

The procedure used is a generic, two-stage method. Firstly, SPI and Gly (weight ratio 1:1) with different percentages of Zn AEDT (5, 10 and 15 wt.%) were homogenized using a mixer Polylab QC (ThermoHaake, Germany). This mixing was performed adiabatically for 10 min at 50 rpm, starting at room temperature (25 ± 2 ºC), following the guidelines of previous studies (Fernandez-Espada et al., 2016a; Jiménez-Rosado et al., 2018). Consequently, the dough-like blends were subjected to an injection molding in a MiniJet Piston Molding System II (ThermoHaake, Germany) to obtain rectangular protein-based bioplastic matrices (60x10x1 cm). The parameters selected in this stage based on previous studies (Fernandez-Espada et al., 2016a; Felix et al., 2014) were a cylinder and mold temperature of 40 and 70 ºC, respectively, an injection pressure of 500 bar (for 20 s) and a post-injection pressure of 200 bar (for 300 s).

Additionally, the bioplastic matrices were subjected to a dehydrothermal treatment, i.e., they were placed in an oven at 50 ºC for 24 h to reinforce their structure, since it was found that without this stage the bioplastic network is not strong enough and it disintegrates immersed into water.

3.3 Characterization of soy protein-based bioplastic matrices

The bioplastic matrices with different percentages of Zn AEDT (5, 10 and 15 wt.%) were characterized in order to evaluate the suitability of the bioplastic matrices as superabsorbent matrices with incorporated micronutrient. In addition, a bioplastic matrix without Zn AEDT was measured as the reference system (Ref.).

3.3.1 *Tensile strength measurements of bioplastic matrices*

The tensile tests were performed in order to evaluate the mechanical resistance of the different bioplastic matrices. The tests were carried out in a mechanical analyzer RSA3 (TA

Instrument, USA) following the ISO standard 527-2:1993 (1993). Therefore, the bioplastic matrices were subjected to an increasing axial stress until breakage at a speed of 1 mm/min, comparing their strain at break (ε_{max}), maximum stress (σ_{max}) and Young's modulus.

3.3.2 *Water uptake capacity and soluble matter loss*

Water uptake capacity and soluble matter loss from the different bioplastic matrices were measured, following the ASTM 180 D570 standard (2005), in order to determine whether the matrices with Zn AEDT incorporated have a superabsorbent character. It is worth mentioning that bioplastic matrices after water absorption are usually called matrices because they lose their bioplastic character, as they lose the plasticizer.

3.3.3 *Scanning electron microscopy (SEM)*

SEM images were captured in order to compare the structures of the different bioplastic matrices before and after the water absorption. Thus, the samples were coated with palladium/gold to improve the quality of the images and subsequently they were observed through a Zeiss EVO electronic microscope (USA) with an acceleration voltage of 10 kV, following the same protocol applied by Orawan et al. (2006).

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

Measurements of inductively coupled plasma-atomic emission spectroscopy (ICP-AES) were made to the different matrices in order to evaluate the amount of zinc that remained in them after the water absorption stage. These measurements were carried out in an ICP SpectroBlue TI (Spectro, Germany), where the samples passed through a plasma torch at a temperature of around 6000 K. Thus, the samples were dissociated into free atoms and ions that emit in characteristic wavelengths, which were measured.

3.3.5 *Compression measurements to matrices*

Compression measurements were made to determine the mechanical resistance of the matrices after a severe water treatment (water uptake capacity test). These measurements were made in compression mode using a mechanical dynamic analyzer RSA3 (TA Instrument) with a cylindrical geometry of 8 mm in diameter. Frequency sweep tests were performed between 0.02 and 20 Hz, at a strain of 0.01% (within the linear viscoelastic range) and room temperature (25 ± 2 ºC) to study the variation of the elastic (E') and viscous (E'') moduli with the frequency.

3.4 Statistical analysis

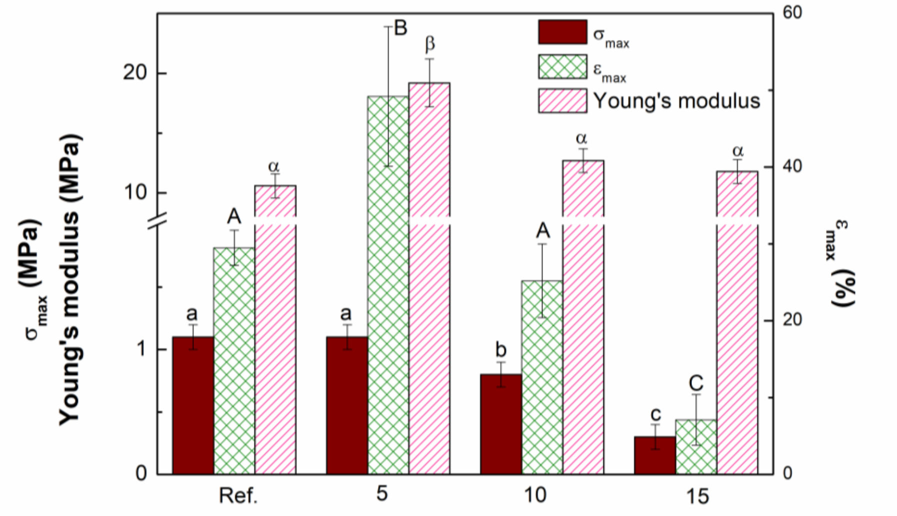
At least three replicates of each measurement were made. Statistical analyses were carried out with t test and one-way analysis of variance ($p \leq 0.05$), for which PASW Statistics for Windows (Version 18 SPSS, Chicago, IL) was used. In addition, the standard deviation of the selected parameters was calculated.

4. Results and discussion

4.1 Tensile strength measurements of bioplastic matrices

The results of tensile strength tests for the different bioplastic matrices are shown in Figure 1. As can be seen, the maximum stress (σ_{max}) of the bioplastic matrices without Zn AEDT and 5 wt.% Zn AEDT did not show significant differences. However, the bioplastic matrices with higher Zn AEDT concentrations (10 and 15 wt.%) showed lower σ_{max} values, which were more pronounced for the system with the highest Zn AEDT percentage. Regarding strain at break (ε_{max}), an increase was observed with the incorporation of 5 wt.% Zn AEDT, which decreased significantly at higher Zn AEDT percentages (10 and 15 wt.%). Finally, the Young's modulus did not present significant differences in the studied systems, except for the bioplastic matrix that contains 5 wt.% Zn AEDT, which improved slightly. This behavior is possibly due to the action of Zn AEDT as filler in these bioplastic matrices, which did not cross over with the protein structure. In addition, it can hinder the crosslinking of the protein, which is also in smaller proportion. Therefore, bioplastic matrices have worse mechanical properties in these cases. However, the 5 wt.% Zn AEDT system seems to be an optimum proportion of filler, since it allows the protein network to develop without obstructing and reinforcing it. Thus, these bioplastic matrices achieved the best mechanical properties.

Figure 1: Tensile strength parameters (maximum stress (σ_{max}), strain at break (ε_{max}) and Young's modulus) for soy protein isolate/glycerol/Zn AEDT bioplastic matrices at different Zn AEDT concentrations (0 (Ref.), 5, 10 and 15 wt.%).

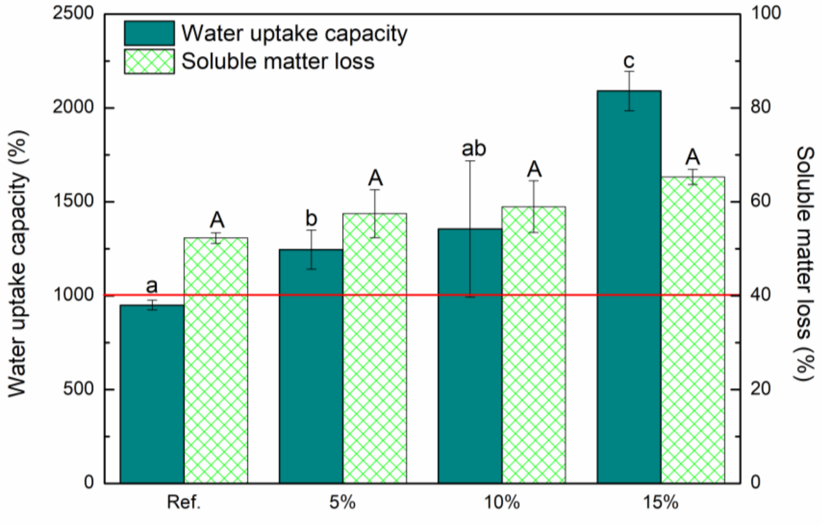


Columns with different letters are significantly different (*p* ≤0.05).

4.2 Water uptake capacity and soluble matter loss

Figure 2 shows both the water uptake capacity and soluble matter loss of the different bioplastic matrices. The bioplastic matrix without salt showed values slightly lower than the limit of the superabsorbent materials (1,000%). However, the incorporation of Zn AEDT increased the water absorption capacity towards superabsorbent values, being more pronounced at higher concentrations. However, the bioplastic matrix with 15 wt.% Zn AEDT, which had the best water uptake capacity, was not resistant out of the water (it even broke down), possibly due to its lower amount of protein, which may have caused the matrix to be less structured. Regarding soluble matter loss, there were no significant differences between the different samples, which suggests that Gly, due its hydrophilic character, was lost in all systems. In addition, the protein/Zn AEDT loss increased when larger amounts of Zn AEDT were incorporated, since these matrices had less Gly, although these systems reached practically the same soluble matter loss. These results may be due to the lower strengthening of the matrices, as mentioned above.

Figure 2: Water uptake capacity and soluble matter loss tests of soy protein isolate/glycerol/Zn AEDT with different Zn AEDT concentrations (0 (Ref.), 5, 10, 15 wt.%).



Columns with different letters are significantly different ($p \leq 0.05$). The red line limits the superabsorbent range.

4.3 Inductively coupled plasma-atomic emission spectroscopy (ICP-AES)

Table 1 shows the amount of zinc present in the bioplastic matrices before and after the absorption stage. There are no significant differences between the amounts of zinc remaining in the matrices after the absorption stage. Therefore, the zinc released in the water absorption stage was more pronounced as more Zn AEDT was incorporated into the initial bioplastic matrix. This fact may be due to the lower strengthening of the matrix, as has already been mentioned, which caused the loss of a greater amount of protein and Zn AEDT.

Table 1: Percentage (wt.%) of zinc in the bioplastic matrices with different Zn AEDT concentrations (0 (Ref.), 5, 10, and 15 wt.%) before and after water absorption tests.

Bioplastic matrix	wt.% Zn before absorption	wt.% Zn after absorption
Ref.	-	-
5 wt.%	9.14^{a}	0.87^{b}
10 wt.%	18.28^{c}	0.88^{b}
15 wt.%	27.42^{d}	0.88^{b}

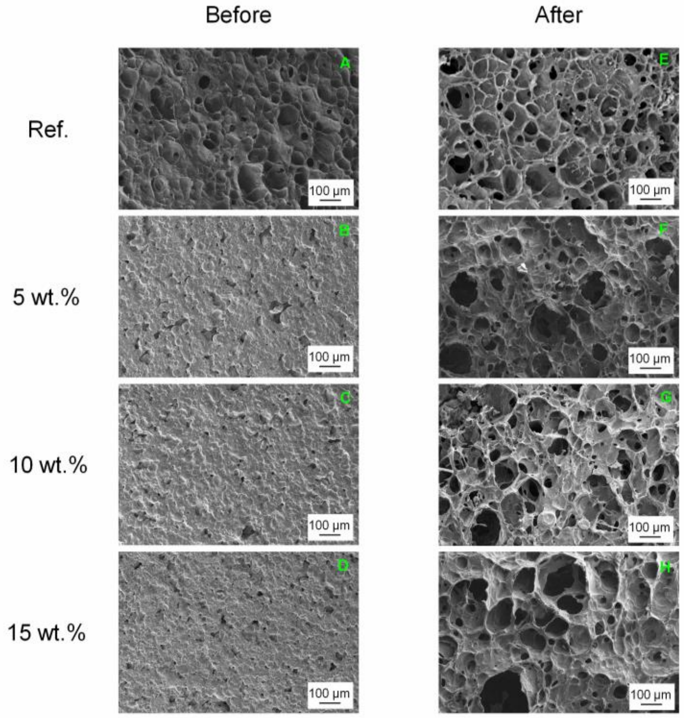
Values with different letters are significantly different ($p \leq 0.05$).

Thus, it is expected that, in all the matrices, most of the zinc would be released in a controlled manner with the water incorporated in the matrix, with this percentage being more pronounced in the matrix with higher Zn AEDT incorporated. Moreover, the small amount of Zn retained in the matrix would be released along with the other nutrients provided by the protein matrix (mainly nitrogen) during its biodegradation.

4.4 Scanning electron microscopy (SEM)

Figure 3 shows the images taken from the different bioplastic matrices before and after the water absorption tests. The images of the bioplastic matrices before the water absorption (3A-D) show that the addition of Zn AEDT to the systems leads to a more homogeneous structure with less pores. This could be due to the greater amount of filler material. On the other hand, the images of the matrices after the water absorption (3E-H) show that the water absorption stage made the matrices develop larger and more heterogeneous pores, which is more evident at higher concentrations of Zn AEDT. This behavior could be due to the greater protein/Zn AEDT loss that took place as more salt was incorporated into the matrix, which was anticipated in the water uptake capacity and soluble matter loss tests (4.2).

Figure 3: SEM images of the bioplastic matrices with different Zn AEDT percentages (0 (Ref.), 5, 10, and 15 wt.%) before and after the water absorption tests.



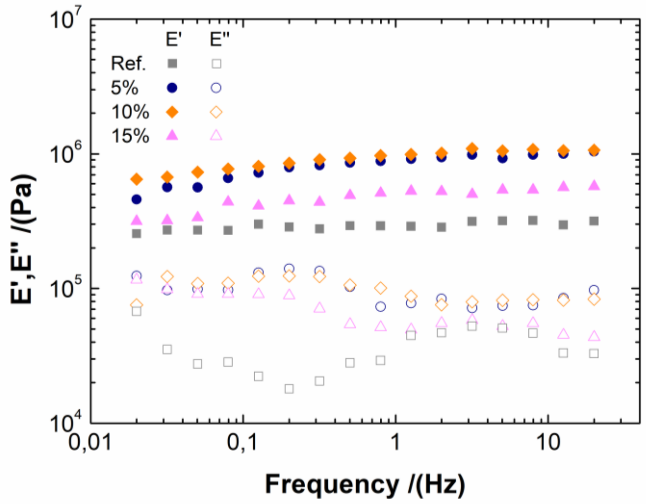
Bioplastic matrices without and with 5, 10 and 15 wt.% Zn AEDT before (A, B, C and D, respectively) and after (E, F, G and H, respectively) water absorption tests.

In this sense, Zn AEDT made the structure develop fewer holes in the bioplastic matrices before the water absorption. However, most of this material was lost in this stage due to the lower protein network formed, making the matrices with greater concentrations of Zn AEDT present more free volume after absorption. Thus, the sample with 5 wt.% Zn AEDT corresponds to the matrix with the lowest free volume, which could explain the improvement of the mechanical properties of this bioplastic matrices. Furthermore, these matrices may have better mechanical properties after absorption.

4.5 Compression measurements of the matrices

Figure 4 shows the mechanical properties of the different matrices after the water absorption tests. As can be observed, the elastic modulus (E’) remained above the viscous modulus (E’’) for all the frequency range studied. Moreover, E’ had a slight dependence on frequency. Thus, all the matrices showed a basically elastic character. In fact, stronger matrices were obtained when Zn AEDT was incorporated, since higher E’ values were observed. It is noted that the matrix with the 15 wt.% of Zn AEDT has the worst E’ comparing with all the matrices with incorporated Zn AEDT. This result, can be explain its disintegration in the water uptake capacity test.

Figure 4: Dynamic frequency sweep tests for soy protein isolate/glycerol and Zn AEDT bioplastic matrices with different Zn AEDT percentages (0 (Ref.), 5, 10, and 15 wt.%).



5. Conclusions

Superabsorbent soy protein-based matrices were developed, showing a great potential for their use in horticulture. In addition, essential micronutrients for crops (zinc) can be incorporated in them.

The incorporation of Zn AEDT to the matrices improved their superabsorbent capacity; however, percentages greater than 5 wt.% Zn AEDT decreased the mechanical properties of the bioplastic matrices due to a lower strengthening of the matrix network. In addition, these worse mechanical properties cause a greater release of zinc in the absorption stage, which must to be taken into account in subsequent release tests conducted in crops in future studies.

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