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5 EFFECT OF PLASTICIZER AND STORAGE CONDITIONS  
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8 ON THERMOMECHANICAL PROPERTIES OF  
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10 ALBUMEN/TRAGACANTH BASED BIOPLASTICS  
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4 **Abstract**  
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8 The effects of composition of the plasticizer fraction and storage conditions on the physical and  
9 thermomechanical properties of egg albumen/tragacanth gum based bioplastics were studied.  
10 Thus, glycerol (G) and water (W) were used as plasticizers at different G/W ratios (1:0, 3:1, 1:1,  
11 1:3, 0:1), keeping the biopolymer fraction always at 60% (w/w). Tragacanth gum was included in  
12 the formulation for its well-known hydrophilic character, as possible future applications of these  
13 bioplastics may be moisture dependent (e.g. Modified Atmosphere Packaging). Moreover,  
14 properties of bioplastics stored at room temperature under no control of relative humidity were  
15 different of those obtained when bioplastics were equilibrated a 53% relative humidity (RH)  
16 atmosphere. This is reflected in the DMTA and tensile tests results, for which water loss in the  
17 samples with the highest water contents (1:3, 0:1) involves very significant increases in  
18 viscoelastic moduli and tensile strength when equilibrated at 53% RH. Glycerol presence when  
19 no RH control was taken promotes water uptake, probably due to an interaction between both  
20 plasticizers, which eventually lead to a greater plastic region in the tensile tests.  
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38 **Keywords:** *albumen; tragacanth; plasticizer; bioplastic; relative humidity*  
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# 1. Introduction

Proteins, biopolymers directly extracted from biomass, are composed of more than 20 different aminoacids that, through numerous intermolecular linkages and interactions, yield a great range of functional properties (Gomez-Martinez et al., 2009; Pommet et al. 2003). In that sense, different types of proteins like wheat gluten, corn zein, soy protein, etc., have recently shown suitability for the manufacture of bioplastics (Zárate-Ramírez et al., 2014; Gomez-Martinez et al. 2013; Gomez-Martinez et al., 2009; Kim, 2008; Mohanty et al., 2005; Tummala et al. 2006; Zheng et al., 2003).

Protein-based bioplastics generally include plasticizers and, sometimes, a disrupting agent in their formulation (Sothornvit and Krochta, 2005; Pommet et al.,2005). Plasticizers, like glycerol or water, are molecules with low molecular weight and volatility that facilitate the bioplastic processing by reducing the intermolecular forces between the polymeric chains, increasing their mobility. With this regard, three main steps must occur during the formation of protein-based bioplastics: a) breaking of the stabilizing intermolecular bonds; b) orientation of mobile polymer chains in the desired shape; and c) formation of new intermolecular bonds that stabilize the three-dimensional network. Furthermore, a procedure in which a dough-like material is obtained by either kneading or extruding a protein/plasticizer blend is referred to as “thermo-plastic”. If the resulting material is further subjected to both heat and pressure, the procedure is called “thermo-mechanical” (Jerez et al., 2007a). Hence, an adequate selection of composition and processing parameters may lead to materials with unique properties (Pommet et al., 2003).

Egg white albumen is a protein mainly composed of ovalbumin (54 wt.%), a monomeric phosphoglycoprotein with four free sulfhydryl groups buried in the protein core. Denaturation of albumen by heat results in the exposure of those groups, accompanied by a decrease in its total content due to oxidation to disulfide bonds (Van der Placken et al., 2005). Consequently,

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4 proteins chains unfold and entangle with neighboring chains and new bonds arise, making  
5 texture change. Due to its functional properties, egg white albumen has traditionally been used  
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7 by the food industry as gelling, foaming, heat setting and binding agent. As a novel alternative to  
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9 the food industry, recent work by Jerez et al. (2007a; 2007b), González-Gutiérrez et al. (2010;  
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11 2011) and Fernández-Espada et al. (2013) has revealed the feasibility of producing highly  
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13 transparent bioplastics from egg white albumen. If compared to other common proteins, those  
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15 bioplastics showed suitable mechanical properties for the manufacture of biodegradable food  
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17 packaging and other plastic stuffs by application of thermo-mechanical methods.  
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22 However, high hydrophilic bioplastics may be required in modified atmosphere packaging (MAP)  
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24 applications, as oxygen scavenging and CO<sub>2</sub> emitting processes are moisture dependent, and  
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26 only occur after water has been absorbed from the food product or package atmosphere. For  
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28 this reason, tragacanth gum, a hydrophilic dried exudate obtained from the stems and branches  
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30 of Asiatic species of Astragalus, may be included in the formulation. Tragacanth gum is a very  
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32 complex heterogeneous anionic polysaccharide of high molecular weight (Weiping and  
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34 Branwell, 2000) consisting of two main fractions: a) a water-insoluble component called  
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36 bassorin, which has the capacity to swell and form a gel; and b) a water-soluble component  
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38 called tragacanthin (Balaghi et al., 2010). Tragacanth gum, accepted since 1961 as GRAS  
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40 (generally recognized as safe, according to FDA) at the level of 0.1-1.3 wt.% (Anderson and  
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42 Bridgeman, 1985), has been used for many years as a stabiliser, thickener, emulsifier and  
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44 suspending agent in the food, pharmaceutical, cosmetic, textile and leather industries, as well  
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46 as in technical applications. It presents high viscosity in aqueous solution at low concentration,  
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48 good suspending action, high stability to heat and acidity and effective emulsifying properties. It  
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50 is also pourable and has creamy mouth feel and good flavour-release properties (Weiping and  
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52 Branwell, 2000), and very long shelf life (Levy and Schwarz, 1958). Preliminary studies carried  
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4 out have pointed out the effect of tragacanth gum on increasing water absorption of the resulting  
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6 protein-based material.  
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9 Moreover, previous results revealed a certain degree of incompatibility between glycerol and the  
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11 protein matrix, which may result in its substantial migration to the bioplastic surface. The  
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13 inclusion of tragacanth gum may prevent this, as it has also shown to prevent glycerol migration.  
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15 In fact, tragacanth gum has been used along with glycerol and water as a tablet binder in the  
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17 pharmaceutical or cosmetic fields (Phillips and Williams, 2000).  
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21 Based on above observations, this research studies the effect of plasticizer on the water uptake  
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23 capacity and thermo-mechanical/tensile properties of albumen/tragacanth bioplastics obtained  
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25 by a thermo-mechanical treatment. Hence, different formulations derived from a fixed  
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27 albumen+tragacanth/plasticizer ratio (60:40), but varying the water/glycerol proportion within the  
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29 plasticizer, were studied. A comparison between fresh samples and those being stored under  
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31 relative humidity (RH) control was also established.  
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## 34 35 **2. Materials and methods**

### 36 37 **2.1. Materials**

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42 Spray-dried egg white albumen (designated EW; with 73 wt.% protein, 6 wt.% ashes and 8 wt.%  
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44 moisture) provided by OVOSEC S.A. (Spain) was used as base material for bioplastics  
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46 manufacture. On the other hand, tragacanth gum (designated TG) (39-42% carbon content) was  
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48 supplied by Sigma-Aldrich (USA). In relation with the plasticizers, glycerol, from Panreac  
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50 Química, S.A. (Spain), and distilled water were designated G and W, respectively.  
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55 As far as formulations are concerned, a biopolymer/plasticizer ratio of 60:40 was set, with the  
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57 protein/gum ratio within the biopolymer fraction being fixed as 2:1, and the glycerol/water ratio  
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59 within the plasticizer varying as 1:0, 3:1, 1:1, 1:3 and 0:1. These formulations allowed the effect  
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4 of the plasticizer on the water uptake capacity and mechanical properties to be evaluated.  
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6 Moreover, the influence of tragacanth addition was evaluated by comparing some of these  
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8 formulations with their counterparts where the protein was the only component within the  
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10 biopolymer fraction. Table 1 provides the final formulations in terms of wt.% of each ingredient.  
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14 With regard to the bioplastics manufacture, this was accomplished by a thermo-mechanical  
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16 process, which includes two stages:  
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19 a) Mixing of ingredients: it was carried out for 10 min in the kneading tool (Rheomix 600p) of a  
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21 torque-rheometer (Polylab, Thermo Haake GmbH, Germany) equipped with two counter-rotating  
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23 rollers turning at 50 rpm (Jerez et al., 2005). Temperature, starting at 25°C, was allowed to  
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25 naturally evolve over this period (no heating/cooling).  
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28 b) Compression-moulding: the resulting dough-like material was subjected to pressure of 100  
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30 bar and temperature of 120 °C for 10 min in a hot-plate press, as described by Jerez et al.  
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32 (2007b). Two types of moulds were used: one to obtain rectangular 3-mm-thick specimens for  
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34 both DMTA and water uptake capacity measurements; and a second one to obtain type IV-  
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36 dumbbell specimens (ASTM D638, 2003) for tensile tests.  
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41 After preparation and before testing, these samples were either wrapped in plastic film and  
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43 stored in sealed glass containers at room temperature (no RH control) or placed in desiccators  
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45 at relative humidity of 53% with a saturated solution of  $Mg(NO_3)_2 \cdot 6H_2O$  at room temperature (RH  
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47 control). Samples were always stored for 24 hours prior any test was conducted.  
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## 50 51 **2.2. Methods**

### 52 53 54 **2.2.1. Water uptake**

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4 Water uptake tests, according to ASTM D570 (2005), were carried out on rectangular probes  
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6 (50×10×3 mm<sup>3</sup>) immersed into distilled water for 24 hours. Three replicates were done for each  
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8 sample, and the water uptake percentage calculated as:  
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$$\text{Water uptake (wt. \%)} = \frac{\text{wet wt.} - \text{reconditioned wt.}}{\text{conditioned wt.}} \times 100 \quad (\text{Eq. 1})$$
  
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17 where: conditioned weight, is the initial weight of the probe; wet weight, refers to the weight of  
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19 the probe just after 24 hours of water immersion; and reconditioned weight, is the final weight of  
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21 the wet sample after 24 hours of drying in an oven at 50°C.  
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### 23 24 **2.2.3. Dynamic Mechanical Thermal Analysis (DMTA)**

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27 DMTA experiments were performed with a RSA3 rheometer (TA Instruments, USA) in dual  
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29 cantilever bending mode on rectangular 50×10×3 mm<sup>3</sup> specimens. Dynamic temperature  
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31 sweeps were performed at a constant frequency of 1 Hz and strains within the linear  
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33 viscoelasticity region. Strain sweep tests were performed to determine the linear viscoelastic  
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35 region (LVR). The heating rate was set at 3 °C/min, within a temperature interval from -30 to  
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37 140 °C. All tests were performed at least three times.  
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### 41 42 **2.2.4. Mechanical properties**

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45 Tensile tests were performed with a MTS Insight 10 kN (USA), according to ASTM D638 (2003),  
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47 with an extension rate of 5 mm/min, at room temperature. An extensometer was used in order to  
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49 accurately register the sample elongation. At least, five tests at least were carried out for each  
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51 dumb-bell shaped type IV sample.  
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### 53 54 **2.2.5. Statistical analysis**

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4 The data were presented as mean  $\pm$  standard deviation (SD) of three (water uptake capacity  
5 and DMTA tests) or five (tensile tests) determinations. A probability value of  $p < 0.05$  was  
6 considered significant.  
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### 10 11 **3. Results and discussion**

#### 12 13 **3.1. Effect of the presence of tragacanth gum on EW/G/W bioplastics**

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16 The effect of the presence of tragacanth gum over the water uptake capacity, viscoelastic and  
17 mechanical properties of albumen-based bioplastics was evaluated through the addition of 20%  
18 (w/w) of tragacanth gum into the biopolymer fraction. A G/W plasticizer ratio equal to 1:1 was  
19 selected as reference.  
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24 On the one hand, important differences in the water uptake capacity are observed when  
25 comparing gum-free and gum-containing specimens. Thus, the inclusion of one third of gum in  
26 the biopolymer fraction (that is, EW/T ratio of 2:1) provokes an increase in the water uptake  
27 capacity of 110.3 % (Figure 1A). On the other hand, figure 1B shows the results obtained from  
28 DMTA tests, revealing the prevalence of the elastic modulus  $E'$  over the loss modulus  $E''$  in the  
29 whole temperature range studied ( $-30$  to  $140$  °C), independently of the presence of the gum.  
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31 But the presence of gum seems to affect the bioplastics mechanical properties, as when  
32 dynamic flexural measurements in the LVR region are conducted, lower values of the elastic  
33 modulus are noticed if the gum is included in the formulation. Also, a rubbery plateau region  
34 appears at temperatures above  $100$ °C when gum is present.  
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52 Finally, tensile tests corresponding to gum-free bioplastics always reveal a larger elongation at  
53 break ( $66 \pm 21\%$ ) and higher tensile strength ( $1.37 \pm 0.06$  MPa) than the corresponding gum-  
54 containing sample ( $17 \pm 2\%$ , and  $0.5 \pm 0.06$  MPa, respectively) (Figure 1C). Thus, the presence  
55 of gum in the formulation seems to result in bioplastics with poorer mechanical properties.  
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4 Moreover, the incorporation of tragacanth gum in the biopolymer may also affect the bioplastics  
5 stability through hydrolysis or degradation reactions. Thus, in Figure 1B, DMTA results obtained  
6 after 15 days of storage are included for both formulations, EW60 and EW40T20, observing  
7 how when tragacanth gum is not present, flexural moduli ( $E'$ ,  $E''$ ) remain virtually unaltered over  
8 that period of time. On the other hand, when the gum is present, moduli values increase after  
9 that storage time, being always lower than those obtained when gum is not present. Thus, it  
10 seems that the presence of tragacanth gum clearly affect the bioplastic stability, which should  
11 be taken into account when assessing the potential of bioplastics containing tragacanth gum for  
12 packaging.  
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25 Then, the presence of tragacanth gum proves to be a key factor in improving the hydrophilic  
26 character of the bioplastics, as may be suggested from the water uptake capacity test results.  
27 This may be desirable in future applications (i.e. Modified Atmosphere Protection, MAP), where  
28 mechanical properties may not be that decisive, though the stability issues should not be  
29 disregarded.  
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### 37 **3.2. Effect of the plasticizer on the thermoplastic processing of** 38 **EW/T/G/W bioplastics** 39 40 41

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43 With the aim to establish a comparative analysis between bioplastics with different  
44 glycerol/water (G/W) ratios, five selected formulations were prepared by fixing 60 wt.%  
45 biopolymer fraction with a EW/T ratio of 2:1, while varying the composition of the 40 wt.%  
46 plasticizer fraction (G/W ratios of 0:1, 1:3, 1:1, 3:1 and 1:0).  
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53 Intimate mixing of the ingredients constitutes the first step in the manufacture of the bioplastics.  
54 Figure 2 shows the evolution of both torque (A) and temperature (B) during the kneading, at 50  
55 rpm, of five albumen/tragacanth/plasticizer blends with different G/W ratios (0:1, 1:3, 1:1, 3:1  
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4 and 1:0). Plasticizer, protein and gum contents of 40, 40 and 20 wt.%, respectively, were kept  
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6 constant for all the systems studied.  
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10 These results show two well-defined regions when water is present (0:1,1:3, 1:1, 3:1): first, an  
11 instantaneous increase in torque up to a maximum value; and then a second region  
12 characterized by a continuous decay down to a steady state value. However, when glycerol was  
13 the only plasticizer (1:0), the torque experienced a slow increase, though not very significant. An  
14 optimum mixing time of 10 minutes was always set in order to obtain a dough-like blend,  
15 suitable for further thermo-mechanical treatments. Other authors reported, for longer mixing  
16 times and similar formulations containing glycerol as plasticizer, a dramatic structural  
17 breakdown, which turns the blend into a granular and heterogeneous material (sudden  
18 decrease in torque down to zero) (Jérez et al., 2007a).  
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30 Accordingly, two different temperature regions, which are related to the above described torque  
31 regions, are found in the mixing process. Thus, temperature increases exponentially during the  
32 first 2 minutes and then starts to equilibrate. In no case, temperature exceeded the plasticizers  
33 boiling points (100 and 290°C, for water and glycerol, respectively). Consequently, no significant  
34 mass loss would be expected.  
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42 When observing the influence of the composition within the plasticizer fraction in the kneading  
43 stage, it may be noticed how both mixing torque and temperature increase with glycerol content  
44 up to a maximum value for the sample containing a G/W ratio of 3:1. However, if the glycerol  
45 content is further increased up to 100 wt.% (G/W ratio of 1:0), torque and temperatures go down  
46 into a minimum value. Thus, formulations containing only one plasticizer, either water (0:1) or  
47 glycerol (1:0), showed much lower values than those where both plasticizers were used  
48 altogether. Interestingly, Gómez-Martínez et al. (2009) also obtained higher torque values for a  
49 sample containing a glycerol/water blend as plasticizer, if compared to samples containing only  
50 one of them. With this regard, some authors (Chen et al., 2009) have analysed the hydrogen  
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4 bonding of glycerol aqueous solutions by means of a molecular dynamics simulation method,  
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6 finding that average numbers of hydrogen bonds per water and glycerol molecule and their  
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8 lifetime depend on the glycerol mole fraction. In our case, it is complicated to find detailed  
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10 explanation to the results obtained, as the hydrogen bonds established between glycerol and  
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12 water molecules occur in a complex matrix composed of protein and gum. However, the  
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14 evolution observed suggests the existence of important interactions between these plasticizers,  
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16 enhanced by a high glycerol concentration.  
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### 21 **3.3. Effect of the relative humidity on EW/T/G/W bioplastics**

#### 22 23 24 **3.3.1. Water uptake capacity**

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28 The water uptake capacity values for EW/T/G/W bioplastics with different glycerol/water ratios  
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30 (G/W ratios of 0:1, 1:3, 1:1, 3:1 and 1:0) are shown in Figure 3, for two different storage  
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32 conditions: no Relative Humidity (RH) control or under RH control (53%). The study of water  
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34 uptake capacity in biopolymer systems is receiving an increasing attention recently because of  
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36 their important applications in the fields of biomedical, pharmaceutical, environmental and  
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38 agricultural engineering (Castro et al., 2008; Buchholz, 1998; Gómez-Martínez et al., 2009).  
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43 When observing those probes without RH control, it seems that higher glycerol contents tend to  
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45 induce greater water uptake after 24 hours. Water uptake average values range from 141.1 to  
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47 235.3% under these conditions, with the lowest value corresponding to the probe without  
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49 glycerol, and the highest value to that with a glycerol/water ratio of 3:1. These results may be  
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51 related to the typical hygroscopic character of low molecular weight polyols, as glycerol  
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53 (Chinachoti and Steinberg, 1984, 1988; Baik and Chinachoti, 2001; Lai et al., 2006).  
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57 On the contrary, no apparent tendency is observed when the probes submitted to RH control  
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59 are studied, with all water uptake values being in a narrower range (from 150.1 to 208%). The  
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4 analysis of samples weight variation after equilibration at 53% RH may shed some light on this  
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6 issue. Thus, those samples with the highest glycerol contents (G/W ratios of 3:1 and 1:0) absorb  
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8 water along storage at 53% RH, consequently gaining weight along storage time (after 24h,  
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10  $14.90 \pm 1.93$  and  $24.00 \pm 1.00\%$ , respectively). On the other hand, samples with higher water  
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12 contents (G/W ratios of 1:3 and 0:1) dry along equilibration (after 24h,  $-14.74 \pm 0.62$  and  $-4.29 \pm$   
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14  $1.32\%$ , respectively). This may explain results shown in Figure 3: a lower water uptake capacity  
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16 was observed when the Relative Humidity (RH) was controlled (53%) for the sample with the  
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18 highest glycerol content, which is possibly related to the gaining of water from the surroundings  
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20 during equilibration. In a similar way, probes with higher water contents that dry under  
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22 equilibration showed a higher water uptake capacity than the corresponding formulations with  
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24 no RH control. Water uptake capacity of the sample with G/W ratio of 1:1 was not altered  
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26 significantly.  
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32 Thus, it seems that the influence of plasticizer is more evident when no RH control is taken, as  
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34 equilibration of samples at 53% RH involves the alteration of the composition of the plasticizer  
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36 fraction in the formulation along storage, seemingly levelling glycerol and water contents.  
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39 Anyway, future research should be carried out to elucidate this.  
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### 41 **3.3.2. Dynamic Mechanical Thermal Analysis**

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45 Figures 4 and 5 show respectively the elastic modulus ( $E'$ ) and loss tangent ( $\tan\delta$ ) from DMTA  
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47 temperature sweep tests, at a constant frequency of 1 Hz, for EW/T/G/W bioplastics with  
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49 different glycerol/water ratios (G/W ratios of 0:1, 1:3, 1:1, 3:1 and 1:0), in the absence of and  
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51 submitted to RH control (A and B, respectively).  
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55 Figure 4A shows the DMTA results ( $E'$  vs. Temperature) for those bioplastics stored under no  
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57 RH control. Qualitatively, they all present very similar curves, showing a drop in  $E'$  value as  
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59 temperature increases from  $-30^\circ\text{C}$  up to around  $75^\circ\text{C}$ , from where a plateau region begins,  
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4 starting at lower  $E'$  values and becoming less developed as glycerol contents increases. This  
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6 may be explained in terms of the well-reported plasticizing effect of glycerol (Coupland et al.,  
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8 2000). Moreover, no increase in  $E'$  is observed after the plateau region is achieved with further  
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10 increasing temperature when glycerol is present, which reveals that the bioplastics thermo-  
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12 setting, caused by protein denaturation, mainly occur during the previous compression-moulding  
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14 process at 120°C.  
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18 When the probes were equilibrated at 53% RH (Figures 4B), the behaviour of  $E'$  vs. temperature  
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20 did not change qualitatively when compared to those stored under no RH control. That is, they  
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22 still show a monotonous decrease in  $E'$  with increasing temperature, and a plateau region at the  
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24 highest temperatures tested. However samples with greater glycerol content (G/W ratio of 1:0  
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26 and 3:1) present lower  $E'$  values when compared to their counterparts without RH control. On  
27  
28 the contrary, samples with G/W contents of 1:3 and, mainly, 0:1 show higher  $E'$  values for the  
29  
30 whole temperature range, than the corresponding probes under no RH control. Again, this may  
31  
32 be related to water migration processes taking place onto or from the probe when they are  
33  
34 stored at 53% RH, which may be reflected in a variation of the weight of the probe after  
35  
36 equilibration. In fact, when the bioplastic containing originally only water as plasticizer (0:1) is  
37  
38 stored under RH control (53%) it apparently dries, finally becoming so hard and brittle that its  
39  
40 flexural viscoelasticity characterization is not feasible.  
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46 On the other hand,  $\tan\delta$ -temperature curves may provide useful information concerning  
47  
48 molecular and/or segmental scale motions in polymers (Zheng et al., 2003). As shown in Figure  
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50 5, all the samples present at around 60°C a well-defined peak (referred to as  $T_{\alpha_2}$ ) associated to  
51  
52 the mechanical gel-glasslike transition temperature of the plasticised egg-albumen (Jerez et al.,  
53  
54 2007a; Sun et al., 2007, 2008; Zárate-Ramírez et al., 2011). However, these peaks became  
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56 wider and less pronounced after the samples were subjected to equilibration under 53% RH  
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58 (Figure 5B). Anyway, independently of the storage conditions studied, glycerol content affected  
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4 the height of the  $T_{\alpha_2}$  peak, as it promotes movement of the polymer chains, resulting in greater  
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6 peaks for samples with a G/W ratio of 1:0 or 3:1. This important increase in the peak height is  
7  
8 generally attributed, in the case of biopolymers, to a reduction in cross-linkage, hydrogen  
9  
10 bonding or hydrophobic interactions (Kalichevsky et al 1993; Di Gioia 1998).  
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14 Additionally, it is possible to distinguish another peak for some samples at much lower  
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16 temperatures (referred to as  $T_{\alpha_1}$ ), which is related to the glass transition of the plasticiser  
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18 (mixture of water/glycerol, in this case). In some cases, this peak is not well-defined or might  
19  
20 even be located below the lowest limit temperature of the DMTA apparatus ( $-40^{\circ}\text{C}$ ).  
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### 24 **3.3.3. Tensile properties**

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27 Figure 6 shows stress-strain curves, from tensile tests, for the five selected bioplastics (G/W  
28  
29 ratios of 0:1, 1:3, 1:1, 3:1 and 1:0) in the absence of and submitted to RH control (A and B,  
30  
31 respectively).  
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35 Tensile stress always shows a monotonous increase as strain becomes larger, until a maximum  
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37 stress value (the so-called tensile strength) at break. Figure 6A reveals that, if probes are stored  
38  
39 under no RH control, both Young's modulus and tensile strength increase with decreasing the  
40  
41 glycerol content. Again, this may be attributed to the plasticizing effect of glycerol (Coupland et  
42  
43 al., 2000). In consequence, the highest Young's modulus and tensile strength values are  
44  
45 obtained when water is the only plasticizer (G/W ratio of 0:1), an observation that matches the  
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47 results from previous viscoelasticity measurements. Interestingly, the maximum value of  
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49 elongation at break is achieved for the sample with a glycerol/water ratio of 1:3.  
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53  
54 On the other hand, the stress-strain curves obtained after equilibration of the probes at 53% RH  
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56 for 24 hours are shown in Figure 6B. As can be observed, the evolution with the glycerol content  
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58 is very similar to that in Figure 6A. However, the samples with glycerol/water ratios of 1:0 and  
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4 3:1 presented much poorer resistance to break than those with no RH control. On the contrary,  
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6 the tensile stress exhibits a dramatic increase for the two highest water contents. Thus, the  
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8 sample with a G/W ratio of 1:3 shows a tensile strength of  $2.25 \pm 0.19$  MPa, in contrast to  $0.70 \pm$   
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10  $0.14$  MPa obtained when the same sample was stored under no RH control. Finally, no test  
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12 could be conducted on the bioplastic containing water as the only plasticizer, as a result of the  
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14 apparent dehydration along equilibration.  
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## 18 **4. Conclusions**

21  
22 This study has focused on how plasticizer composition (glycerol and/or water) and storage  
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24 conditions, more specifically control of Relative Humidity (RH), affect the viscoelastic and tensile  
25  
26 properties of egg albumen based bioplastics. Most of the bioplastics considered in the present  
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28 work included tragacanth gum in their formulation, which has been proved to confer them a  
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30 higher water uptake capacity.  
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33 Equilibration of the bioplastics under controlled atmosphere at 53% RH is seen to promote  
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35 water absorption/desorption processes, depending on their original water content, significantly  
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37 altering their viscoelastic/tensile properties, as well as their water uptake capacity. Bioplastic  
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39 including both plasticizers at the same concentration generally show similar results,  
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41 independently of the storage conditions.  
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44 When no RH control is taken, the effect of the plasticiser content is more evident, generally  
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46 reaching higher water uptake values, and lower viscoelastic moduli and tensile properties as  
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48 glycerol content increases.  
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51 Then, these results support the fact that plasticizer composition is a key factor to consider in the  
52  
53 manufacture of bioplastics, also being the Relative Humidity of the surrounding media in the  
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55 final application an important parameter to consider in the formulation stage.  
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## 59 **5. Acknowledgements**

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## Figure Captions

Fig. 1. Influence of tragacanth gum ratio (0 or 20 wt.%) on water-uptake capacity values (A); DMTA profiles at 1 Hz after 1 day and 15 days (B) and stress-strain ( $\sigma$ - $\epsilon$ ) relationship (C) for thermo-mechanically processed egg white bioplastics without RH control with glycerol/water ratio 1:1.

Fig. 2. Evolution of torque (A) and temperature (B) during the mixing process of different bioplastic formulations.

Fig. 3. Water uptake values for albumen-tragacanth gum bioplastics with different glycerol/water ratios (0:1, 1:3, 1:1, 3:1 and 1:0). ( $\square$ ) without RH control; ( $\blacksquare$ ) with RH control.

Fig. 4. Evolution of  $E'$  along temperature at 1 Hz of thermo-mechanically processed egg white-tragacanth bioplastics containing different glycerol/water ratios (0:1, 1:3, 1:1, 3:1 and 1:0) not submitted to RH control (A); and submitted to RH control (B)

Fig. 5.  $\tan\delta$  vs. temperature profiles for thermo-mechanically processed egg white-tragacanth bioplastics containing different glycerol/water ratios (0:1, 1:3, 1:1, 3:1 and 1:0) not submitted to RH control (A); and submitted to RH control (B)

Fig. 6. Influence of glycerol/water ratio on stress-strain ( $\sigma$ - $\epsilon$ ) relationship for the egg white-tragacanth bioplastics. (A) not submitted to RH control; (B) submitted to RH control.

**Table 1.** Formulation of the protein-based bioplastics

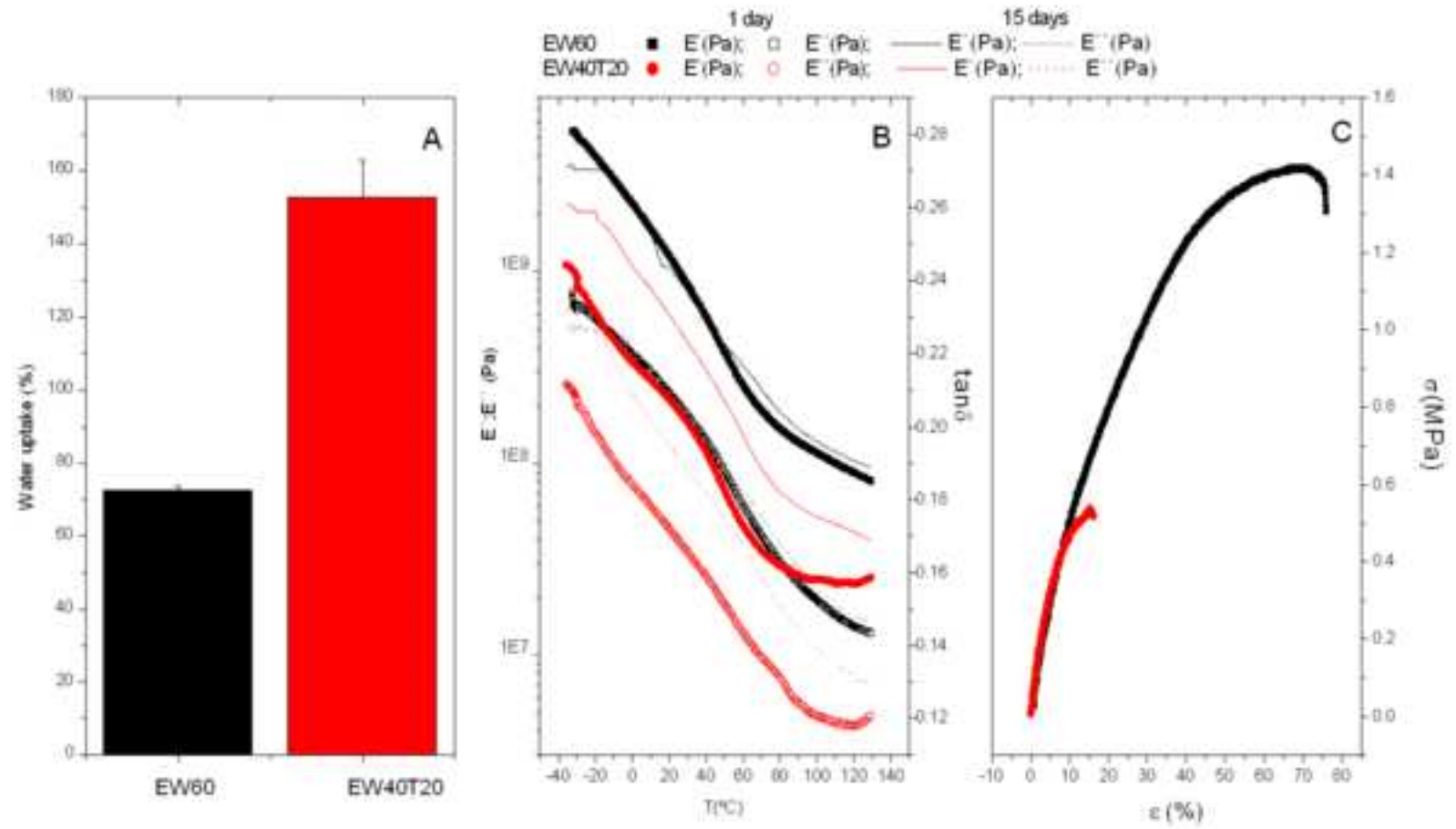
	<b>TG-free formulations</b>	<b>TG-containing formulations</b>				
	<b>G/W</b>	<b>G/W</b>				
	<b>1:1</b>	<b>0:1</b>	<b>1:3</b>	<b>1:1</b>	<b>3:1</b>	<b>1:0</b>
<b>EW (wt.%)</b>	60	40	40	40	40	40
<b>TG (wt.%)</b>	0	20	20	20	20	20
<b>W (wt.%)</b>	20	40	30	20	10	0
<b>G (wt.%)</b>	20	0	10	20	30	40

**Table 2.** Tensile properties (Young Modulus, E, and maximum stress,  $\sigma_{\max}$ , of egg white albumen bioplastics with different Glycerol/Water ratios in the plasticizer content

	No RH control		RH control	
G/W	E (MPa)	$\sigma_{\max}$ (MPa)	E (MPa)	$\sigma_{\max}$ (MPa)
<b>0:1</b>	1.07 ± 0.65	1.71 ± 0.65	-	-
<b>1:3</b>	0.26 ± 0.01	0.70 ± 0.14	0.60 ± 0.02	2.25 ± 0.19
<b>1:1</b>	0.19 ± 0.06	0.75 ± 0.21	0.15 ± 0.03	0.31 ± 0.04
<b>3:1</b>	0.17 ± 0.02	0.34 ± 0.06	0.05 ± 0.00	0.14 ± 0.04
<b>1:0</b>	0.16 ± 0.04	0.33 ± 0.14	0.05 ± 0.00	0.10 ± 0.06

Figure

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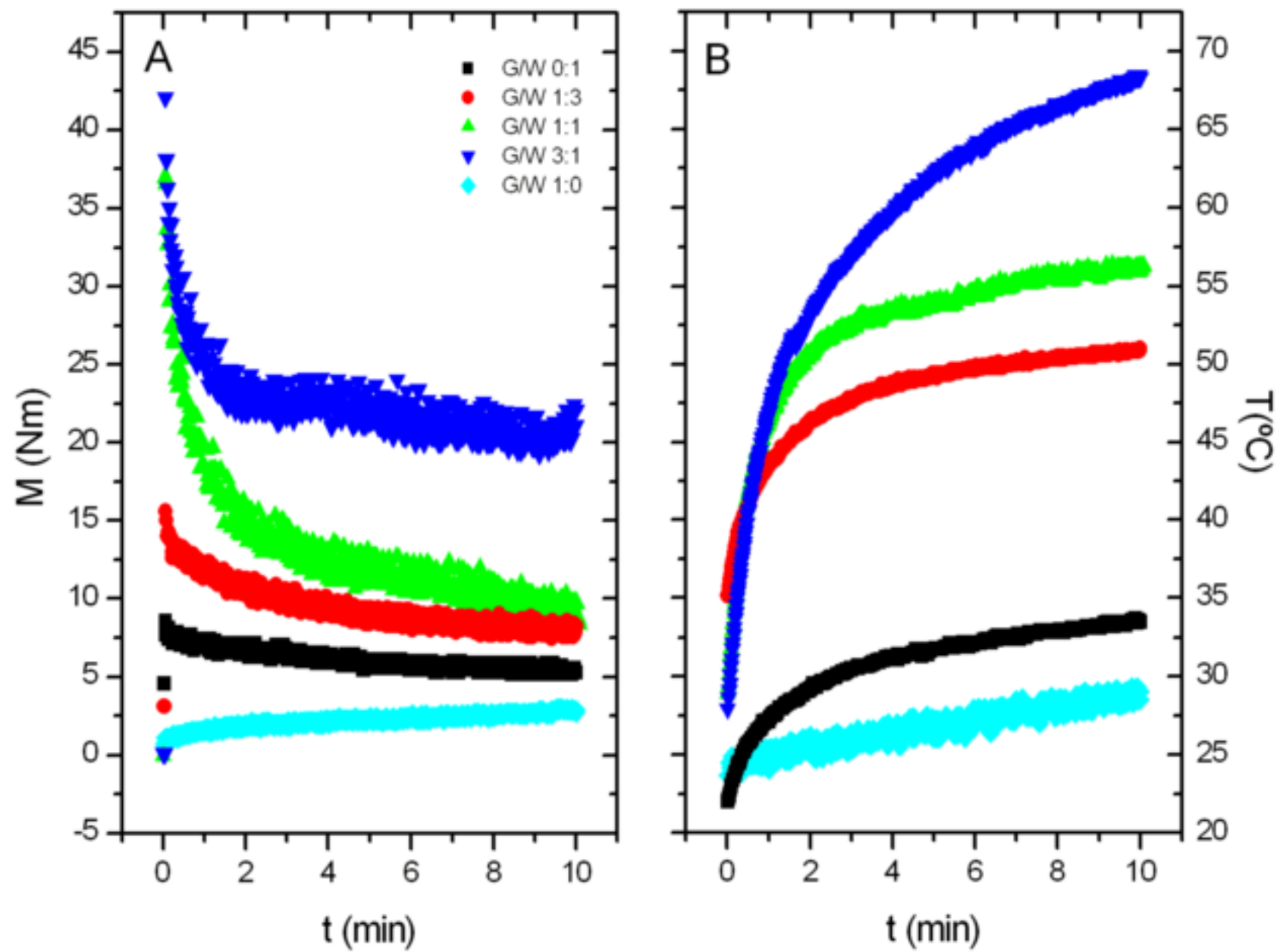


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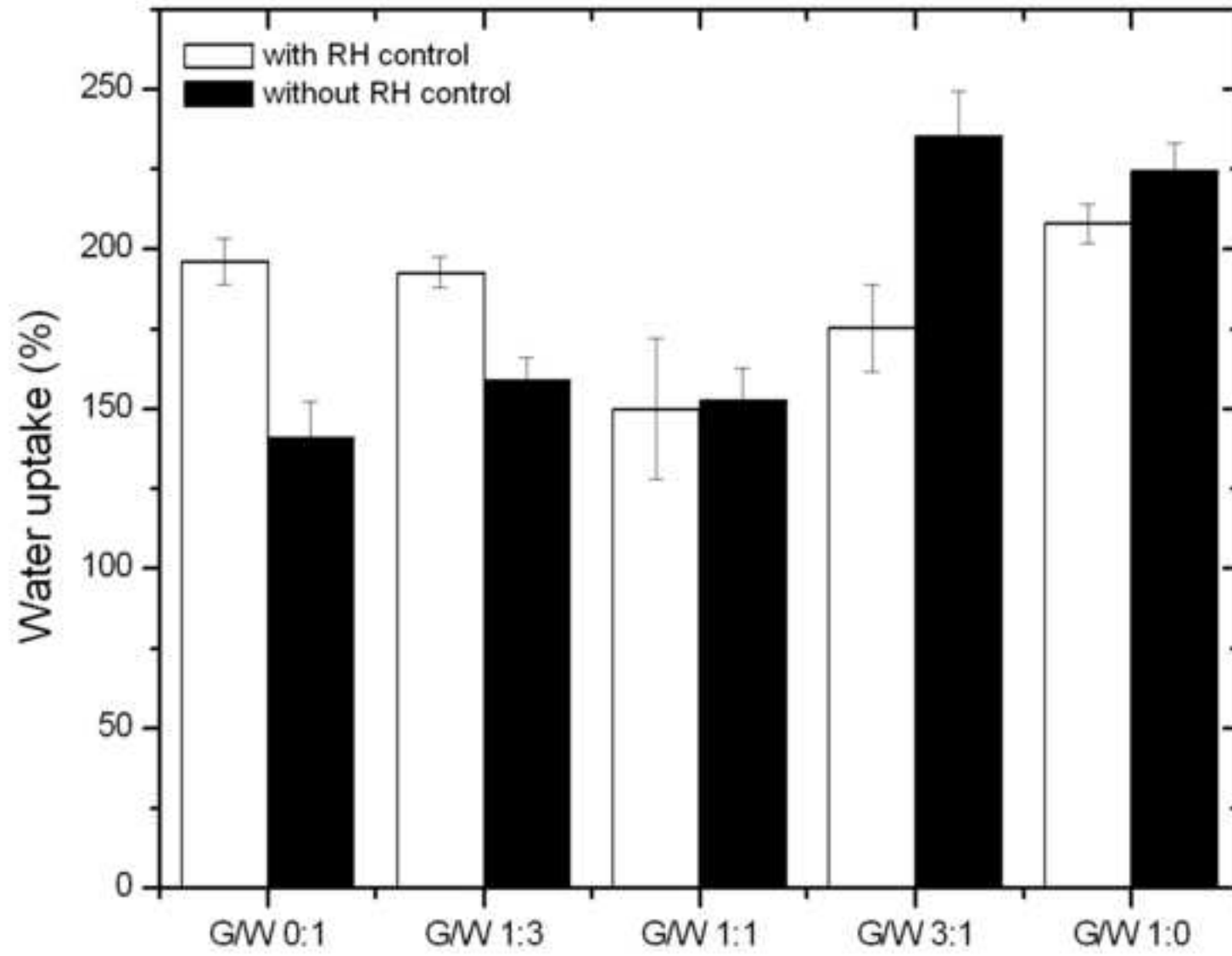


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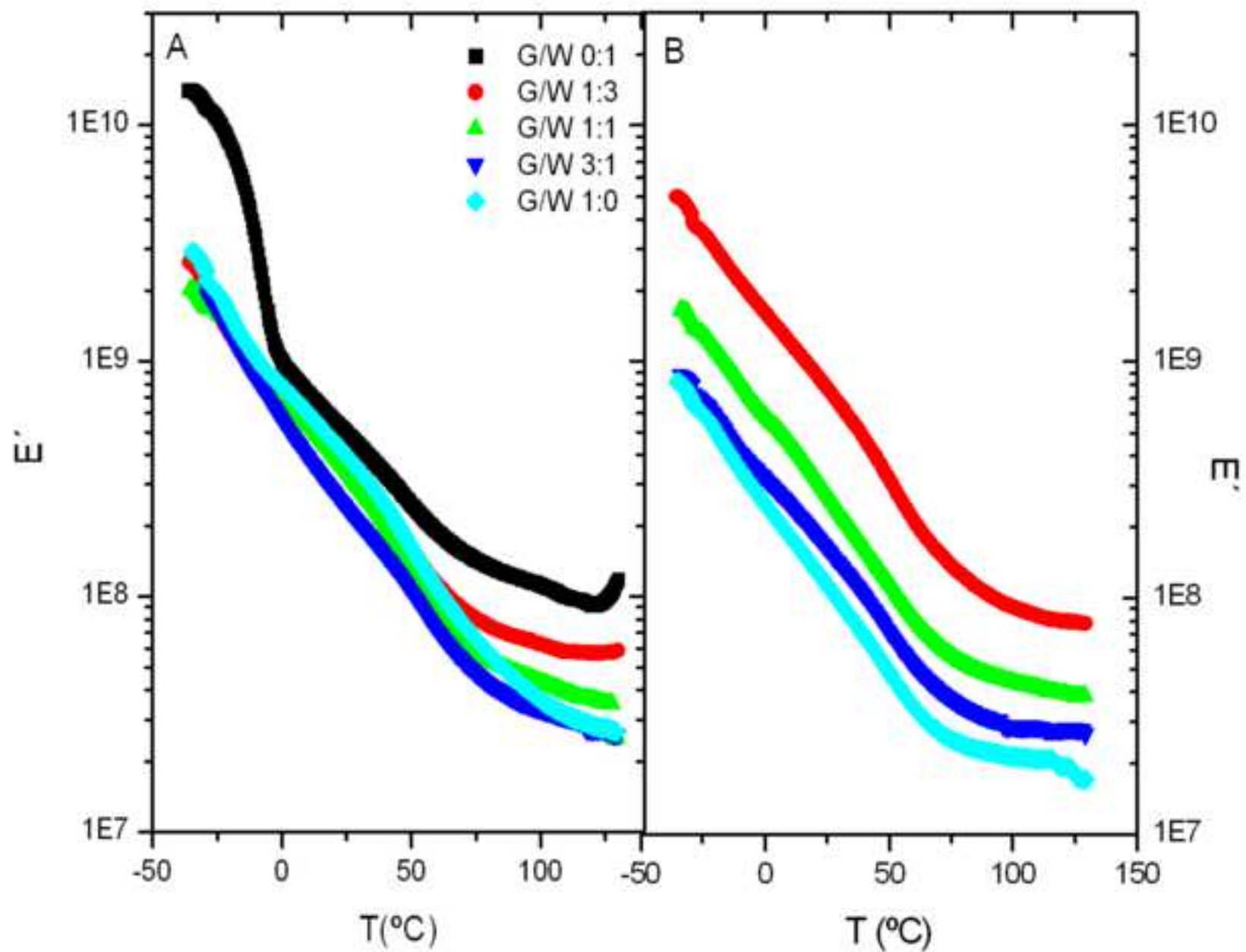


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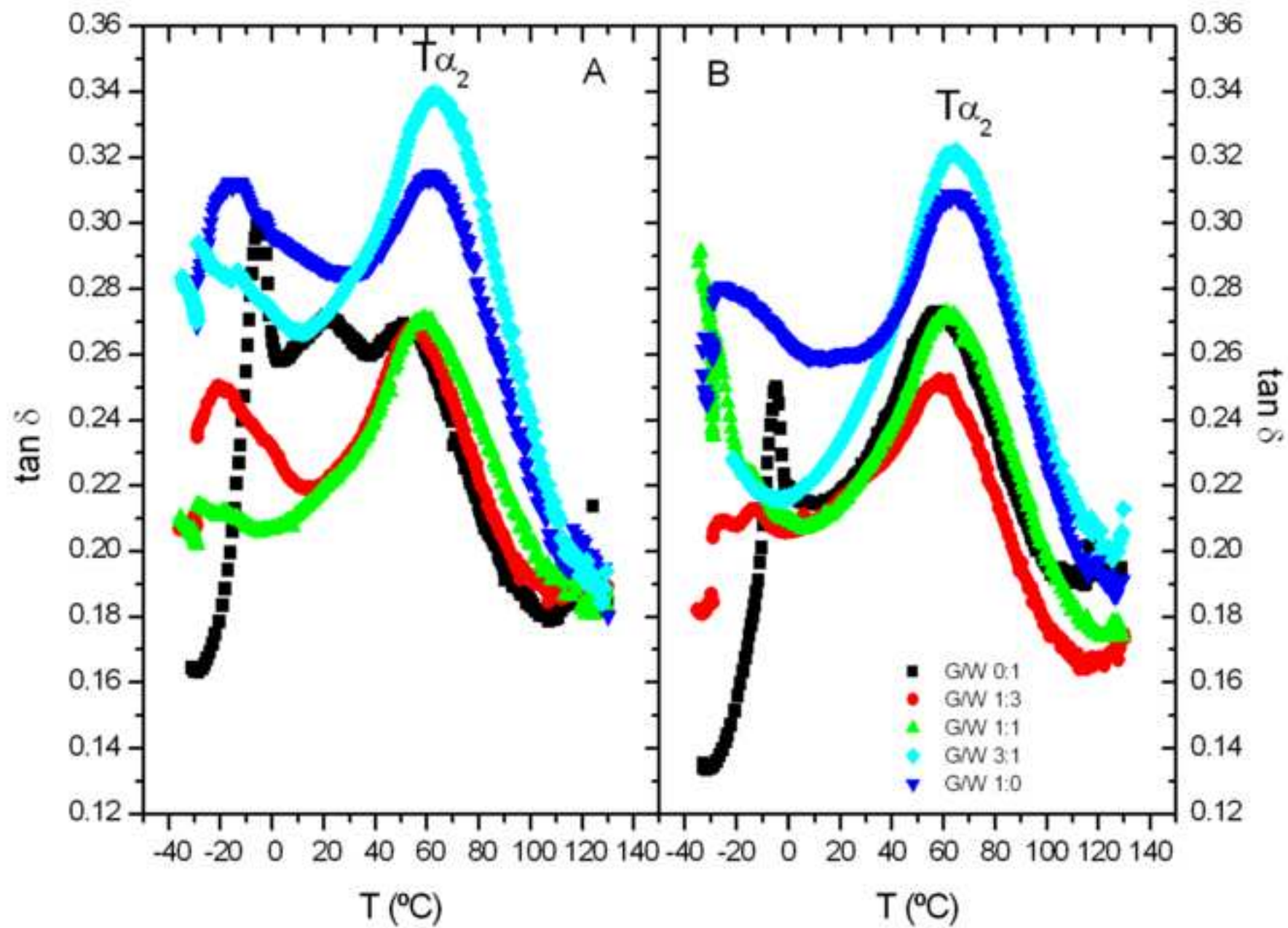
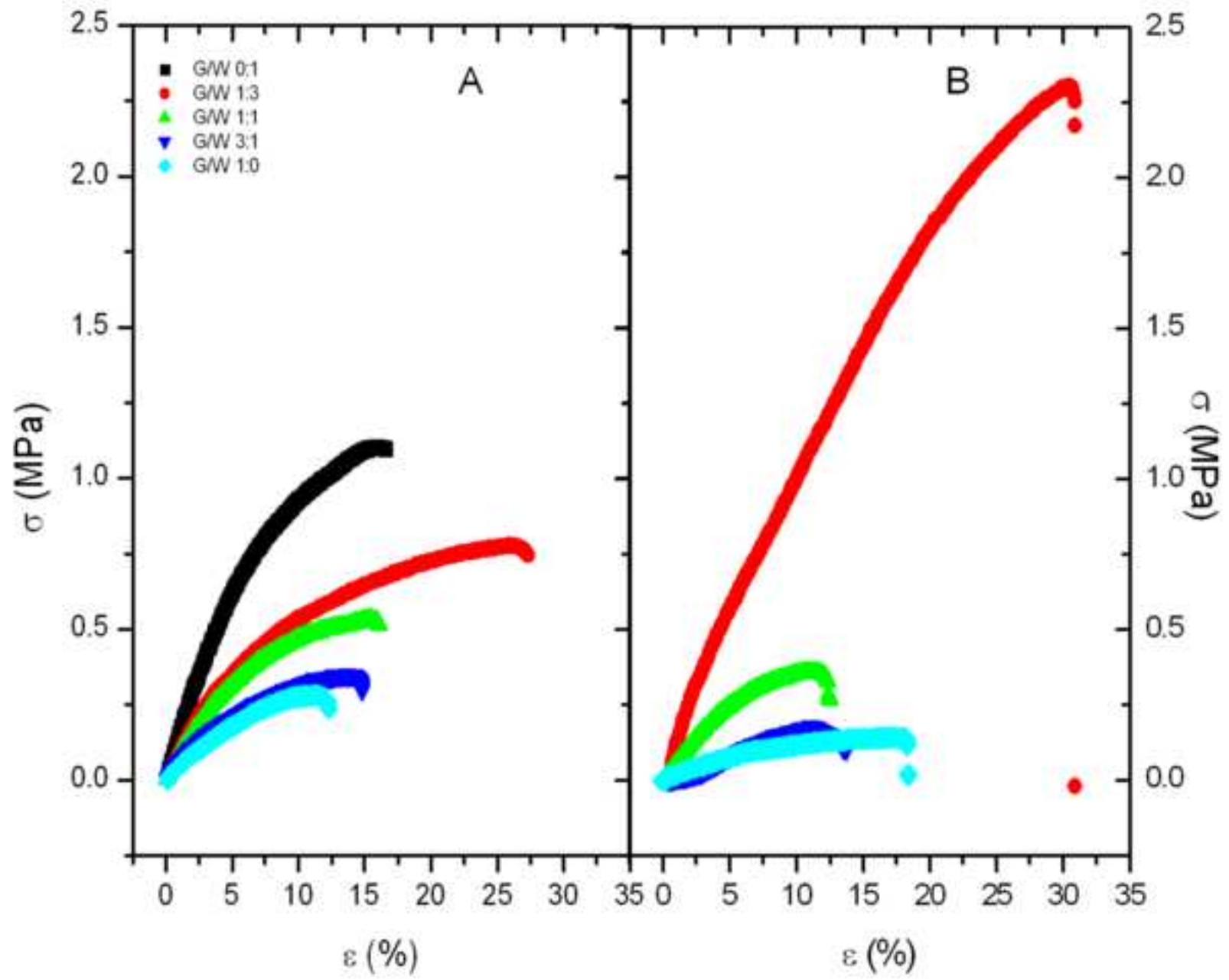


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