Effect of plasticiser on the morphology, mechanical properties and permeability of albumen-based nanobiocomposites

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ABSTRACT

This paper delves into the role plasticisers play in the formulation and processing of bioplastics and nanobiocomposites, trying to understand their effect on nanoclays dispersion and, consequently, on mechanical and gas barrier properties of protein-based nanobiocomposites. Egg white protein/montmorillonite clay nanobiocomposites were obtained by thermomechanical processing plasticised with varying molar concentration of different components (water, glycerol, polyethylene glycol). The extent of dispersion of the filler was evaluated by X-ray diffraction and transmission electron microscopy. Tensile tests and solid-state rheological measurements were conducted to evaluate glass transition temperature and thermomechanical behaviour of plasticised protein-clay nanobiocomposites, whereas gas permeability tests were used to study their gas barrier properties. The results showed that the samples plasticised by a blend of 1:1 glycerol/water presented the most exfoliated structures, resulting in an improvement in gas barrier and mechanical properties. Morphological analyses combined with tensile and permeability tests have shown a lesser effect of polyethylene glycol

of 300 molecular weight (PEG 300) on the exfoliation extent into such nanobiocomposites. Moreover, the larger size of PEG 300 does not allow the formation of a structure as compact as in the case of water and glycerol, as a consequence of an apparent phase separation, leaving more spaces that facilitate the diffusion of gases through the material.

Keywords: Plasticiser, protein, albumen, nanoclay, nanobiocomposite, permeability.

Abbreviations:
PEG: polyethylene glycol
NBC: nanobiocomposite
EW: egg white protein
MMT-Na: natural sodium montmorillonite
OMMT: organo-modified montmorillonite
W: water
G: glycerol

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1. Introduction

The management of plastic waste has long been a matter of concern to the scientific community and industry. However, the magnitude of this problem affecting both public health and nature makes society more and more aware that this is one of the greatest challenges to be faced by humanity during the 21st century. The need to find a truly sustainable alternative is imperative and in this the context, the option of using biodegradable bioplastics from renewable sources as an alternative to conventional plastics of fossil origin is gaining strength

every day. Raw materials such as carbohydrates, lipids and proteins have been the main sources for obtaining biodegradable and renewable bioplastics for many years (Bernard Cuq, Gontard, & Guilbert, 1998; Song & Zheng, 2008). In particular, protein-based bioplastics turned out to be among the rates of fast-degrading polymers (Domenek, Feuilloley, Gratraud, Morel, & Guilbert, 2004). Moreover, proteins have been extensively used in the development of bioplastic materials because of their capability to form three-dimensional structures with different interactions and bindings that lead to a wide range of functional properties (Bernard Cuq et al., 1998). An example of this is egg white protein (albumen), which is composed mainly of ovalbumin. When this protein is heated, the free sulfhydryl sulphides located in its nucleus are exposed and oxidized to form disulphide bonds. Through covalent bonds such as these and other weak ones (ionic, hydrogen bonding and Van der Waals), the three-dimensional structure of the polymer matrix is created (Fernández-Espada, Bengoechea, Cordobés, & Guerrero, 2013; Abel Jerez, Partal, Martínez, Gallegos, & Guerrero, 2007; Pommet, Redl, Morel, Domenek, & Guilbert, 2003). Albumen has been demonstrated to be a very interesting raw material for obtaining bioplastics focused on applications where transparency is important, without neglecting mechanical performance (Bernard Cuq et al., 1998; Diañez, Martínez, & Partal, 2016; A. Jerez, Partal, Martínez, Gallegos, & Guerrero, 2007). In addition, egg white protein can be easily processed by moulding and both the mixing and compression stages are carried out at temperatures lower than those required for processing most polymers and biopolymers (A. Jerez et al., 2007).

However, despite the many advantages of these materials, they still have some deficiencies that prevent them from being competitive with conventional plastics in many of their applications. For this reason, the addition of certain types of nanoparticles to the polymer matrix for obtaining nanocomposites is a way of providing bioplastics with even more interesting and valuable features, without affecting their biodegradability and renewability.

It is already well known that the morphology obtained with the addition of nanoparticles is totally determinant in the mechanical (J. E. Lee & Kim, 2010), thermal (Mohanty & Nayak, 2012) and barrier properties (Sanchez-Garcia, Lopez-Rubio, & Lagaron, 2010) of the materials obtained. Specifically, in the case of laminar nanoparticles, the most significant improvements are observed when an exfoliated structure is achieved, in which the clay plates have been completely separated from each other and are uniformly dispersed through the polymeric matrix (Sharma, Malik, & Jain, 2018; Zhu et al., 2019).

In previous study (Diañez et al., 2016) we determined that the macromolecular structure of egg white-based materials was affected by the nature of the nanoclays added. Moreover, the molecular/macromolecular compatibility between the clay layers and the egg white matrix appeared as the key parameter governing the nanostructure, and therefore, the mechanical properties and the water absorption capacity of resultant nanobiocomposites.

Another indispensable component in the formulation of bioplastics and biocomposites are plasticisers, which have been proved to be a determining factor in the final properties of plastics, bioplastics and nanocomposites (Chivrac, Pollet, Dole, & Avérous, 2010; Hopkins, Stone, Wang, Korber, & Nickerson, 2019; Lara & Salcedo, 2016). Plasticisers are usually small molecules that are located between the polymer chains, facilitating their mobility, increasing free space and reducing interactions between them. This results in the material being easier to process, more flexible and less brittle, lowering its glass transition temperature and considerably increasing its elongation capacity (Athamneh, Griffin, Whaley, & Barone, 2008; B. Cuq, Gontard, Cuq, & Guilbert, 1997; R. S. Lee, Pranata, Ustunol, & Almenar, 2013; Song & Zheng, 2008; Vieira, Da Silva, Dos Santos, & Beppu, 2011). However, beyond all these well-known aforementioned effects, it is of great interest to evaluate the influence of plasticisers, on the dispersion of nanoparticles in the polymeric matrix. To create a packaging concept for optimal preservation of food, different parameters such as the thermal and mechanical properties, humidity uptake, and barrier properties must be taken into account in order to

avoid loss of nutritional content, off-flavors, color changes, oxidation processes, and spoilage. Knowing the influence of plasticizers on those parameters is very important to develop customized packaging materials (El Miri et al., 2018). Bio-based films could be a good alternative to prevent deterioration for many food products because they often possess excellent oxygen barrier properties. Unfortunately, plasticisers usually increase gases permeability. Previous studies have indicated that gas permeability increases proportionally with plasticiser content (Arvanitoyannis, Nakayama, & Aiba, 1998; Sothornvit & Krochta, 2000). However, although the use of plasticiser to modify the permeability of different films has been reported, the interaction of them and albumen-based nanobiocomposites requires additional elucidation, since the finding of a plasticiser that favours the correct dispersion of nanoparticles into this matrix could compensate for this negative effect and help to obtain materials with improved barrier properties.

Cuq et al. (1997) found that differences in plasticising ability of substances with similar chemical nature were actually due to different molar content when comparing on mass basis substances with different molecular weight. Previously, Donhowe and Fennema (1993b) had stated that these effects of the chemical nature of plasticisers are only significant when the differences are substantial, such as when comparing glycerol with a much higher molecular weight PEG (Donhowe & Fennema, 1993).

The aim of this research was to find a plasticiser with the most suitable formulation to favour the dispersion of nanoclays in albumen bioplastic matrix and, consequently, to improve the mechanical behaviour and barrier properties of the obtained material. To accomplish this objective, two studies were performed. In the first one, the molar content of the plasticiser was varied, keeping the mass content constant. To this end, different proportions of two plasticisers of similar characteristics (and probably the most widely used) such as water and glycerol, were studied. Subsequently, in the second study, the plasticiser capacity of PEG 300,

129 a large plasticiser that has already been successfully used in the plasticisation of other
130 proteins, was evaluated.
131
132 **2. Experimental**
133 ***2.1. Materials***
134 The spray-dried egg white albumen (EW) used was provided by OVOSEC S.A. (Spain). Glycerol
135 from Guinama (Spain), PEG 300 from Manuel Riesgo, S.A. (Spain) and distilled water were used
136 as protein plasticisers. Regarding the nanoparticles, two selected montmorillonites from
137 Southern Clay Products, Inc. (USA) were used: a) Cloisite® Na^+ (MMT-Na) (natural sodium); and
138 b) Cloisite® 30B (OMMT) (organo-modified).
139 ***2.2. Samples preparation and characterisation***
140 *2.2.1. Samples formulation*
141 A plasticiser/protein mass ratio of 0.4/0.6 was always maintained (Diañez et al., 2016; López-
142 Castejón, Bengoechea, García-Morales, & Martínez, 2015, 2016), for both bioplastics and
143 nanobiocomposites (NBCs) preparation, where the plasticiser consisted of a mix of glycerol (G),
144 polyethylene glycol and distilled water (W) in different proportions. To obtain protein/clay
145 composite materials, natural and organo-modified clay nanoparticles were added to all the
146 formulations, at 3 and 0.5 wt %, respectively. These types of nanoparticles and their
147 proportions have been selected from the results obtained in a previous work (Diañez et al.,
148 2016). The final overall compositions are shown in Table 1.
149 *2.2.2. Thermoplastic processing*
150 During the thermoplastic processing step, all ingredients were mixed, for 10 min at room
151 temperature, in the kneading tool (Rheomix 3000p) of a torque-rheometer (Polylab, Thermo
152 Haake GmbH, Germany) equipped with two counter-rotating rollers turning at 50 rpm (Dealy,
153 1982), except for the case of the NBC containing PEG, for which the mixing process was
154 manually stopped when the sample reached 40 ^{o}C, to avoid protein degradation due to

155 excessive heat-induced protein aggregation (A. Jerez et al., 2007; Pommet et al., 2003;
156 Verbeek & van den Berg, 2010).
157 In any case, the process can be considered adiabatic, since neither heating nor cooling was
158 supplied to the kneading chamber. The values of both torque and temperature were
159 continuously monitored and recorded throughout mixing process. Specific mechanical energy
160 (SME) transferred to the material was then calculated from these torque data, by the following
161 equation (Redl, Morel, Bonicel, Guilbert, & Vergnes, 1999):

$$SME = \frac{\omega}{m}\int_0^{t_{mix}} M(t)\partial(t) \qquad [1]$$

162 where ω (rad/s) is the mixing speed, m (g) is the sample mass, M(t) (N·m) the torque and t_{mix}
163 (s) the mixing time.
164 *2.2.3. Compression-moulding*
165 Both EW bioplastics and NBCs were moulded by compressing the dough-like material resulting
166 from the thermoplastic processing, at 120 ^{o}C and 100 bar gauge pressure, using different
167 moulds to obtain different types of samples: according to ASTM dimensions (ASTM, 2003) for
168 tensile tests, thin (1.25 mm) sheets for permeability measurements (ASTM, 2009) and 50x10x3
169 mm^3 rectangular specimens, for others. Samples thus obtained were stored at 53% RH and
170 room temperature.
171 *2.2.4. Dynamic Mechanical Thermal Analysis (DMTA)*
172 DMTA tests were performed with a Seiko DMS 6100 (Seiko Instruments, Japan), in double
173 cantilever bending mode. Storage and loss modulus, E' (elastic response) and E" (viscous
174 response), respectively, were determined by this technique as a function of temperature. From
175 these data, damping factor (tanδ) was calculated as tanδ = E''/E'. All temperature sweeps were
176 carried out at a constant frequency of 1 Hz and a stress of 400 Pa, within linear viscoelasticity
177 (LVE) region. A heating ramp of 2 ^{o}C/min was set between 25 and 180 ^{o}C.
178 *2.2.5. Tensile tests*

179 Tensile tests were carried out with a Shimadzu AG-IS testing machine (Shimazdu, Japan) at a
180 single cycle of 50 mm/min, stretching to the breaking point to determine the Young's modulus
181 and maximum tensile strength and strain of the samples, meeting the ASTM D638-10 Standard
182 Test Method for Tensile Properties of Plastics.

183 *2.2.6. X-ray diffraction (XRD)*

184 X-ray diffraction assays were conducted in a Bruker D8 Advance X-Ray Diffractometer (Bruker
185 AXS, Germany) with a monochromatized CuKα radiation at 40 kV and 30 mA was used over a
186 scanning range (2θ) from 2 to 30°, a step size of 0.05°, and a count time of 15 s per step. The
187 clay gallery separation or d-spacing was calculated from Bragg's law using XRD results (Rosa &
188 Auriemma, 2013):

$$d = \frac{n\lambda}{2\sin\theta} \qquad [2]$$

189 where d is the spacing between layers of the clay, n is a whole number, λ the wavelength of X-
190 ray and θ the angle at the maximum point of the mean peak in the spectra.

191 *2.2.7. Transmission electron microscopy (TEM)*

192 Specimens for TEM observation were cut from bioplastic and NBC blocks at -120 °C, using a
193 Leica EM UC7 Ultramicrotome (Leica Microsystems GmbH, Germany), equipped with a glass
194 knife, to obtain sections with thickness between 70 and 90 nm. Transmission electron images
195 were taken with a Zeiss Libra 120 microscope, at an acceleration voltage of 80 kV.

196 *2.2.8. Permeability to gases (O_2 and CO_2)*

197 For permeability measurements thin sheets (1.25±0.23 mm), 10 cm diameter, were analysed in
198 a manometric gas permeability tester (Lyssy L100-5000, Systech Instruments Ltd, UK). The
199 instrument operated according to the following measuring method: evacuating a measuring
200 chamber down to a defined pressure and measuring the time required for a small pressure
201 increase within two limits depending on the permeability of the sample. A plastic film of
202 known permeability provided by the instrument manufacturers was used as standard for later
203 permeability calculations. The samples were affixed to a self-adhesive sample card, which was

inserted into the measuring chamber, separating the upper and lower sides of the chamber. A cooling water thermostat was used to keep the temperature at 23 ºC. Dehumidified oxygen and carbon dioxide were obtained from high pressure bottles equipped with a two-stage pressure reducing station. The permeability was calculated in mL/m^2 day. Results were obtained after getting 9 readings with identical values for each sample. The measuring method conformed ASTM D1434-82 and ISO 15105-1 standards.

2.2.9. Statistical Analysis

The results obtained from the different measurements are reported as an average value of all replicates, more or less the standard deviation (SD), as a measure of variability. At least, five replicates were measured in tensile tests and three in others. In addition, in the permeability measurements, each of the three replicates was considered valid only after obtaining nine identical measures, as commented in the previous paragraph.

Significant differences were determined by one-way ANOVA analysis, followed by post-hoc Tukey's HSD test (Astatsa online software: https://astatsa.com/OneWay_Anova_with_TukeyHSD/) at significance level of 95% ($p < 0.05$).

3. Results and discussion

The plasticisers investigated in this study represent different chemical composition and molar mass, thus providing the opportunity to explore the effects of these factors on dispersion of nanoclays and NBC final properties.

3.1. Glycerol/water-based plasticisers

In the first section of the results we have evaluated the plasticising effect of water, glycerol and their blends at different proportions, based on mole of plasticiser per mass of protein (100 g).

3.1.1. Thermoplastic processing

During thermoplastic processing of NBCs, protein, plasticisers and nanoclays, are mixed together using a torque rheometer, which allows control of mixing variables such as time, initial temperature and rotors speed, as well as continuous measurement of the torque and temperature values reached during process. It is important to consider that on the same mass basis in NBC with different glycerol/water ratios, those NBCs with a higher content of water (lower molar mass than glycerol) would have a greater molar content of plasticiser. Thus, the behaviour during mixing of blends plasticised, with several proportions of water and glycerol, is plotted as a function of plasticiser molar concentration in Figure 1. It is easily noticeable that all mixing-related parameters studied (SME, maximum torque and temperature increment) were intimately related to each other, showing a nearly identical evolution with the increase of plasticiser molar content. It can be clearly seen that there is a maximum in all cases for a molar content of 2.21 mol plast./100 g prot., corresponding to the system plasticised with an equal mixture of water and glycerol (NBC-50G50W). A lower plasticiser molar content (blends with higher amounts of glycerol, NBC-100G and NBC-75G25W) seems to result in less friction between the components, using less mechanical energy and also leading to a lower temperature increase during mixing. In fact, there are practically no differences between them. On the other hand, from the maximum value mentioned above, a gradual decrease in the energy, maximum torque and temperature increase associated with mixing can be observed as the plasticiser molar content increases.

The occurrence of this maximum for the sample (NBC-50G50W) in the analysed mixing variables indicates that the molecular amount of these plasticisers can greatly affect the properties of the resulting dough. In other studies, researchers reported that formulations of bioplastics containing a glycerol/water blend as plasticiser presented also higher torque and temperature values if compared to samples containing only one of them (Gómez-Martínez, Partal, Martínez, & Gallegos, 2013). This increase was associated to the formation of hydrogen bonds established between glycerol and water molecules depending on the glycerol mole

256 fraction (Chen, Li, Song, & Yang, 2009). Moreover, strong intermolecular interactions between
257 hydrophilic glycerol and water with hydrophilic-negatively charged-clay surfaces can be
258 expected and, due to the strong polar–polar interactions between protein, plasticisers and clay
259 surface, we could expect a competition mechanism among them (Nedi, Di Maio, & Iannace,
260 2012).
261 Although it is not possible to predict the plasticiser efficiency based on the
262 torque/temperature behaviour during mixing, the formation of a cohesive blend is
263 characterized by a torque increase (Redl et al., 1999). Thus, we suggest that there is an optimal
264 plasticiser molar amount that allows a better dispersion of the nanoclays, and hence a change
265 in consistency of the material is observed.
266 *3.1.2. Morphology*
267 In order to evaluate the influence of plasticiser composition on the morphology of these
268 materials, nanoclays and NBCs were analysed by X-ray diffraction. The presence of high
269 intensity peaks indicated the existence of a repetitive organised structure, where the mean
270 dimension, d_{001} (Figure 2), depends on both formulation and processing of these materials and
271 can be obtained by using Bragg's Law, which inversely relates the diffraction angle and the d-
272 spacing, d_{001}. Therefore, the smaller the diffraction angle is, the bigger separation exists
273 between different layers of the material. When no intense peaks appear, the material can be
274 considered as essentially amorphous. Based on this fact, these measures can also be used to
275 evaluate the degree of dispersion of the clay nanoparticles in the biopolymer matrix. If, after
276 processing a NBC, the nanoparticles still maintain their characteristic structure in the form of
277 stacked platelets, a peak appears whose calculated d_{001} will correspond to the distance
278 between those platelets. However, if the nanoclays have completely dispersed (forming what
279 is known as an exfoliated structure), this peak should disappear. In the intermediate situation,
280 in which the polymer chains have been able to penetrate the interlaminar space of the clays,

281 causing the expansion of the structure (intercalated morphology), a displacement of the peak
282 towards smaller diffraction angles is observed, which implies greater d_{001} values.
283 In this case, it can be observed that the peaks corresponding to the original nanoparticles (Fig.
284 2a) do not appear for the NBCs including both plasticisers. Therefore, it can be considered that
285 the structure of the nanoclays has been successfully broken or, at least, transformed into a
286 different one from that it had in their native state.
287 Noteworthy is that the nanocomposites, when glycerol or water was the only plasticiser
288 (Figure 2b), showed a diffraction peak located at 5.05° corresponding to a d_{001} value of 17.48 Å
289 for NBC-100G sample and at 5.45° corresponding to a d_{001} values of 16.20 A for NBC-100W
290 sample, while there is a peak that appears repeatedly at the same angle, equivalent to a d_{001} of
291 15.77 Å, that can be observed for bioplastic without nanoparticles (EW-GW) and NBCs, as long
292 as the plasticiser is a mixture of water and glycerol (Figs. 2c, d and e). This peak did not occur
293 when the plasticiser was only water or glycerol and became especially intense for both EW-GW
294 bioplastic and NBC-25G75W NBC, revealing a more repetitive and orderly structure of these
295 samples. The occurrence of this extra peak (at $2\theta = 5.6°$, $d_{001} = 15.77$ Å) could be ascribed to
296 the formation of a characteristic W/G/protein crystalline structure. (Anglès & Dufresne, 2000;
297 Mathew & Dufresne, 2002). The intercalated and exfoliated nanostructure was confirmed by
298 TEM images. Figure 3 is representative of TEM images of the albumen–clay nanocomposites
299 with different plasticisers in the formulation. Clay platelets were well dispersed, giving rise to a
300 fairly uniform and mainly exfoliated structure, with the presence of some intercalated clay
301 clusters (Figure 3a). This image highly agrees with the NBC-100W diffractogram, in which only
302 a narrow low-intensity peak can be observed, which was also at smaller angles (greater d_{001})
303 than that of the most abundant nanoclays (MMT-Na). On the other side, the image
304 corresponding to the NBC-100G sample (Figure 3b) showed a heterogeneous material, in
305 which areas practically free of nanoclays and other areas where they accumulate can be
306 differentiated, forming a multilayered nanostructure, with some single clay platelets, meaning

lower delamination and dispersion of platelets for the NBCs with only glycerol in the formulation. Meanwhile, more single, disordered clay platelets can be seen in Fig 3c, indicating that more exfoliated structures were obtained for a blend of plasticiser at 1:1 glycerol/water ratio. In fact, a good dispersion of the clay, whether the clay was organically modified or pristine, was generally observed in TEM images for the nanocomposites containing both plasticisers. Although only NBC-50G50W seemed to be XRD silent (an absence of peaks compared to the strong peak at 11.70 Å (7.55°) for MMT-Na and 18.39 Å (4.8°) for OMMT), the TEM results revealed exfoliated and intercalated structures.

3.1.3. Tensile tests

The values of Young's modulus, ultimate strength and strain at break obtained for NBCs subjected to tensile load, at a constant speed of 50 mm/min are presented in Figure 4. With regard to the tensile strength, a maximum was observed when the water to glycerol ratio was 1:1 (NBC-50G50W). This can be attributed to the homogeneous dispersion on the nanoclay as well as the preferable interaction between the nanoclays and the protein matrix. Such improvements are well known in exfoliated nanocomposites for other polymers also and attributed to the higher reinforcing effect of layered fillers (Pandey et al., 2005). When the molar content increases, due to the higher proportion of water in the plasticiser blend, the tensile strength of the NBCs drops and remains almost constant.

The presence of the plasticiser eased the relative movement of the polymer chains, making the material capable of withstanding greater deformation before breaking. In this way, as the molar content of plasticiser increased, and water was present as a plasticiser, the resulting materials acquired a better elongation capacity, which seemed to be steadily shifting. However, the NBC with a glycerol:water ratio of 1:3 (NBC-25G75W: 2.96 mol plast./100g protein) had a non-expected elongation at break of 137.1%. In the case of nanocomposite containing only glycerol (NBC-100G) as plasticiser, the reduction in strain could be attributed to the fact that, due to the higher molecular weight of glycerol, this system has the lowest

333 molar plasticiser content of all those considered in this study, not being sufficient for the
334 material to reach its maximum plasticiser efficiency in terms of increasing of strain (Mangavel,
335 Barbot, Guéguen, & Popineau, 2003).
336 Young's modulus, on the other hand, presents the inverse trend. Precisely when the plasticiser
337 content decreases and the mobility of the polymer chains is reduced, the material offers
338 greater resistance to deformation, causing the Young's modulus to increase, as can be seen in
339 Figure 4.
340 *3.1.4. Dynamic Mechanical Thermal Analysis (DMTA)*
341 The protein-based NBCs were subjected to dynamic temperature sweeps ranging from 25 to
342 180 ^{o}C, in bending mode, obtaining the results presented in Figure 5a and Table 2.
343 Regardless of their composition, all samples present a similar thermomechanical behaviour,
344 with a damping factor (tanδ) curve with two maximal values which can be related to gel-
345 glasslike transition and degradation of the egg white protein, respectively (Dianez et al., 2016;
346 González-Gutiérrez, Partal, García-Morales, & Gallegos, 2011), being the former used to
347 determine the glass transition temperature, T_g (Table 2). In general, the higher the NBC
348 plasticiser molar content, the lower the resulting T_g value. This is a typically and well-known
349 effect and it is, in fact, one of the main applications of plasticisers (B. Cuq et al., 1997; Lodovico
350 di Gioia and Stephane Guilbert, 1998).
351 Tanδ-temperature curves may provide useful information concerning molecular and/or
352 segmental scale motions in polymers (López-Castejón et al., 2016; Zheng, Tan, Ran Zhan, &
353 Huang, 2003). Figure 5a shows the damping factor of NBCs between room temperature and
354 180 ^{o}C. As, commented early, all of them presented the same general shape, characterised by
355 a tanδ curve with two maximums. However, several differences can be observed depending on
356 the plasticiser used in each case. For instance, the samples with a G/W mass ratio of 1:0 or 3:1
357 (NBC-100G and NBC-75G25W, respectively) presented the largest peaks, as a consequence of
358 increased movement of the polymer chains provoked by high contents of glycerol. This

359 important increase in the peak size is generally attributed, in the case of biopolymers, to an
360 increase in the percentage of amorphous phase of the material (Pouplin, Redl, & Gontard,
361 1999). Moreover, the T_g values were the highest for these samples, due to the resulting T_g of
362 the glycerol-water mixture (Zhao, Cao, & Wang, 2015).
363 At low temperatures (below about 80 ºC), it can be clearly seen that a higher water content in
364 moles resulted in a more elastic response, being the damping factor lower, what agrees with
365 visual examination and manipulation of these materials, as well as with a lower susceptibility
366 to temperature increase. This is the case of the NBC-100W, which underwent the slightest
367 variation over the entire temperature range studied. Those variations with increasing water
368 content are attributed to the motional restriction of protein chains. This phenomenon could be
369 explained by a better chain alignment, which subsequently contributes to a more ordered
370 system when water content is progressively increased.
371 Furthermore, as happened with thermoplastic processing, NBC-100G and NBC-75G25W
372 samples behaved in a very similar way, especially at temperatures above 80 ºC.
373
374 ***3.2. PEG as plasticiser***
375 In this second part of the sudy, the mass of plasticiser per mass of protein is used as criteria to
376 elucidate the effect of the plasticiser molecular weight. For this purpose, a comparison was
377 made between the best plasticiser blends from the first screening results, in terms of
378 dispersion of the nanoclay and its effect on the properties, with the addition of a larger
379 hydrophilic plasticiser: PEG 300. According to the results presented so far, the NBC-50G50W
380 has shown the best dispersion of the nanoclay in the protein matrix, so this will be the system
381 chosen for comparison with PEG 300.
382 *3.2.1. Thermoplastic processing*
383 Figure 6 shows the specific mechanical energy, maximum torque and temperature rise
384 associated with the mixing of a NBC plasticised with a mixture of PEG, glycerol and water

(50/25/25 wt.%). Also included are the data corresponding to the NBC plasticised with an equal blend of water and glycerol, which had provided some of the most noteworthy results described in the previous section. Both are also compared with their respective bioplastic matrices without any nanoparticles added. In this case, systems plasticised only with water and glycerol showed a behaviour characterised by a sudden initial rise to a maximum, then a partial drop occurs followed by a stabilisation of the torque values (Diañez et al., 2016). However, when PEG was added to the mix, the initial rise was more progressive and could only be detected after 10 min of mixing (Gómez-Martínez et al., 2013). As a consequence, there was a huge difference between the energy required for thermoplastic processing with and without PEG as a plasticiser. Water/glycerol-plasticised bioplastic needed about 9 kJ/kg of energy during mixing, rising to almost 14 kJ/kg when 3.5 wt.% of nanoclays was added. However, when PEG was added to the plasticiser mixture, these values dropped to just around 1 kJ/kg.

However, in the PEG plasticised systems, the increase in torque values caused by the addition of nanoparticles was much greater than for the G/W mixture. As a consequence, the temperature increase was also greater, to the extent that thermal degradation of the protein and macroscopic phase separation occurred, behaviour already observed in previous studies (Gómez-Martínez et al., 2013). When this happens, systems obtained after thermoforming tend to exhibit poor mechanical performance, especially in terms of tensile strength and elongation capacity. Thus, in order to avoid this deterioration, mixing was manually stopped for this system, when it reached 40 ºC.

The dough-like material obtained after this process showed a suitable homogeneous appearance, at the same time that the energy necessary to perform the mixing was considerably reduced in comparison with the other samples and, consequently, the temperature increment, what is very important to avoid protein degradation.

Overall, these results show that intermolecular interactions between protein surfaces and clays' tactoids are modified by the blend of plasticisers selected (Nedi et al., 2012). The reduction of the mixing-related parameters observed for PEG-plasticised samples could be attributed to less compatibility of this plasticiser with the protein and nanoclays compared to the water/glycerol blends. It might be due to a combined effect of steric hindrance and different distribution of the number of hydrophilic groups per PEG molecule, which influences the ability of the hydrophilic plasticiser molecules to share hydrogen bonds with the protein network (Gómez-Martínez et al., 2013).

3.2.2. Morphology

The XRD pattern of the samples NBC-50G50W and NBC-50PEG25G25W and their corresponding bioplastics without nanoclays are represented in Figure 2c and 2d. As can be observed, the nanobicomposite formulated with PEG showed an intensive peak at lower angles than native Na-MMT, equivalent to a d_{001} of 17.66 Å. The presence of this diffraction peak reveals that these materials are mainly intercalated. Its counterpart without nanoclays showed only a narrow but low intensity peak, attributed as previously mentioned to the water/glycerol mixture. On the other hand, the XRD patterns of the NBC-50G50W changed dramatically in comparison with the montmorillonite clays, without any sharp peak. In fact, the peaks at 7.55° (d_{001} = 11.70 Å) and 4.8° (d_{001} = 18.39 Å), corresponding to the two types of nanoclays added, disappeared when the nanoparticles were introduced into the protein bioplastics, and large amounts of the clay platelets were exfoliated and randomly distributed in the protein matrix, as it was discussed previously. These results demonstrate that the presence and type of plasticisers greatly affect the formation of nanostructured materials. When PEG is present in the sample, the degree of clay exfoliation decreases. This also could mean that the blend of water and glycerol facilitates stronger interactions between the protein and clay surfaces (Nedi et al., 2012; Pasini Cabello, Takara, Marchese, & Ochoa, 2015).

435 The intercalated and exfoliated nanostructure proposed was confirmed by TEM images, where
436 images of the protein-clay nanocomposites with different combination of plasticisers can be
437 seen (Figure 3). More single, disordered clay platelets indicate that more exfoliated structures
438 were obtained (Figure 3c), while ordered, multilayered nanostructure and some single clay
439 platelets, means lower delamination and dispersion of platelets for samples with PEG (Figure
440 3d).
441 *3.2.3. Tensile properties*
442 In general, better tensile properties can be obtained when PEG is not used as a plasticiser
443 (Table 3). NBC made only with water and glycerol had the most common change in the
444 response to traction when these nanoparticles were added to a polymeric matrix (Diañez et
445 al., 2016). Compared to the original bioplastic, the NBC becomes stiffer and offers greater
446 resistance to deformation, but it had less ability to elongate before breaking. On the other
447 hand, by using PEG as a plasticiser, the effect of the nanoclays on the tensile properties of the
448 material was greatly reduced and no significant change in tensile strength was observed. This
449 lack of a reinforcement effect would imply weak clay-protein-plasticiser intermolecular
450 interactions, since the higher the affinity between inorganic fillers and the polymer matrix, the
451 higher the strengthening obtained (Gao, 2004; Zare, 2016; Zare, Fasihi, & Rhee, 2017). The
452 Young's modulus and elongation capacity were slightly increased and decreased, respectively,
453 so that the trend observed for the G/W mixture was maintained. These results could suggest
454 that the PEG was not really plasticising the protein, but forming a polymer/polymer blend with
455 it, instead. In this case, only water and glycerol would act as plasticising agents, what could be
456 a reason for the better elongation capacity of EW-GW sample, since it contained a larger
457 proportion of plasticiser than the others.
458 When the sample has an equal blend of water and glycerol content as plasticisers (NBC-
459 50G50W), the nanocomposite showed significant improvements in tensile strength and
460 modulus as compared to their corresponding systems without nanoparticles. The elongation at

461 breakage decreased but still remained at a sufficient level for flexibility of the composites. The
462 nanoparticles were indeed effective at improving the mechanical properties of this protein
463 system.

464 *3.2.4. Dynamic Mechanical Thermal Analysis (DMTA)*

465 Values of T_g obtained from DMTA measurements of bioplastics and NBCs, with and without
466 PEG as plasticiser are presented in Table 3. By looking at that it is possible to confirm how both
467 PEG and nanoclays caused a drop in T_g. As an example, for samples plasticised only with water
468 and glycerol, the addition of 3.5 wt.% of nanoparticles caused a decrease in T_g of more than 17
469 °C: from almost 73 °C (72.8±3.4 °C) for the EW-GW sample to 55.5 °C for NBC-50G50W.
470 Bioplastic containing PEG, on the other hand, passed through the glass transition at a
471 temperature even lower than NBC-50G50W: 46 °C, which drops to 43 °C for its NBC NBC-
472 PEGGW. Thus, 19.3 wt.% PEG and 3.5 wt.% nanoclays over the final composition are capable of
473 causing a drop in T_g of almost 30 °C, what could mean a decisive improvement in the
474 processing ability of the material. Water/glycerol and PEG play different plasticising roles in
475 the bioplastics and nanobicomposites, according to their affinity. PEG with less hydroxyl
476 groups per molecule will present lower affinity with polymeric network as molecular weight is
477 higher. According with Cabello et al. (2015), the lower hydrogen bonding capacity, coupled
478 with the larger size of PEG, causes it not to be easily placed between protein chains and
479 interact with them, as water and glycerol do. This suggests an apparent phase separation, in
480 which PEG acts more as a component of a polymeric blend than as a plasticiser (Pasini Cabello
481 et al., 2015). As a result, these systems are more rigid and brittle than their water/glycerol
482 plasticised counterparts, as can be confirmed in both DMTA (lower tanδ values) and tensile
483 tests (reduced tensile strength and elongation at break).

484 *3.2.5. Permeability to gases*

485 The oxygen and carbon dioxide permeability values of bioplastics and NBCs with and without
486 PEG as a plasticiser can be used to give more information about the morphology obtained with

487 the addition of nanoclays, apart, of course, from providing information of great interest for
488 possible end uses (Table 4). The greater the degree of dispersion of the nanoclays in the
489 polymer matrix, the more obstacles a gas molecule encounters in its diffusion path, making it
490 longer. This is known as the tortuous path effect (Lebaron, Wang, & Pinnavaia, 1999).
491 A comparison of the results of EW-50G50W and NBC-50G50W shows a clear reduction in the
492 permeability of the NBC to both types of gases, but especially to CO_2, a molecule bigger than
493 O_2, which can be reduced to less than 4% of the value recorded for the bioplastic matrix. This
494 indicates that the occurrence of exfoliation was very helpful for improving the barrier
495 properties of the film.
496 Nanomaterials can influence the barrier properties by causing changes in the protein matrix
497 itself. If the nanoclay-protein interactions are favourable, the protein chains in the proximity to
498 the nanomaterials can be partially immobilized. Therefore, the gas molecules that migrate
499 through these interfacial areas will have attenuated movement (Müller et al., 2017).
500 Observed permeability values for O_2 and CO_2 were, in general, low, similar to other biobased
501 films and within the range reported for low-density polyethylene (Petersen, Nielsen, & Olsen,
502 2001). The exception was NBC-PEGGW, which presented very high values. Overall, when PEG
503 was present, the permeability increased dramatically as has been observed for other
504 biopolymers (Sothornvit & Krochta, 2000). This could be due to the fact that the larger size of
505 PEG 300 does not allow the formation of a structure as compact as in the case of water and
506 glycerol, leaving more spaces that facilitate the diffusion of gases through the material. It is
507 known that permeability is independent of thickness, which was not uniform for all the
508 samples. Consequently, small differences could be expected related to this factor.
509 Considering the O_2:CO_2 permeability ratio, a very important factor for several food packaging
510 applications, EW-PEGGW, with 1:7, might be useful for packaging of highly respiring foods.
511 However, more experiments are needed for evaluating other aspects like water vapour
512 permeability, interactions between the food product and the packaging material as well as the

effect of storage conditions (temperature, RH, etc.) on the mechanical and physical properties of these materials.

4. Conclusions

The effect of plasticiser composition and its molar concentration (glycerol and/or water and PEG 300) on albumen bioplastics/nanobiocomposites permeability and mechanical properties were compared. In general, for egg white-based-bioplastics and nanobiocomposites, the presence of glycerol favours the disorder or amorphous character of the structure. On the contrary, water gives a more ordered and compact material. Additionally, the best characteristics are obtained when the nanobiocomposites includes both plasticisers. Particularly, the addition of an equal blend of water and glycerol showed significant improvements in tensile strength, which can be due to the homogeneous dispersion of nanoclay and the improved affinity between the nanoclay and the protein matrix. The dispersion state of nanoparticles affects nanobiocomoposites gas barrier properties. With that plasticiser mixture (NBC-50G50W), a clear reduction (to at around 4% for CO_2 and 30% for O_2, compared to the non-filled matrix), in the gas permeability of the nanobiocomposite was recorded. It could be related to the ability of the plasticisers to supply hydrogen bonds to the proteins. In contrast, with PEG present in the plasticiser mixture, the degree of clay exfoliation decreases and the tensile properties of the material was greatly reduced. It could be due to the high molecular weight of PEG or to the limited compatibility with the protein, forming a possible phase separation and a heterogeneous clay distribution. It could explain the lack of improvement in mechanical properties and even the observed decline in the gas barriers properties.

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697

Sample	Overall composition (wt.%)						Plasticiser composition (wt.%)			mol plast./ 100g protein
	EW	PEG	G	W	MMT-Na	OMMT	PEG	G	W	
NBC-100G	57.9	0.0	38.6	0.0	3.0	0.5	0	100	0	0.72
NBC-75G25W	57.9	0.0	29.0	9.7	3.0	0.5	0	75	25	1.47
NBC-50G50W	57.9	0.0	19.3	19.3	3.0	0.5	0	50	50	2.21
NBC-25G75W	57.9	0.0	9.7	29.0	3.0	0.5	0	25	75	2.96
NBC-100W	57.9	0.0	0.0	38.6	3.0	0.5	0	0	100	3.70
EW-GW	60.0	0.0	20.0	20.0	0.0	0.0	0	50	50	2.21
EW-PEGGW	60.0	20.0	10.0	10.0	0.0	0.0	50	25	25	1.22
NBC-PEGGW	57.9	19.3	9.7	9.7	3.0	0.5	50	25	25	1.22

698 *Al samples labelled with the code GW contain an equal blend of water and glycerol*

699 **Table 1.** Final compositions of the samples studied.

Sample	mol plast./100g prot.	T_g [°C]
NBC-100G	0.72464	59.2±3.4
NBC-75G25W	1.4694	55.8±4.0
NBC-50G50W	2.21417	55.5±2.5
NBC-25G75W	2.95894	53.0±1.7
NBC-100W	3.7037	50.5±1.0

Table 2. Glass transition temperature (T_g) for different mole of plasticiser/protein weight ratios.

706

Samples	Tensile strength [MPa]	Young's Modulus [MPa]	Strain at break [%]	T_g [°C]
EW-GW	6.1±0.5^a	48.7±4.3^a	174.3±30.5^a	72.8±3.4^a
NBC-50G50W	8.1±0.1^b	104.6±7.7^b	97.5±1.0^b	55.5±2.5^b
EW-PEGGW	5.2±0.4^c	41.1±1.0^a	125.4±23.4^b	46.0±0.8^c
NBC-PEGGW	5.0±0.1^c	52.9±9.6^a	88.9±23.3^b	43.0±1.6^c

707

708 **Table 3.** Effect of PEG addition on thermomechanical properties of bioplastics and NBCs.

709 Different superscript letters in values of the same column indicate statistically significant

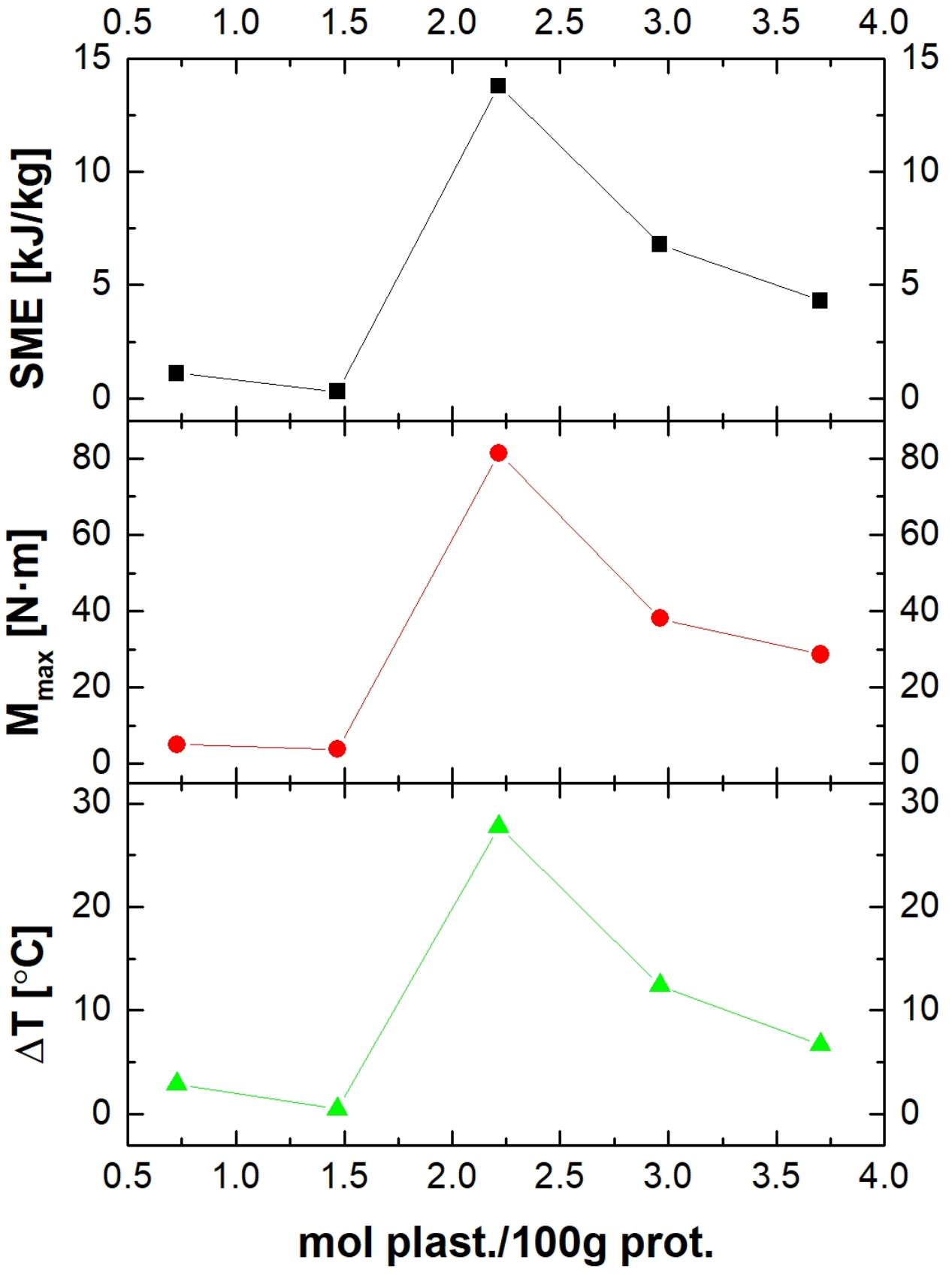
710 differences ($p<0.05$).

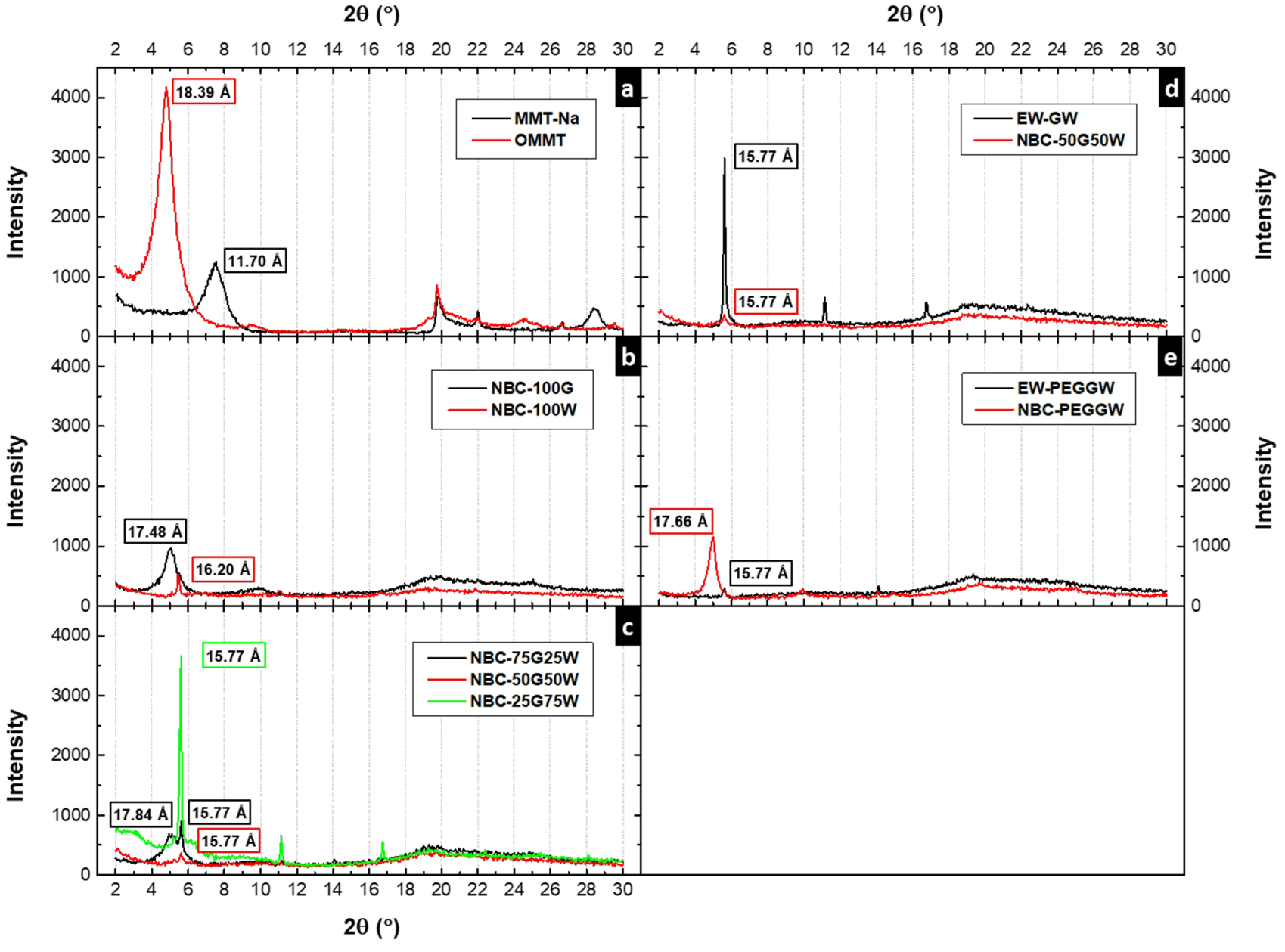
712

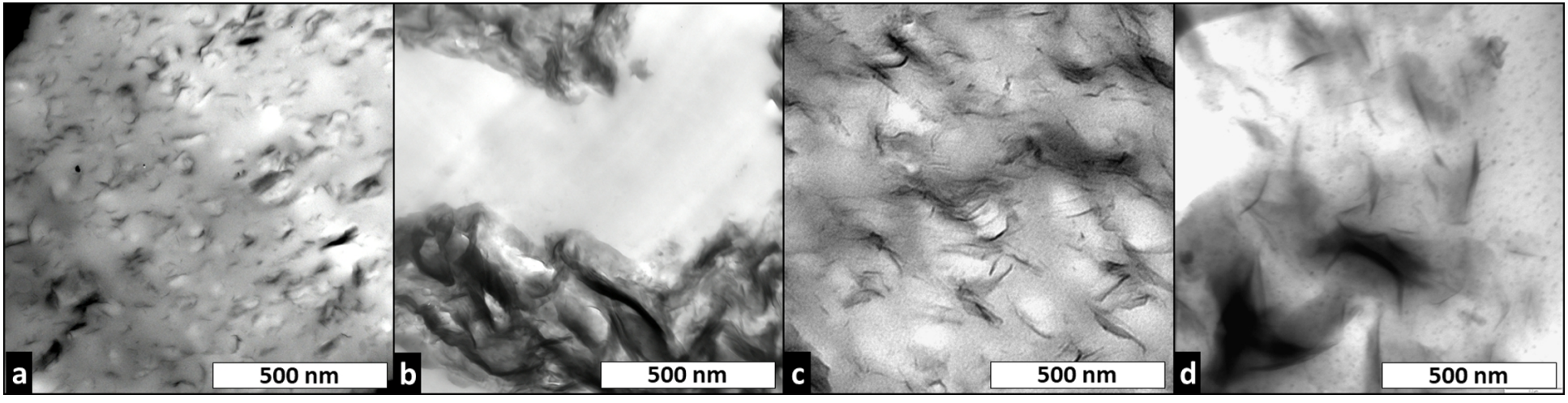
Sample	CO_2 (ml/m^2day)	O_2 (ml/m^2day)
NBC-50G50W	3307.67	7201.60
EW-GW	84653.00	24012.33
EW-PEGGW	113565.00	16007.22
NBC-PEGGW	98173.00	144089.00

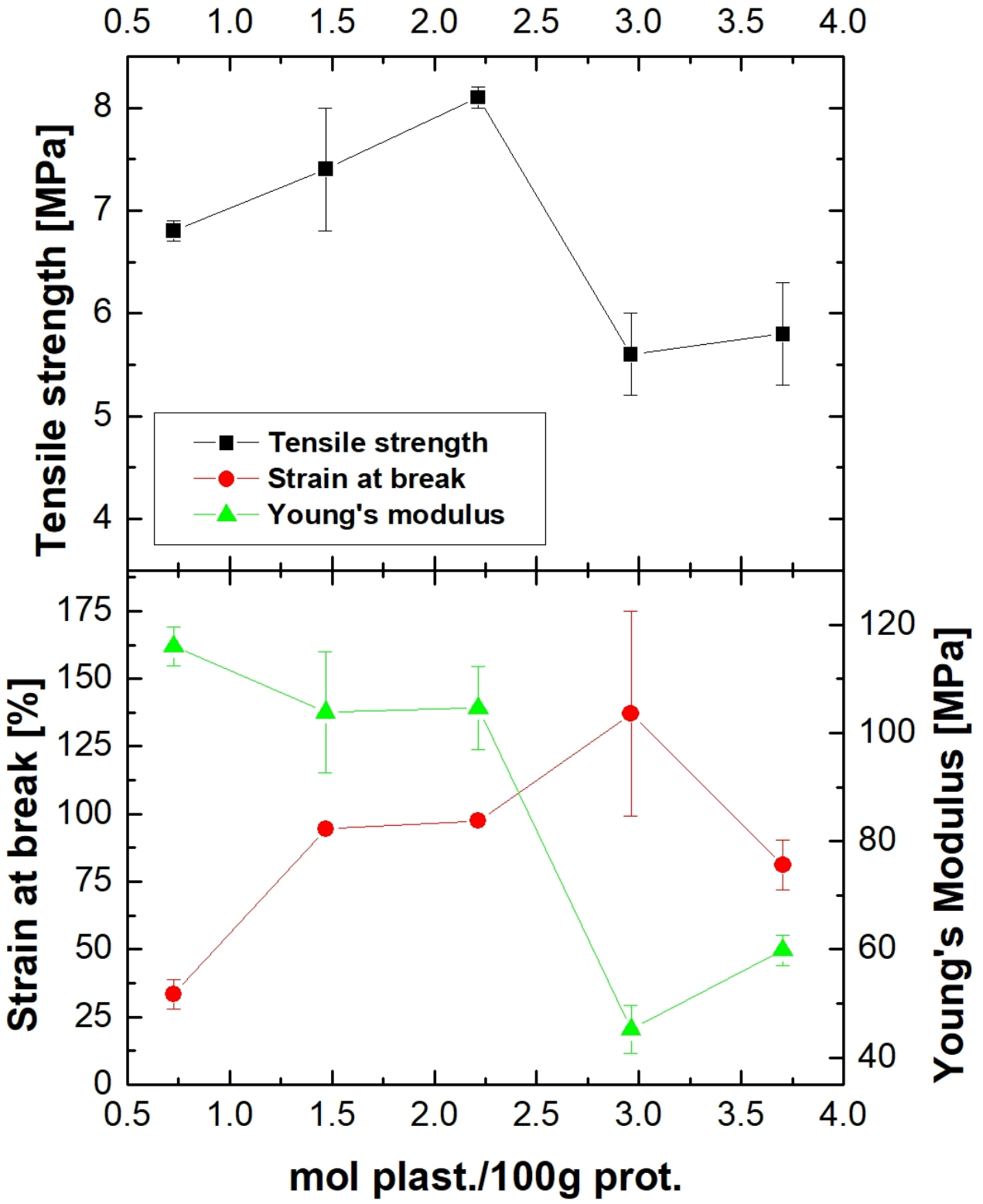
713

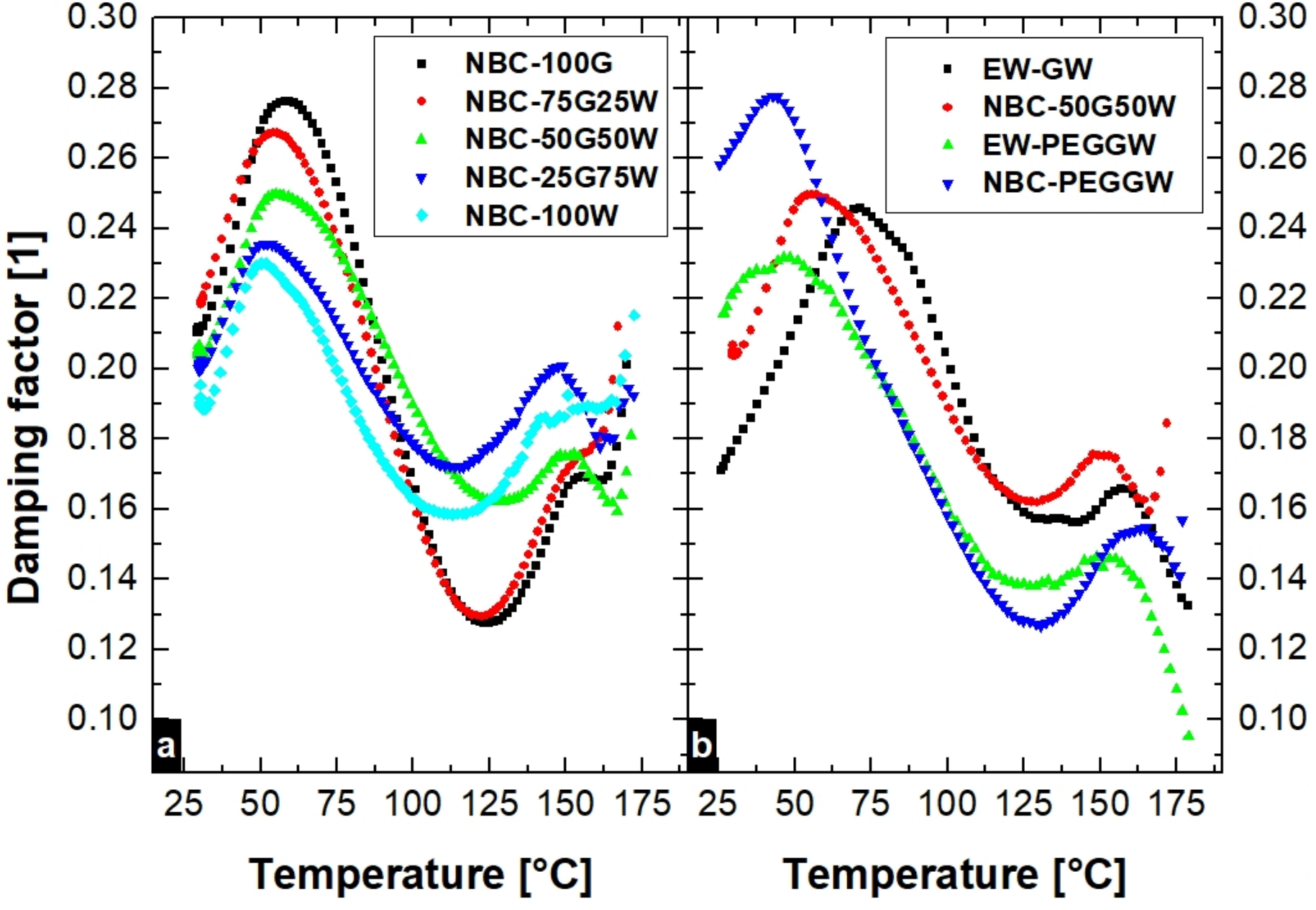
714 **Table 4.** Gas permeability results obtained for selected bioplastics and NBCs.

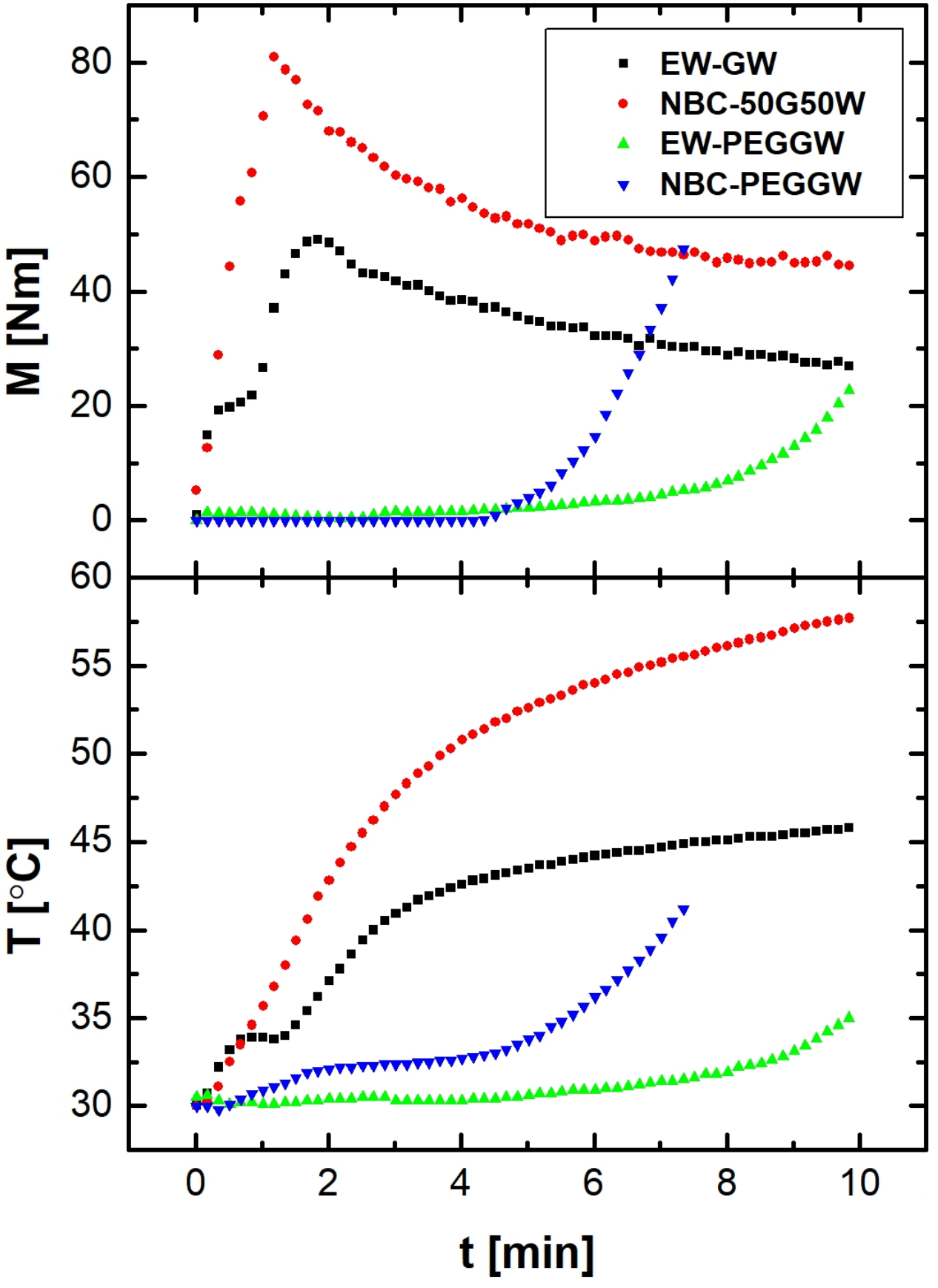

0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
SME [kJ/kg]
0
5
10
15
M max [N·m]
0
20
40
60
80
ΔT [°C]
0
10
20
30
mol plast./100g prot.


2θ (°)
Intensity
a
MMT-Na
OMMT
18.39 Å
11.70 Å
b
NBC-100G
NBC-100W
17.48 Å
16.20 Å
c
NBC-75G25W
NBC-50G50W
NBC-25G75W
15.77 Å
17.84 Å
15.77 Å
15.77 Å
d
EW-GW
NBC-50G50W
15.77 Å
15.77 Å
e
EW-PEGGW
NBC-PEGGW
17.66 Å
15.77 Å


a
500 nm
b
500 nm
c
500 nm
d
500 nm


Tensile strength [MPa]
Tensile strength
Strain at break
Young's modulus
Strain at break [%]
Young's Modulus [MPa]
mol plast./100g prot.


Damping factor [1]
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
NBC-100G
NBC-75G25W
NBC-50G50W
NBC-25G75W
NBC-100W
a
25
50
75
100
125
150
175
Temperature [°C]
EW-GW
NBC-50G50W
EW-PEGGW
NBC-PEGGW
b
Temperature [°C]


EW-GW
NBC-50G50W
EW-PEGGW
NBC-PEGGW
M [Nm]
T [°C]
t [min]
80
60
40
20
0
60
55
50
45
40
35
30
0
2
4
6
8
10