Material characterisation

Wheat gluten films obtained by compression molding

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ABSTRACT

Edible films based on plasticized wheat gluten protein were prepared by intensive mixing followed by compression molding. The effects of water and glycerol, which were selected as plasticizers for the wheat gluten, as well as the processing conditions (mixing time and molding temperature) on the physical and mechanical properties of the films were evaluated. The resulting films were characterized in terms of moisture sorption, total soluble matter, water vapor permeability, dynamic mechanical and tensile properties. It was found that plasticizer type and concentration had a dominating effect on mechanical properties and WVP, while other physical properties remain almost non-affected. Moreover, the effect of the added plasticizer (glycerol) on the film properties strongly depends on natural presence of water in commercial gluten (9% as is). On the other hand, the pressing temperature affected the final properties of the films more than the mixing time because the former influences the final cross-linking degree of the protein network. Processing temperatures higher than 100 °C led to darker films that would be discarded by consumers if they were used as food packaging.

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1. Introduction

Materials made from naturally occurring polymers could be sustainable alternatives to traditional petroleum-based plastics. Thus, in recent years, research attention has turned to developing biodegradable and/or edible packaging made from natural polymers, often from waste products of agriculture or fishing. Such polymers may be protein, lipid or polysaccharide based [1], and the physical properties of the resulting films can be highly variable, depending on the type of polymer employed. Protein-based materials have been explored as potential packaging materials because of their good barrier properties against oxygen (dry conditions) and aroma compounds [2]. Proteins are thermoplastic heteropolymers of both polar and non-polar amino acids that are able to form numerous intermolecular linkages and undergo various interactions, allowing a wide range of potential functional properties [3]. Among the numerous protein sources proposed for the preparation of biodegradable films, wheat gluten has been taken into account due to its interesting viscoelastic and film-forming properties, ability to cross-link on heating, low water solubility, low cost and availability as a co-product of the wheat starch industry [2,4,5]. Wheat gluten proteins consist mostly of monomer gliadins and polymer glutenins in roughly equal amounts by weight [6]. Glutenins are composed of discrete polypeptide subunits linked together by interchain disulfide bonds to form high molecular weight polymers. Consequently, gluten proteins cover a broad range of molecular masses up to several million daltons. Both disulphide and hydrogen bonds play a key role in the structure and properties of wheat gluten proteins [6,7].

In general, two technological processes are used to make protein-based materials: a wet process, commonly

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referred to as casting, based on dispersion or solubilization of the protein, and a dry process based on the thermoplastic properties of proteins at low water concentrations [6,8]. From economical and environmental viewpoints, thermomolding or, more specifically, high-temperature compression or injection molding is an interesting way of producing rigid gluten-based materials since it is fast and requires no solvent [4,9]. Exposing wheat gluten to high temperatures results in important changes in the types and degree of covalent cross-linking within the molecular network [4] which, in the absence of or low concentrations of plasticizer (typically water or glycerol), vitrifies on cooling into a rigid material. To control the brittleness of protein-based materials and to lower their shaping temperature, the addition of a plasticizer is generally required. These small molecules interact with the polymer chains, thereby lowering forces holding the chains together [6,10]. As a result, an extended and softened polymer matrix is obtained. Hence, plasticizer insures the formation of freestanding films and helps avoid chipping or cracking of the film during subsequent handling and storage, which could decrease film barrier properties [3].

Film packaging based on wheat gluten can improve food storage, mainly as a result of its ability to act as barrier to water, preventing dehydration, and to oxygen and light, reducing lipid oxidation. It can also be used to incorporate functional agents such as antimicrobial, antioxidants, probiotics, etc. to food products [11]. However, even though there is some research focused on the effects of molding temperature, mixing time and type and content of plasticizers on the properties of wheat gluten plastics [12], the processing conditions selected to obtain the films differ significantly according to the literature. For example, a group of researchers published the characterization of films obtained by mixing gluten with glycerol at room temperature [8,9,12–16] using glycerol contents (based on the total weigh of the dough) ranging from 25 to 40% [9]; 29.6% [8]; 25.9% [12,13]; 18.6 to 51.9% [14]; 27.1% [15]; 34.64% [16] and then molding the dough at 90 or 130°C [9]; 80 to 150 °C [8]; 65 to 125°C [12,13]; 120°C [14]; 80 or 120 °C [15]; 25°C (to study the effect of thermo-mechanical treatments [16]). Pommet et al [6] prepared wheat gluten blends with 5 different plasticizers (water, glycerol, 1,4-butanediol, octanoic acid and lactic acid) in a mixing chamber at 80 and 60 °C, for hydrated and dry gluten, respectively, and then the blends were compression-molded at 100 or 130 °C. Furthermore, water vapor permeability, as well as the properties related to water affinity and the color of the films, are very important aspects to be taken into account when designing food packaging, since they impact directly in the food shelf life or in the consumer preferences. These aspects are directly related to glycerol content and processing temperature [9,12,15]: increasing glycerol content increases WVP, while increasing processing temperature decreases WVP but leads to darker films, while consumers prefer transparent or light colored films that enhance the appearance of food.

Thus, the aim of this work is to study the effect of the processing parameters and plasticizer type (glycerol and/or water) and concentration on the physical and mechanical properties of gluten films obtained by thermoplastic processing, using relatively low plasticizer concentrations and processing temperatures to increase the water vapor barrier properties while keeping the light color of the films. Moreover, the effect of the added plasticizer (glycerol) on the film properties was evaluated on films prepared from commercial gluten "as received" (i.e. containing ~9% moisture) and dried before compounding. Additionally, water vapor absorption was measured by conditioning the films at 95% RH, which is a realistic situation to evaluate the packaging efficiency in wet foods (i.e. fresh fruits).

2. Materials and methods

2.1. Materials

Wheat gluten, food grade, with a moisture content of $9.08 \pm 0.10\%$ (determined in triplicate by drying in a convective oven at 105 °C for 2 days) provided by Dietetics Los Pinos (Mar del Plata, Argentina) and glycerol, technical grade, purchased from DEM Chemicals (Mar del Plata, Argentina) were used to produce the films.

2.2. Methods

2.2.1. Preparation of films

The wheat gluten powder was mixed with glycerol (15, 20 and 25 wt.% referred to the total mass) in a laboratory Brabender-type intensive mixer of 50 cm^3 capacity with counter-rotating roller-type blades, which was operated at 50 rpm and 80 °C for 5 or 10 minutes. The paste was formed into films by pressing it at 80 or 100 °C and 100 kg/m^2 for 10 minutes in a hydraulic press. Two sheets of Mylar® (0.5 mm thickness) were used to separate the paste from the platens and a framework of the same material was placed between them to avoid loss of polymeric material.

2.2.2. Characterization of the films

Film thickness: The thickness of the film was measured with a 0–25-mm manual micrometer with an accuracy of ± 0.01 mm at four random locations for each film.

Tensile properties: Prior to running mechanical tests, films were conditioned for 48 h at $50 \pm 5\%$ RH at 23°C, by placing them in a humidity chamber. Tensile tests were performed at room temperature (23 ± 2 °C) using an Instron Universal Testing Machine model 8501. The specimens were cut according to the ASTM D1708. Crosshead speed was set at 5 mm/min. The maximum strength (σ_m), elongation at break (ε_b) and elastic modulus (E) were calculated as described in ASTM D638.

Dynamic Mechanical Analysis (DMA): Wheat gluten films were tested in a rheometer (Anton Paar, Physica MCR 301) operated in torsion mode at 1 Hz, in the temperature range −50 to 150 °C, at a heating rate of 5 °C min^{-1}. The dimensions of the samples were $50 \times 10 \times 0.5$ mm^3. The samples were subjected to a cyclic strain of 0.1%, this being sufficiently small to guarantee that the mechanical response was within the linear viscoelastic range. The set up was used to determine the storage modulus G′, the loss modulus G″ and the ratio of these two parameters, $\tan \delta = G''/G'$.

Surface hydrophobicity: This value was estimated by the sessile drop method, using optical contact angle measurement carried out with a homemade instrument. A droplet of ethylene-glycol (Aldrich Co.) (5 μL) was deposited on the film surface with an automatic piston syringe. The drop image was photographed using a digital camera. An image analyzer was used to measure the angle formed between the surface of the film in contact with the drop, and the tangent to the drop of liquid at the point of contact with the film surface. All the samples were conditioned at 50% RH for three days before the test. Seven parallel measurements were performed for each film at 20 ± 2 °C.

Water vapor permeability (WVP): prior to the test, the films were placed in a chamber for 3 days, at 67% RH and 20 ± 2 °C, to ensure equilibrium conditions. Then, film specimens were sealed in acrylic permeation cups (5 cm diameter) containing dehydrated Cl_2Ca (0% RH). The cups were weighed at 3 h intervals over a 72 h period. A fan located inside the chamber was used to move the internal air ensuring uniform conditions at all test locations. Linear regression was used to fit the data, weight vs. time, and to calculate the slope of the resulting straight line in $g.s^{-1}$. WVP of the films, expressed as $[g.m/(Pa.s.m^2)]$, was calculated as follows:

$$WVP = \Delta W \, y \, [A \, \Delta t \, (p_2 - p_1)]^{-1} \qquad (1)$$

where ΔW is the weight of water absorbed in the cup (g), Δt the time for weight change (thus $\Delta W/\Delta t$ is the slope calculated from a plot of cup weight vs. time), A is the exposed area of the film (m^2), y is the film thickness (m), and $p_2 - p_1$ is the vapor pressure difference across the film (Pa), which was calculated based on the chamber temperature and the relative humidity inside and outside the cup.

Total soluble matter (*TSM*): TSM was expressed as the percentage of film dry matter solubilized after 24 h immersion in distilled water. Two different methods for this determination were used and compared. The first one (DRY) was previously used in several studies on films from various proteins [17–23]. In this case, three specimens from a cast film were weighed (sensitivity of 0.0001 g) and dried in an air-circulating oven at 105 °C for 24 h to determine their initial dry matter (m_0). Subsequently, the dried film pieces were placed in 50 ml beakers containing 30 ml of distilled water and stored in an environmental chamber at 25 °C for 24 h with occasional gentle stirring. Traces of sodium azide were also added to inhibit microbial growth. Undissolved dry matter (m_f) was determined by removing the film pieces from the beakers, gently rinsing them with distilled water, and drying them in the air-circulating oven (105 °C for 24 h). The weight of solubilized dry matter was calculated by subtracting the weight of insolubilized dry matter from the initial weight of dry matter. For the second method (WET), dry matter and soluble matter were not determined on the same film specimens to avoid cross-linking by heating of the samples prior to storage in water [24]. Instead, three film specimens from a film were directly immersed in water (25 °C for 24 h) as described above and, subsequently, oven dried (105 °C for 24 h) to determine solubilized dry matter (m_f). At the same time, additional samples (not previously immersed in water)

were oven dried (105 °C for 24 h) in the same conditions to determine the initial moisture content of the film (m_0). This value was used to calculate the initial dry weight of the specimens.

The TSM was calculated as follows:

$$TSM \ (\%) = \frac{m_0 - m_f}{m_0} * 100 \qquad (2)$$

Moisture sorption: the films, previously dried at 60 °C for two days in a vacuum oven, were placed inside an environmental chamber maintained at 23 ± 2 °C and 95% relative humidity (RH) by using a glycerol-water solution (20 wt.% glycerol), to obtain water sorption kinetics. Samples were removed at regular time intervals and weighed with a precision of ± 0.0001 g. The equilibrium moisture content of the samples was calculated as:

$$Meq \ (\%) = \frac{Mf - Mi}{Mi} * 100 \qquad (3)$$

where *Meq* is the moisture content of the sample at equilibrium expressed in dry basis [%]; *Mf* is the weight of the sample at equilibrium [g] and *Mi* is the initial dry weight of the sample [g].

Scanning Electron Microscopy (*SEM*): The surface and the cross-section of the films (obtained by cryo-fracture after immersing samples in liquid nitrogen) were analyzed using a scanning electron microscope (JEOL, model JSM-6460 LV, Tokyo-Japan). For this purpose, the pieces of the films were mounted on bronze stubs using double-sided tape and then coated with gold, before being observed under the microscope.

Statistical analysis: Data values were statistically analyzed by one-way analysis of variance (ANOVA). Differences between pairs of means were assessed on the basis of confidence intervals using the Tukey test. The least significance difference was $p < 0.05$. In the tables, different superscripts (a–c) within the same column indicate significant differences between samples.

3. Results and discussion

3.1. Effect of the processing conditions

The processing conditions for obtaining wheat gluten films by intensive mixing followed by compression molding were selected by comparing the final mechanical and physical properties of film produced with 25 wt% glycerol. Contrary to thermoplastic polymers, gluten viscosity does not decrease on heating but rather levels off or increases due to cross-linking reactions [25,26]. Moreover, it is worth noting that the blends processed in the intensive mixer harden quickly once removed from the equipment, probably because moisture evaporated during this step performed at 80 °C, which make it difficult to evenly distribute the paste in the mold to be placed in the press. Thus, the films were pre-formed manually immediately after removing the dough from the mixer and were then hot pressed. Also, pressing temperatures higher than 100 °C were discarded since plates with a uniform brownish color (as opposed to the slightly yellow color) were obtained

(consumers prefer transparent or light colored films, as indicated previously). This darkening was probably a consequence of higher network density, extensive aggregation, temperature effects on pigments and Maillard reactions, as reported previously [9,27].

Table 1 shows the tensile properties of the films as a function of the processing parameters. It is clear that increasing mixing time, as well as pressing temperature, leads to less deformable films, as can be seen by comparing the elongation values of samples 2 and 3 with respect to the elongation at break of sample 1. However, the effect of temperature is more pronounced, and thus films pressed at 100 °C had higher elastic modulus and tensile strength than those obtained at 80 °C, which can be attributed to the ability of gluten proteins to undergo disulfide bonding on heating, which leads to the formation of a three-dimensional macromolecular network [6,8,9].

The extent of cross-link rearrangement and aggregation depends on, e.g., process temperature, plasticizer content and type, time and shear rate [9]. According to Sun et al. [12], glutenins and gliadins are unfolded on heating up to 75 °C, which facilitates the sulfydryl-disulfide interchange. The proteins are then 'locked' into the denatured state on cooling. For glutenins, cross-linking reactions occur above 60–70 °C, whereas for gliadins the reactive zone is clearly around 90 °C [15,28]. Thus, at 80 °C, cross-linking reactions may have occurred for some glutenins but not for gliadins, whereas at 100 °C both glutenins and gliadins may undergo aggregation reactions [15,28]. Thus, the sulfydryl-disulfide interchange allows gliadins and glutenins to recombine by the formation of intermolecular covalent bonding, which results in an increase of mechanical strength and Young's modulus, as shown in Table 1. However, another outcome of the formation of this cross-linked and dense protein network is that it restrains the extensibility of the network strands, leading to a decrease of the elongation at break.

Gluten proteins characteristically have a high content of glutamine residues (around 35%) that are involved in hydrogen bonding [29], which also plays a key role in the structure and properties of the films obtained. In order to confirm these effects, additional tests were performed. Table 2 shows the equilibrium moisture content of samples conditioned at high relative humidity (95%), the contact angles measured on film surfaces using a polar solvent (ethylene-glycol) and the total soluble matter, obtained from the wet and dry methods. In this case, it is seen that both the contact angle, which indicates a more hydrophilic character of the film surface as lower is its value, and the equilibrium moisture content are statistically similar for all

Table 1
Tensile behavior of gluten films containing 25% glycerol.

ID	Mixing time (min)	Pressing temperature (°C)	E (MPa)	σ_m (MPa)	ε_b (%)
1	5	80	$15.08^a \pm 4.24$	$1.86^a \pm 0.53$	$57.5^a \pm 7.2$
2	5	100	$22.58^b \pm 4.36$	$2.00^a \pm 0.59$	$39.8^b \pm 11.1$
3	10	80	$17.60^{ab} \pm 2.48$	$1.96^a \pm 0.53$	$37.7^b \pm 6.3$

[a,b]Means within a column which are not followed by a common superscript letter are significantly different ($p < 0.05$).

Table 2
Physical behavior of gluten films containing 25% glycerol.

ID	Contact angle (°)	Meq (%)	TSM (dry), %	TSM (wet), %
1	$38.0^a \pm 3.1$	$68.14^{ab} \pm 5.40$	$24.94^b \pm 1.40$	$28.31^a \pm 0.49$
2	$36.8^a \pm 3.0$	$65.61^b \pm 5.19$	$24.06^b \pm 0.34$	$25.60^b \pm 0.25$
3	$37.3^a \pm 3.9$	$72.34^a \pm 2.70$	$26.61^a \pm 0.34$	$28.53^a \pm 0.20$

[a,b]Means within a column which are not followed by a common superscript letter are significantly different ($p < 0.05$).

processing conditions. Moreover, when samples reached equilibrium moisture content at 95%RH, exudation of glycerol was observed (confirmed by FTIR measurements of the exudates, not shown). The equilibrium water absorption represents a competition between the force exerted on the gluten network by water molecules penetrating into it and that developed by the elongation of the cross-linked gluten network resulting from water penetration [4]. For some polymeric matrices, including gluten bioplastic, the equilibrium water absorption attained by submersion of test samples in water provides a measure of the degree of cross-linking [4]. However, in this case, due to the important standard deviation of the equilibrium moisture content measurements, these results are not conclusive. Moreover, in a related work Angellier-Coussy et al. [15] concluded that the accessibility of water vapor to the hydrophilic sites in thermo-molded wheat gluten films obtained at 80 °C or 120 °C was not significantly different whatever the processing temperature, meaning that both films had the same "capability" of water vapor sorption. On the other hand, gluten films are relatively insoluble in water because of the low content of ionized polar amino acids, the numerous hydrophobic interactions between the apolar amino acids and the presence of disulfide bonds [3]. Moreover, on heating, two different phenomena occurred concomitantly: the denaturation of proteins (unfolding) and the covalent cross-linking reactions inducing an increase in the insoluble protein fraction [15], thus relatively low water solubility is anticipated for thermoformed gluten films, which is the case in this work. In fact, our own data showed that the TSM is only slightly higher than the glycerol content of the samples (25%), which could be considered as an indication that plasticizer was not trapped in the protein network. Nevertheless, all swollen materials kept their integrity after TSM tests. Sun et al. [12] noticed that their wheat gluten films plasticized with water, glycerol or butanediol were 30% water soluble (dry weight loss after swelling test) and confirmed that in these cases all the plasticizer dissolved in water during sample immersion. Furthermore, it is also seen that samples 1 and 3, both compressed at 80 °C, exhibit statistically higher values of wet TSM than sample 2, which confirms that additional cross-linking is obtained by forming the films in the press at 100 °C. Dry TSM values are lower than the corresponding wet ones, indicating that the drying step (105 °C for 24 h) also contributes to increase the cross-linking of the protein, confirming the important effect of the selected processing temperatures. Similar results can be found in the work of Domenek et al. [30].

Fig. 1 shows, as an example, SEM images of sample 2, conditioned at room humidity (~50%RH) and at 95%RH. It is
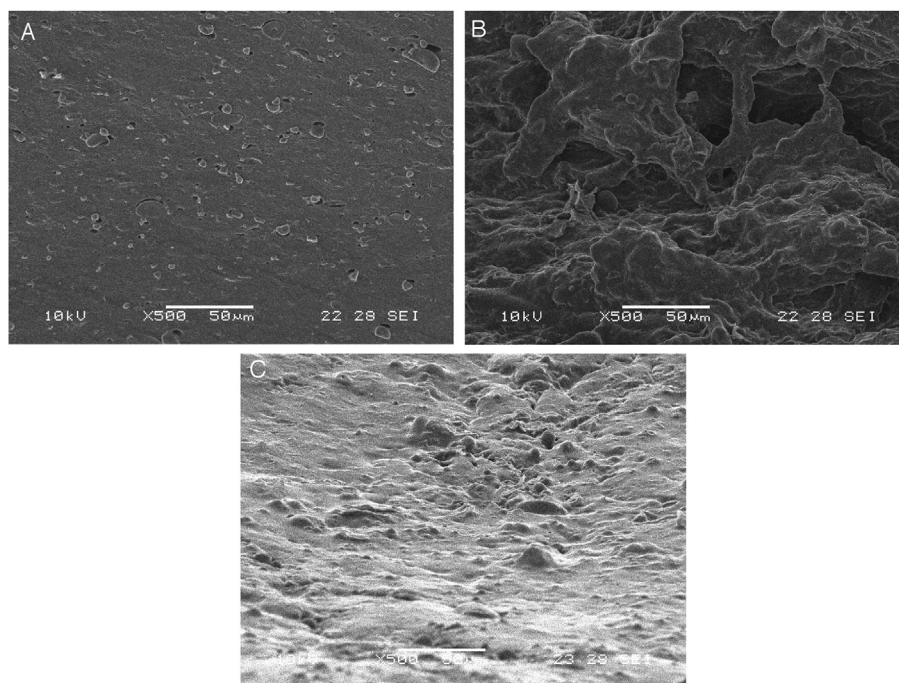
Fig. 1. SEM pictures of sample 2. A: Conditioned at 50%RH, transversal section; B: Conditioned at 95%RH, transversal section; C: Conditioned at 95%RH, film surface.

clear that moisture sorption induces severe changes in the microstructure of glycerol-plasticized wheat gluten films: water is a plasticizer for gluten so the higher its concentration the higher is the mobility of polymer chains. Moreover, as was previously indicated, glycerol migration occurred at high water activities. Thus, the even and compact transversal section of the film conditioned at 50 % RH (Fig. 1A) becomes a relaxed, uneven and porous structure in extremely humid conditions (Fig. 1B). These changes affect not only the thickness, leading to swollen samples, but also affect the surface of the films, as can be seen from Fig. 1C.

According to the previous observations, the films prepared to study the effect of plasticizer content were obtained using a mixing time of 5 min and a pressing temperature of 100 °C.

3.2. Effect of the plasticizer content − mechanical and dynamic mechanical properties

Once the processing parameters were selected, the effect of the plasticizer type and concentration was investigated. The data presented in Table 3 corresponds to the tensile behavior of films prepared with "as received" wheat gluten (i.e. without previous drying, moisture content ~9%), except in the case of sample 2-20D, which was prepared with dried gluten (i.e. very low moisture content, dried in a vacuum oven at 60 °C for 48 h), in order to address the effect of a unique plasticizer. From Table 3, as well as our own observation during the preparation of the films, it can be concluded that the effect of the plasticizers is much more important than the changes in the processing parameters. In fact, the moisture contained in the wheat

gluten powder greatly reduced the torque necessary to process the mixture gluten-glycerol, and facilitates its posterior manual pre-forming. Comparing the tensile properties of samples 2−20 and 2−20D (both containing 20% glycerol), it is surprising to see that the film obtained by processing dried wheat gluten is less stiff and more deformable than sample 2−20 (p < 0.05), as if the plasticizing effect of the glycerol is enhanced when used as unique plasticizer. Moreover, in these cases the tensile strength of both samples is comparable (p > 0.05). On the other hand, samples produced from wet gluten presented the expected behavior: tensile modulus and strength increases and elongation decreases as glycerol concentration decreases.

The mechanical behavior of protein-plasticizer systems could be affected by different contrary effects: 1) the presence in the protein matrix of the easily deformable plasticizer rich domains that lead to material plasticization producing a decrease in rigidity and strength, and an increase the elongation at break; 2) molecular interactions

Table 3
Tensile behavior of gluten films containing different amounts of glycerol and water.

ID	Water	Glycerol concentration (wt%)	E (MPa)	σ_m (MPa)	ε_b (%)
2−25	Yes	25	22.6[c] ± 4.4	2.00[c] ± 0.59	39.8[b] ± 11.1
2−20	Yes	20	95.2[b] ± 18.9	4.92[b] ± 1.00	28.2[c] ± 6.3
2−15	Yes	15	325.7[a] ± 35.6	8.30[a] ± 1.16	21.2[c] ± 5.6
2−20D	No	20	48.9[c] ± 11.2	4.37[b] ± 0.39	53.2[a] ± 3.1

[a,b,c]Means within a column which are not followed by a common superscript letter are significantly different (p < 0.05).

that could enhance the structural bonds in the polymer network leading to an increase in the tensile strength but decreasing its ability to stretch. Moreover, in the present case, the cross-link of the wheat gluten that leads to the formation of a protein network takes place during the thermoforming process and needs the denaturation of proteins (heat activate process) that leads to their unfolding and then to the covalent cross-linking reaction. Our theory is that the water contained in the "non dried" gluten facilitates the unfolding of the protein chains, by increasing its mobility during processing, and thus facilitates wet samples (2−25, 2−20 and 2−15) to achieve higher cross-linking with respect to sample 2−20D. Other researchers found similar results in samples containing different moisture levels and no glycerol [31,32]. Moreover, Irissin-Mangata et al. [3] affirmed that water is the most ubiquitous and uncontrollable plasticizer of wheat gluten protein because of its ability to modify the structure of this natural polymer, which supports our theory. This hypothesis explains correctly the trend observed in Table 3: for samples prepared from wet gluten, the rigidity decreased and the elongation at break increased with glycerol concentration because the plasticizing effect increases while the cross-linking degree remains constant. On the other hand, the low cross-linking degree of the films prepared from dried gluten compensates the important reduction in the total content of plasticizer (only glycerol, not water) leading to less stiff and more deformable films. Moreover, according to Sun et al. [12], aggregation of wheat proteins through disulfide bonding is facilitated by increasing temperature and moisture content, affirmation that also supports our hypothesis.

To further study the relative importance of the contribution of water and/or glycerol in plasticizing gluten films, dynamic mechanical tests were carried out and the results are shown in Fig. 2. Fig. 2A shows that the storage modulus of all samples decreases continuously, although with different slopes, as temperature increases. For samples prepared from wet gluten, the storage modulus decreases as glycerol concentration increases in the whole temperature range, which is the expected behavior in plasticized polymeric materials, since plasticizers change the polymer chains three-dimensional organization, decreases attractive intermolecular forces and increases free volume and chain mobility [4−6,33]. Actually, in plasticized polymers, the polymer−polymer physical cross-links (hydrogen bonds and/or hydrophobic interactions) are replaced by polymer−plasticizer interactions [6]. However, it can be seen that the behavior of the sample containing 25% glycerol is more erratic (noisy and sometime puzzling G′ values) than those of the other specimens, especially at temperatures higher than 50 °C, since the film becomes so soft and flexible that the equipment signal becomes inaccurate due to sample partial collapse, as was seen in related research work [6]. In fact, the sample failed completely before reaching the highest imposed temperature (150 °C). On the other hand, the storage modulus of the film prepared from dried gluten is the highest at low temperatures (up to 13 °C), but then becomes lower than that of the wet sample with 15% glycerol, thus, presenting an overall different trend that could be ascribed to a different level of cross-linking.

One of the most visible effects of the plasticizers added to polymeric systems is the decrease of the glass transition temperature of the polymer [6,15]. In fact, the change in polymer Tg as a function of the molar content of the plasticizer is a classical measure of plasticizer efficiency. From the tan δ plot (Fig. 2B), it can be seen that wheat gluten films exhibit two wide transitions: the low temperature one attributed to the glycerol rich domains while the high temperature one is associated with the protein rich zones, which is typical of phase separated systems and was noted in related works [12,34,35]. The peak observed at higher temperatures could be related to the α-relaxation of plasticized wheat gluten, and can be interpreted as motions of the side chains of protein linked to glycerol/water by hydrogen bonding [12], and thus appears as a wide peak with maximum in temperature between 60 and 135 °C, depending on the total plasticizer content of the sample. The great α-transition width may reflect a wide variety of degree of order within gluten proteins, reflecting the high heterogeneity of gluten protein molecular weights ranging
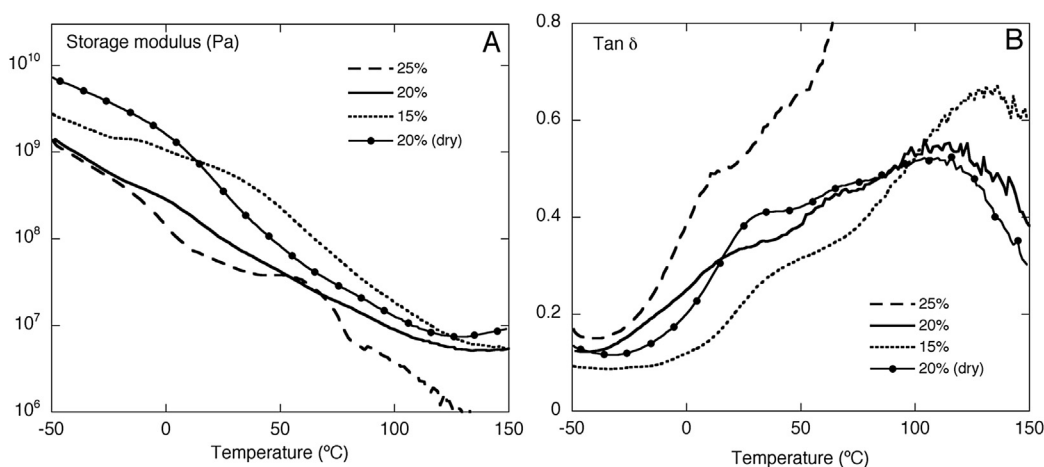

Fig. 2. Storage modulus (G′) and tan δ curves for wheat gluten films prepared with different content of plasticizers.

from 30,000 to millions [12]. In fact, closer observation of this wide peak reveals the existence of a shoulder that appears at lower temperature as the glycerol concentration is raised, and can be attributed to the Tg of plasticized glutenin, (40–90 °C for 25–15% glycerol samples, respectively), while the main peak to a plasticized gliadin, (60–132 °C for 25–15% glycerol samples, respectively), as indicated by Jerez et al. [25] and Matveev [36,37]. It is also interesting to note that the temperatures of these transitions are similar for both samples containing 20% glycerol and prepared from wet or dry gluten, which indicates that the plasticization of the protein is mainly due to the glycerol, with just a marginal (or no) contribution of water, in contrast to the observations of Sun et al. [12]. Actually, Sun et al. [13] studied the effect of cross-linking types on the properties of thermo-molded gluten plastics containing glycerol as a plasticizer and found that cross-linking through disulfide bonding results in a high degree of phase separation, giving rise to a high glass transition temperature of the gluten-rich phase.

The lowest temperature transition associated with the glycerol relaxation [13,26] appears in the range 13–30 °C, suggesting that glycerol mobility was more restricted in these samples due to interactions between components as plasticizer content decreases. In this case, the effect of water is considerable, as reflected by the important change in the peak temperature when dry or wet gluten was used to prepare the films (i.e. ~29 °C vs. ~17 °C), which probably indicates that the moisture contained in the "as received" gluten migrates to the glycerol rich phase during the mixing/molding steps. Moreover, the moisture contained in the samples evaporates faster at higher temperatures, and thus the effect of water in the high temperature transitions is lower.

SEM images of the cryogenic fractured transversal section of the gluten films prepared with different total plasticizer concentration and conditioned at 50 and 95 %RH are shown in Fig. 3. The fractured surfaces of the samples conditioned in a relatively dry ambient (50%RH) are quite similar, with slight differences: the 25% glycerol film exhibit a rather featureless and smoother surface and the film prepared from dried gluten a more fragile feature (fissures and cracks are seen) than those containing 15 or 20% glycerol. Moreover, voids are observed in all samples. In principle, voids and sharp cracks may act as stress concentrators that in turn induce early fracture, wherein cracks are normally more serious than voids [4]. However, in this case, the sample presenting cracks is the film obtained from dried gluten, which showed the highest elongation at break indicating that, although these defects are present and may contribute to the mechanical properties, their effect seemed less important than cross-linking. Similar conclusions were obtained by Jansens et al. [4].

On the other hand, the differences described previously were enhanced when the samples are saturated with moisture. As indicated in the preceding section, the migration of plasticizer in the film containing 25% glycerol leads to a very open and uneven structure, with a succession of ripples and valleys, which can be related to a more ductile material. The fractured surfaces of the films containing 15 and 20% glycerol become even and smooth due

to the combined plasticizer action (water + glycerol), and the transverse section of the film obtained from dried protein confirmed its more brittle nature at low temperature by showing a continuous succession of ridges produced during cryogenic fracture.

3.3. Effect of the plasticizer content – moisture sorption, TSM and WVP

Table 4 presents the contact angle, equilibrium moisture content, TSM and WVP values for samples prepared with wet gluten and different glycerol contents. These measurements were not performed on the films obtained from dried gluten, since they lost cohesion after a few days of preparation. Again, contact angle values do not present a clear trend with glycerol concentration and all values are statistically similar ($p < 0.05$), indicating in all cases a quite hydrophilic film surface. The average values for equilibrium moisture content show the anticipated increase with increase of plasticizer concentration, although the differences are minor and the standard deviations quite large, meaning no statistical differences. From a general point of view, it is interesting to note that the TSM values are lower than those reported for other protein based films, for example, 35–65% solubility (DRY method) was obtained for triticale (a hybrid between wheat and rye) plasticized with glycerol based films [18] obtained by casting, while 30–36% TSM (DRY) was measured for compression molded soy protein isolate (SPI) based films, blended with gelatin and plasticized with glycerol [38]. In the present case, TSM increases with increasing glycerol content ($p < 0.05$) but, unexpectedly, for samples containing 15 and 20% glycerol, the soluble mass is almost 5% higher than the plasticizer concentration, in disagreement with was observed in the previous section, which can probably be attributed to an increase in the soluble or low molecular weight protein concentration in the gluten rich-phase. In this sense, it is interesting to note that, while the tan δ peak height for the low temperature transition increases as plasticizer content increases, the height of the high temperature peak is higher for the sample plasticized with 15% than for the counterpart containing 20%, while the complete curve for the 25% sample could not be obtained. The increase in the height of the tan δ peak is related to the increase in the volume fraction of glycerol/gluten undergoing the transition [6]. Probably, protein chain scission in the less plasticized samples occurred as result of the intensive mixing during processing, since higher torque was necessary to effectively mix low glycerol content blends. In this respect, Pommet et al. [6] indicates that, during mixing, gluten de-aggregates due to shear and, in some cases (when they used acid compounds as plasticizer to prepare wheat gluten films), disaggregation induced by shear effects competed with or even prevailed over thermal aggregation due to high temperature. Once more, the TSM values determined from samples dried at 105 °C are higher than wet ones, indicating that the drying step at high temperature also contributes to gluten aggregation.

WVP is reduced dramatically ($p < 0.05$) by decreasing the glycerol concentration from 20 to 15%, which is in agreement with the role of an efficient plasticizer (i.e.
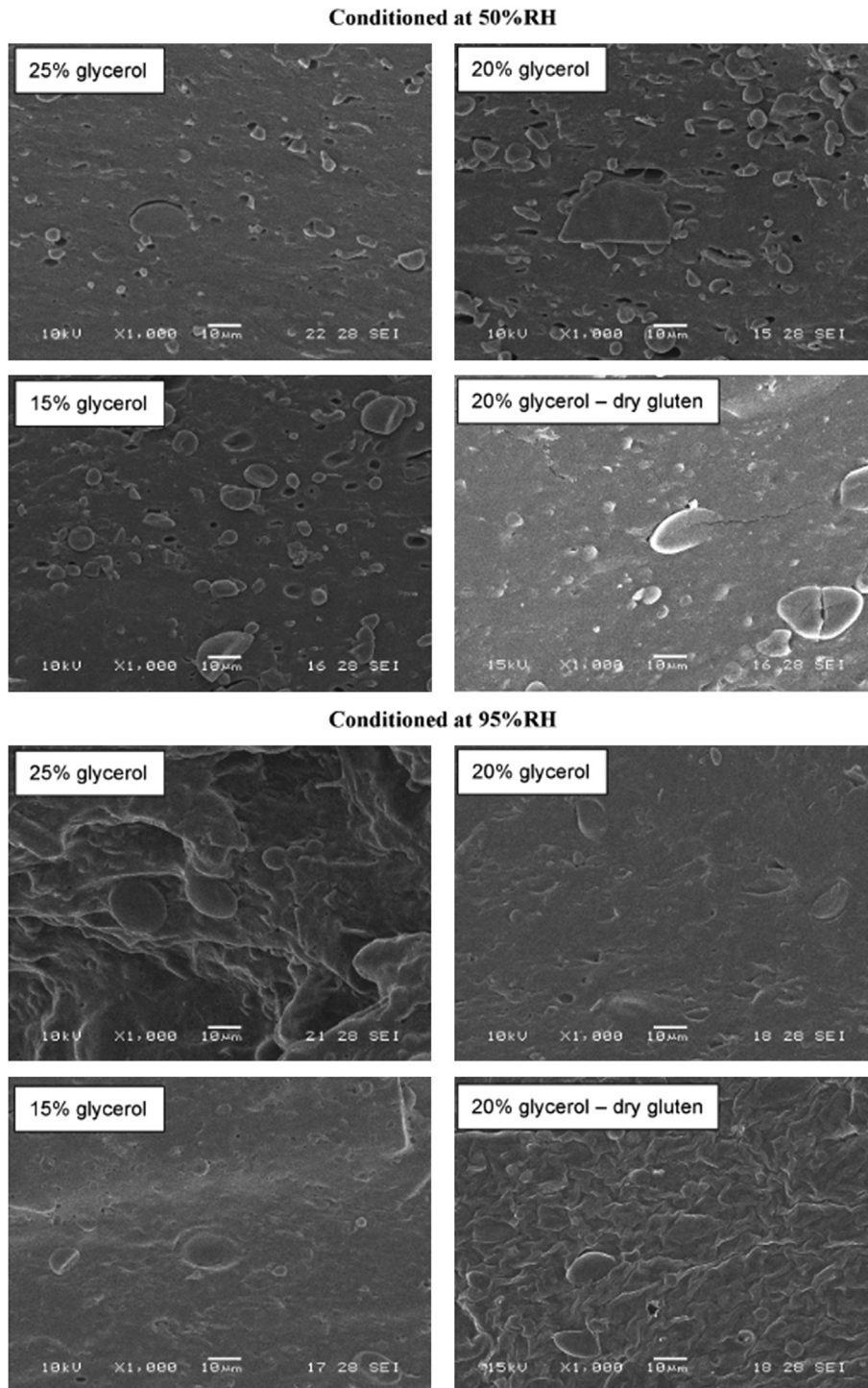
Fig. 3. SEM pictures of samples 2–25, 2–20, 2–15 and 2–20D (transversal section) conditioned at 50%RH or 95%RH.

decreases the bonding forces that holds the protein chains together, leading to a more open and permeable structure). Moreover, in this case, the change is really substantial considering that for a 5 wt% reduction in the glycerol concentration the WVP decreased by 45%.

4. Conclusions

A dry process (intensive mixing of the protein with plasticizer followed by compression molding) was used to obtain edible films based on wheat gluten protein. It was

Table 4
Physical behavior of gluten films containing different glycerol concentrations.

ID	Contact angle (°)	Meq (%)	TSM (dry) %	TSM (wet) %	WVP x 10^{10} (g.m/Pa.s.m^2)
2–25	$36.8^a \pm 3.0$	$65.61^a \pm 5.19$	$24.06^a \pm 0.34$	$25.60^a \pm 0.25$	–
2–20	$33.7^a \pm 3.5$	$63.44^a \pm 1.88$	$22.91^b \pm 0.23$	$24.38^b \pm 0.24$	$1.75^a \pm 0.06$
2–15	$35.9^a \pm 5.4$	$63.07^a \pm 1.17$	$20.01^c \pm 0.02$	$21.58^c \pm 0.35$	$0.80^b \pm 0.06$

[a,b,c]Means within a column which are not followed by a common superscript letter are significantly different (p < 0.05).

found that effect of molding temperature on the final mechanical and physical properties of the films is more pronounced than that of the mixing time, because gluten protein undergoes additional disulfide bonding during heating, leading to a more cross-linked polymeric network. This observation was also confirmed by performing TSM tests in dry and wet conditions. However, the plasticizer type and content were the dominating factors that lead to the most important variations in the final properties of wheat gluten films. In this case, films prepared from "as received" gluten and 25% glycerol exudes the latter when conditioned at high relative moisture. As expected, mechanical performance (tensile modulus and strength) decreased as glycerol concentration increased, while elongation at break increased. However, samples prepared from previously dried gluten deviate from this trend, which indicates that they reached a different level of cross-linking during processing. The glass transition temperatures of both glycerol and protein rich phases decreased with increase of glycerol, the latter being almost unaffected by the initial moisture content of the gluten. Unexpectedly, physical properties such as contact angle and equilibrium moisture content slightly change with increasing glycerol concentration, although plasticizer concentration has a dominating effect on TSM and mainly on WVP.

Thus, it can be concluded that plasticized wheat gluten films prepared by mixing the humid ("as received") protein with no more than 20 wt% glycerol for 5 min at 80 °C and then pressing the paste at 100 °C can be obtained with a reasonable balance of final properties while keeping the light color.

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