Properties of triticale protein films and their relation to plasticizing–antiplasticizing effects of glycerol and sorbitol

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ABSTRACT

Triticale protein films were prepared and the influence of glycerol and sorbitol on their functional properties was investigated. At equal plasticizer concentration glycerol-plasticized triticale films had higher moisture content at a given relative humidity than did sorbitol films. Films plasticized with sorbitol exhibited higher solubility than glycerol-plasticized films. Mechanical properties of films are strongly affected by relative humidity and considerably varied with the type and concentration of plasticizer used. Glycerol exhibited better plasticizing effect for triticale films. Tensile strength, Young's modulus and puncture force of the films decreased as the glycerol content increased. The increase in the tensile strength and Young's modulus of the films containing low levels of sorbitol was ascribed to the antiplasticization of the films. Films prepared with sorbitol had lower water vapor permeability values but tended to be stiff and in some cases too brittle for tensile measurements. Sorbitol plasticization of the triticale protein films approached saturation at the plasticizer level of 50%. This work contributes to the understanding of the plasticizing–antiplasticizing effects of glycerol and sorbitol on properties of protein films and may be useful in the handling of these films as new food packaging systems, in accordance with the specific requirements of potential users.

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1. Introduction

Biopolymer films derived from natural products that can be used as food packaging have received considerable research interest during the last years resulting from ecological concerns. This has led to the use of several renewable materials like proteins, as a promising biodegradable material. Proteins are heteropolymers that are able to form numerous intermolecular linkages and undergo various interactions and have been used as film-forming agents (Irissin-Mangata et al., 2001). During film formation, proteins may associate and dissociate in different ways, depending on experimental conditions. The three-dimensional networks are dependent of the intra and intermolecular interactions among proteins, but also from interactions between proteins and the constituents of films like plasticizers. To date, numerous protein sources have been proposed for the preparation of films (Aguirre et al., 2011; Bamdad et al., 2006; Bodnár et al., 2007; Gillgren et al., 2011; Valenzuela et al., 2013). A particular interest has been focused on the use of triticale protein to prepare films. Triticale (x *Triticosecale* Wittmack)

is the hybrid between wheat (*Triticum* ssp) and rye (*Secale* ssp). This cereal may be suitable for grain production, forage, energy, bioethanol production (Jørgensen et al., 2007; Pejin et al., 2009) and film formation (Aguirre et al., 2011). It possesses the yield potential of wheat and the adaptation capacity of rye to a dry environment (Aguirre et al., 2006). Also, the nutritional and functional properties of triticale proteins have been investigated (Pérez et al., 2003). The versatility that triticale offers as a grain, a forage and as a biofuel feedstock adds to the economic viability that sustains the interest in the crop. Although triticale is a suitable grain for human diet, the overall food market for triticale is very small. Current triticale varieties do not possess the milling and baking characteristics to be competitive with wheat for use in bread and pasta products. Triticale flour proteins showed suitable film-forming capacity for the formulation of biodegradable films with properties comparable to that of other edible films and could be used as a component of new biopolymeric films (Aguirre et al., 2011).

In addition to the biopolymer, a major component of protein-based films formation is the plasticizer. From a mechanical point of view, the effect of a plasticizer is to increase the flexibility, extensibility, workability and ductility of a biopolymer by decreasing its mechanical resistance. Plasticizers can interfere with protein chain-to-chain hydrogen bonding, reducing the intermolecular forces and increasing the mobility of polymeric chains, and are generally used

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to improve the mechanical properties of films. The plasticizer's composition, size and shape influence its ability to disrupt protein-chain hydrogen bonding, including its ability to attract water to the plasticized protein system. Water is also an effective plasticizer for protein films. Polyols are often cited as good plasticizers for protein-based materials due to their ability to reduce intermolecular hydrogen bonding while increasing intermolecular spacing.

The phenomena of plasticization and antiplasticization have begun to receive increasing attention from scientists and technologists (Chang et al., 2006; Suppakul et al., 2013).

The objective of the present research was to study the influence of glycerol and sorbitol as plasticizer agents and to evaluate the effect of their concentration and of different relative humidity conditions on mechanical and water vapor parameters of triticale proteins films.

2. Materials and methods

2.1. Material

Triticale (variety Buck TK 205) flour (moisture content, $13.25 \pm 0.02 \, g \, 100 \, g^{-1}$, protein content, $8.88 \pm 0.02 \, g \, 100 \, g^{-1}$, ash content, $0.61 \pm 0.02 \, g \, 100 \, g^{-1}$, particle size: pass through a US Standard Sieve No 100) was donated by Campeloni Semillas S.A. (Córdoba, Argentina). All chemical reagents used in this research were purchased from Sigma–Aldrich Chemie Gmbh (Munich, Germany) and were of analytical grade.

2.2. Proteins-rich film-forming solution preparation

Protein fractions were extracted from triticale flour. Initially, albumins and globulins were extracted from 50 g of flour using 0.25 L of NaCl solution ($5 \, g \, L^{-1}$) by stirring ($20 \, °C$) for 1 h and centrifuged at $10,000 \times g$ for 15 min. Supernatant was discarded. The precipitate was dispersed in 0.25 L of an ethanol solution (70 mL/100 mL), stirred 1 h ($20 \, °C$), and centrifuged at $10,000 \times g$ for 15 min ($20 \, °C$). The supernatant was collected. The precipitate was discarded.

2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Protein fraction was analyzed by SDS-PAGE under reducing and non-reducing conditions with a discontinuous buffered system (Aguirre et al., 2011), using a 4% stacking gel and 12% separating gel. Samples were prepared in sample buffer at pH 6.8 with or without disulfide bond cleaving 2-mercaptoethanol (2-ME). Protein molecular weights were estimated using Bio-Rad SDS-PAGE Molecular Weight Standard Broad Range. A Mini Protean II unit (Bio Rad Laboratories, CA) was used. Images of scanned gels were analyzed with the "Gel Submenu" of the ImageJ software (available at http://rsbweb.nih.gov/ij/). Areas under peaks were calculated by the software after each peak is marked and selected. Each peak is then labeled with the percentage of area under each peak with respect to the total area of the measured peaks. This analysis is performed in triplicate lanes.

2.4. Film formation

Films were prepared with triticale proteins with a final 7.5 g of protein/100 mL film forming solution. The plasticizers studied were:

- Glycerol: (Molecular weight 92.03) $C_3H_8O_3$, straight chain.
- Sorbitol: (Molecular weight 182.17) $C_6H_{14}O_6$, straight chain.

Glycerol or sorbitol was added at different concentrations (g plasticizer/100 g protein) as plasticizer, and stirred for 20 min. Measured volumes (20 mL) of the film-forming solution were poured onto a horizontal flat silicon tray (12 cm diameter) to allow water and ethanol to evaporate. Films were dried at 40 °C in an oven with air circulation. Dry films were peeled off the casting surface and preconditioned in an environmental chamber at 25 °C and 32%, 52% or 68% relative humidity (RH). RHs were obtained using saturated salt solutions of $MgCl_2$, $Mg(NO_3)_2$ and KI respectively, for at least 72 h prior to testing. The thickness of the films was determined with a micrometer (average of 6 measurements). The mean thickness ($200 \pm 50 \, \mu m$) was used to calculate the mechanical and barrier properties.

2.5. Moisture content (MC)

MC of triticale films was determined according to Aguirre et al. (2011). Triplicate measurements of MC were conducted for each type of film and an average was taken as the result.

2.6. Total soluble matter (TSM) in water

Total soluble matter was defined by the content of dry matter (dm) that was lost after 24 h immersion in water. TSM was determined according to Aguirre et al. (2011).

2.7. Tensile test

Tensile strength (TS), Young's modulus (EM) and percent of elongation at break (%E) were determined using a texture analyzer TA.XT2i (SMS, Surrey, England). Films were cut into 20 mm wide and 50 mm long strips, and mounted between the grips of the texture analyzer. The initial grip separation was set at 30 mm and the crosshead speed at 1.0 mm/s. TS (force/initial cross sectional area) and %E (percentage of the change of the original length of the specimen between the grips at break) were determined directly from the stress × strain curves using the software Texture Expert V.1.22 (SMS). EM was calculated as the slope of the initial linear portion of the curve.

2.8. Puncture test

The force at the breaking point of the triticale films were determined by a puncture test using a texture analyzer TA.XT2i (SMS, Surrey, England). The films were fixed on a still flat surface with a 10 mm diameter hole and perforated with a P/2N probe (needle probe), moving at 1 mm/s until the film broke. All determinations were made five times. Mean and standard deviations were calculated.

2.9. Water vapor permeability (WVP)

WVP was measured gravimetrically according to the method reported by Aguirre et al. (2011). Each film sample was sealed over a circular permeation cup containing silica gel (desiccant at relative humidity (RH) 0%). The environment within the cabinet was held at constant RHs and controlled temperature (25 °C). The RH inside the cell was always lower than the outside, and water vapor transport was determined from the weight gain of the permeation cell. Cups were periodically weighted and water vapor transfer rates (WVTR, $g \, m^{-2} \, s^{-1}$) of films were determined from the slope of weight gain versus time plots using: WVTR = $(\Delta m \, A^{-1} \, \Delta t^{-1})$, where Δm is weight gain of permeation cell (g), A is the exposed area and

Δt is time. Water vapor permeability (WVP, $g\,m^{-1}\,s^{-1}\,Pa^{-1}$) were calculated using the following equation:

$$WVP = \frac{WVTR \cdot X}{\Delta P}$$

where X is film thickness and ΔP is vapor partial pressure difference (Pa) across the film.

2.10. Statistical analyses

Measurements of each property were in triplicates for moisture content, water solubility and water permeability and in five replicates for mechanical properties. Statistical data was analyzed using Microsoft Excel 2003. Analysis of variance (ANOVA) was carried out to test mean differences. Student's t-test was applied to compare averages of properties with a level of 95% confidence interval.

3. Results and discussion

3.1. Protein characteristics, SDS-PAGE

For this study we grouped the proteins analyzed into 3 groups (Fig. 1). According to the mobility of the markers we used the molecular weight (MW) of the first group (A) of proteins is higher than 116 kD. The second group (B) corresponds to those proteins with MW that range from 31 kD to less than 116 kD. The third group (C) corresponds to those located on the lower half of the 12% separating gel. Their estimated MW is within the range of 6.5 kD and 31 kD. Fig. 1 summarizes the main findings regarding the relative proportions of the three protein groups (A, B, C), with a significant proportion (45.03%) of proteins of the A-group. When sample is
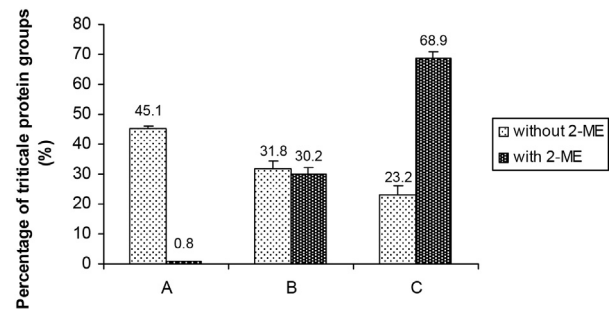
Fig. 1. Percentage of groups of triticale flour proteins determined by SDS-PAGE of non-reduced and reduced (with 2-ME) solutions. Group A = proteins with MW higher than 116,000 D; Group B = proteins with MW between 31,000 D and 116,000 D; Group C = proteins with MW between 6500 D and 31,000 D. 2-ME: 2-mercaptoethanol.

treated with the reducing agent 2-ME there are significant change in the relative proportion of the protein group. For practical purposes proteins of group A disappear when treated with 2-ME. Group C proteins increase after treatment with 2-ME. Thus, the aggregates on the top of the gel (group A) were likely the result of the formation of disulfide bonds between subunits through sulfhydryl–disulfide interchange reactions. Disulfide bonds were cleaved by reduction with 2-ME into proteins of smaller MW.

3.2. Moisture content (MC)

Fig. 2 shows the moisture content (MC) of triticale films. Films plasticized with glycerol or sorbitol presented higher MC at 68 than at 52% or 32% RH regardless of plasticizer
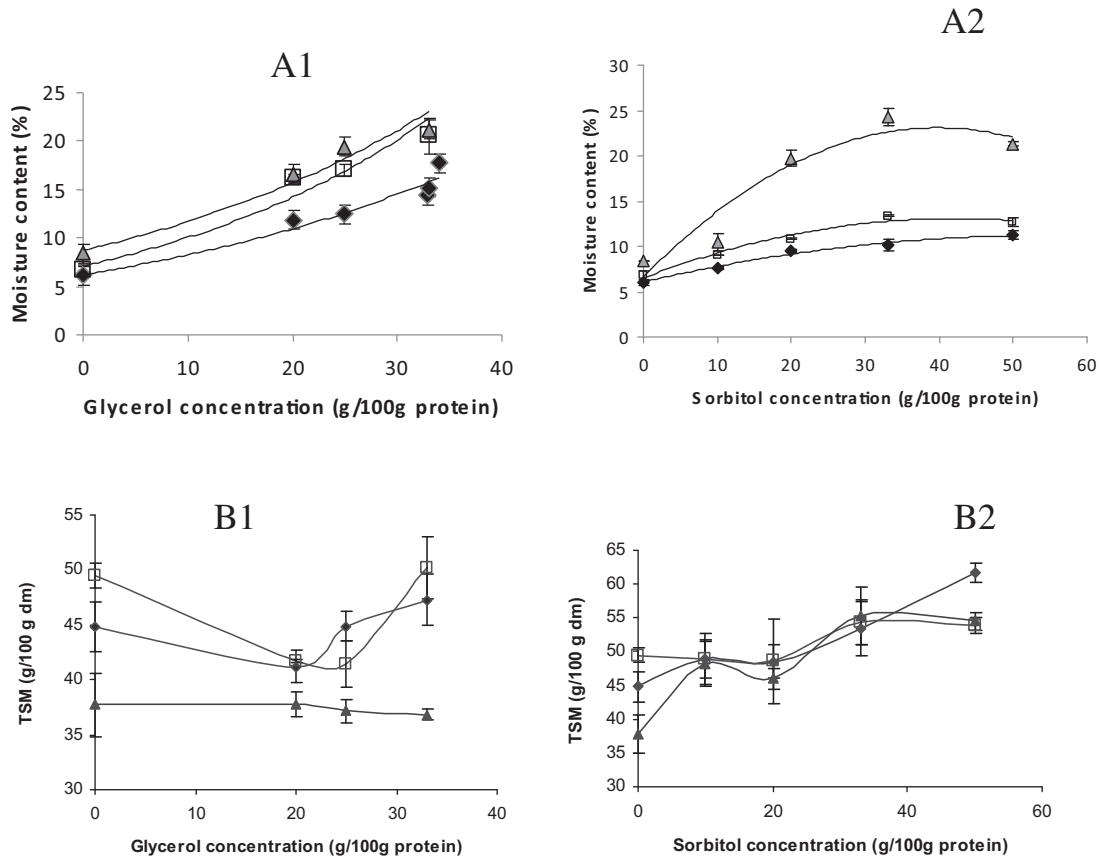
Fig. 2. Moisture content (A1, A2) and total soluble matter (TSM, B1, B2) of triticale films conditionated at 32%, 52% and 68% RH as a function of plasticizer concentration. ♦32% RH, □52% RH, ▲68% RH. Bars represent standard deviation.

Table 1
Mechanical properties of triticale protein films.

Plasticizer	Concentration (g/100 g protein)	Tensile strength (Mpa)	Elongation at break (%)	Young's modulus (Mpa)	Puncture force (N)
At 32%RH					
Glycerol[*]	20	2.90 ± 0.03^a	9.67 ± 1.25^a	147.59 ± 0.71^a	1.71 ± 0.15^a
	25	1.65 ± 0.08^b	76.65 ± 11.24^b	24.82 ± 9.06^b	1.03 ± 0.21^b
	33	0.33 ± 0.09^c	196 ± 15.23^c	3.75 ± 0.80^c	0.52 ± 0.11^c
Sorbitol[**]	20	nd	nd	nd	0.64 ± 0.13^d
	50	1.73 ± 0.50	0.54 ± 0.01	179.61 ± 9.67	0.76 ± 0.17^d
At 52%RH					
Glycerol[*]	20	1.15 ± 0.13^a	21.17 ± 3.57^a	36.01 ± 8.79^a	1.31 ± 0.29^a
	25	0.57 ± 0.05^b	134.84 ± 9.87^b	6.15 ± 1.10^b	0.40 ± 0.05^b
	33	0.11 ± 0.02^c	250.00 ± 12.44^c	0.28 ± 0.05^c	0.45 ± 0.07^b
Sorbitol[**]	10	0.95 ± 0.27^d	0.62 ± 0.06^d	159.19 ± 7.67^d	1.28 ± 0.07^d
	20	nd	nd	nd	0.86 ± 0.26^e
	33	3.34 ± 0.98^e	0.28 ± 0.04^e	349.36 ± 56.67^e	2.36 ± 0.20^f
	50	1.21 ± 0.71^d	0.85 ± 0.50^d	124.08 ± 86.30^d	0.99 ± 0.32^{de}
At 68%RH					
Glycerol[*]	20	nd	nd	nd	0.24 ± 0.13
Sorbitol[**]	20	0.85 ± 0.06^d	4.59 ± 0.33^d	30.21 ± 0.28^d	0.52 ± 0.14^d
	33	3.49 ± 1.02^e	0.6 ± 0.09^e	249.92 ± 30.10^e	0.45 ± 0.09^d
	50	0.77 ± 0.20^d	1.82 ± 0.50^f	50.05 ± 20.63^d	0.73 ± 0.26^d

Reported values are means ($n = 5$) $\pm$ standard deviation. nd: no data for some films was obtained due to its extreme brittleness or stickiness.

[*] Different letters as superscripts in a column (a,b,c) indicate significant differences ($p < 0.05$).

[**] Different letters as superscripts in a column (d,e,f) indicate significant differences ($p < 0.05$).

content. Plasticizer concentration affects the moisture content of triticale films. Films with a higher concentration of plasticizer absorbed more water at a given relative humidity value. Increased amount of water in films with high plasticizer concentration and high RH was due to the appearance of interactions between water and excess of plasticizer. Higher levels of plasticizer increased the film moisture content because of its hydrophilicity, which presented hydroxyl groups capable to interact with water by hydrogen bonds. Glycerol plasticized triticale films showed higher capacity to absorb water at all concentrations and at all RH conditions measured. The experimental values obtained can be adjusted by Eq. (1),

$$MC = Ae^{Bx} \tag{1}$$

where x is the glycerol concentration (g/100 g protein) and A and B are empiric parameters, whose values were calculated by non-linear regression: $A = 6.2291$, $B = 0.0282$, with $R^2 = 0.9663$; $A = 7.1081$, $B = 0.0347$, with $R^2 = 0.9653$ and $A = 8.7187$, $B = 0.0294$, with $R^2 = 0.9711$ for 32%, 52% and 68% RH respectively.

The influence of sorbitol concentration on MC was not as important as that of glycerol. Thus, sorbitol concentration did not affect significantly MC at 52% RH. Similar results were found by Osés et al. (2009) who studied the variation of MC in whey protein films plasticized with different quantities of sorbitol. The experimental values obtained in the present study with sorbitol can be adjusted by Eq. (1'),

$$MC = Ax^2 + Bx + C \tag{1'}$$

where x is the glycerol concentration (g/100 g protein) and A, B and C are empiric parameters, whose values were calculated by non-linear regression: $A = -0.0017$, $B = 0.1879$, $C = 60,797$ with $R^2 = 0.9872$; $A = -0.0037$, $B = 0.3104$, $C = 65,378$ with $R^2 = 0.9781$ and $A = -0.0104$, $B = 0.8258$, $C = 66,649$ with $R^2 = 0.9057$ for 32%, 52% and 68% RH respectively. Although sorbitol has more hydroxyl groups to interact with water by hydrogen bonds, glycerol has higher water affinity.

3.3. Total soluble matter (TSM) in water

Solubility of film in water is an important property of edible films. Water insolubility and water resistance are usually required

for potentially commercial films (Ying et al., 2007). However, the opposite is desirable when the intent is to design a package with premeasured dry food amounts to be dissolved in water before consumption (Perez-Gago and Krochta, 2001). After 24 h incubation in deionized water, the triticale films did not maintain their integrity (visual examination). The solubility values of triticale plasticized films and a comparison with films that did not contain plasticizer are shown in Fig. 2. The TSM were in the range of 37.71–61.66%.

The lost matter of unplasticized films could be attributed to low molecular peptides and non-protein compound (e.g. starch) remaining in the film after casting (Hernández-Muñoz et al., 2004). TSM of plasticized films were largely due to the plasticizer which have great affinity to water, hence they easily migrates to water when they come in contact with that. An increase in plasticizer content led to an increase in TSM in water.

The results of glycerol-plasticized films conditionated at 68% RH could be explained by the overlapping plasticizing effect of water. The effect of glycerol (more hydrophilic than sorbitol) was reduced in the presence of large amounts of water.

3.4. Tensile tests

The values of the mechanical properties of triticale films are shown in Table 1. As the table shows, triticale films are strongly affected by relative humidity (RH). As RH increased, TS and EM decreased, while elongation increased in all films, showing how water acts as a plasticizer in hydrophilic films. Films stored at higher RH contain higher MC than films at lower RH.

Plasticizer type and concentration produced differences in mechanical properties. At very low concentration of glycerol (<20%) the films became too brittle to handle. At a high concentration of glycerol (>33%) the sturdiness of the films is lost and they could not be mounted in the texture analyzer. When the samples were plasticized with glycerol at concentrations between 20% and 33% it was noted that an increase in the content of plasticizer led to a decrease in mechanical resistance (decrease in TS), an increase in elasticity (decrease in EM) and an increase in extensibility (increase in %E). Like most other protein films, the addition of glycerol made the film more ductile, which indicates that glycerol takes the place between protein molecules and interferes with the intra-molecular forces leading to the formation of polymer–plasticizer interactions

to the detriment of polymer–polymer interactions. Similar changes in the mechanical properties were observed in wheat gluten films (Irissin-Mangata et al., 2001) and whey protein films (Bodnár et al., 2007).

Triticale films plasticized with sorbitol were stiffer than those containing glycerol. Elongation of sorbitol films remained low at all RHs (Table 1). The results obtained by mechanical measurements indicate that there are two types of behavior in triticale films plasticized with sorbitol. Sorbitol did not have the classic effect of a plasticizer on the mechanical properties at lower concentrations, by inducing rigidification of the film through a lowering of the %E and an increase of TS and EM. This behavior shows similarities to antiplasticization, a phenomenon observed in some synthetic polymers (Gaudin et al., 2000). Conceptually, antiplasticization refers in this context strictly to the mechanical properties of polymer–diluent blend (Seow et al., 1999).

The addition of plasticizer to a polymer generally increases free volume and segmental mobility. Selective inter-polymer bonds may be weakened or broken, leading to a reduction in elastic modulus, which is the conventional macroscopic effect of a plasticizer. However, the incorporation of plasticizer in polymers can produce effects opposite to those expected (Seow et al., 1999). This has been termed "antiplasticization" and occurs below a "plasticization threshold" that must be exceeded before the conventional plasticizing effect on physical properties occurs.

The cause of antiplasticization has not been clearly elucidated and several mechanisms may be involved, but it would appear that local molecular mobilities play a preponderant role in the mechanisms involved. The free volume theory recognizes the plasticizer as the component that can impart a greater free volume per volume of material due to an increase in the proportion of end groups. The phenomenon observed in this study could be due to the disappearance of local molecular mobilities, and the appearance of the intrinsic mobility of sorbitol. Sorbitol might not be able to position itself effectively between polymer segments, and therefore might not be able to form sufficient hydrogen bonds with the protein chains. Above 33% of concentration and at equilibrium RH of 52%, sorbitol began to exert its positive plasticizing effect due to the appearance of clusters of sorbitol which allow some mechanical plasticity. These clusters were not large enough to form a new phase. But, above 50% of sorbitol TCL films showed sorbitol crystals on the surface. This occurs when films are over-plasticized, and may be attributed to at that high concentrations, sorbitol was not compatible with triticale proteins and an excess of sorbitol causing phase separation and physical exclusion of plasticizer. The phase separation between sorbitol and the triticale proteins was due to the high molecular weight of sorbitol.

In general, antiplasticization has been hypothesized to involve a combination of several factors. Even, some contradictory results illustrate the fact that different types of material might respond differently to the presence of low levels of diluents (Chang et al., 2006). The present work recognize that the types of interactions in triticale proteins films containing two different diluents would be highly complex.

Glycerol and water plasticized synergistically, resulting in films that are more flexible. The differences in the distribution of mechanical properties between a film with glycerol or sorbitol but with similar water content, indicated that the water behaves differently in the presence or glycerol or sorbitol. The plasticizing effect of glycerol and sorbitol could also be explained in terms of their MW or the number of hydroxyl groups in its chemical structure.

The higher the MW of the employed plasticizer, the lower the observed elongation of the triticale films. Similar tendency was found in alginate-calcium films (Olivas and Barbosa-Cánovas, 2008). Turhan and Sahbaz (2004) found that methylcellulose-PEG

films had a lower % of E as PEG molecular weight increased, and concluded that is due to a heterogeneous film structure arising from the decrease in solubility and miscibility of high molecular weight plasticizers.

To evaluate the effect of MW and chemical structure of glycerol and sorbitol on mechanical properties of triticale protein films, Fig. 3 compares TS and EM with the concentration of plasticizer (g 100 g^{-1} protein), the number of hydroxyl groups per mole (three for glycerol and six for sorbitol) and with the mole fraction (mol plasticizer 100 g^{-1} protein). From our results, comparing with the % of plasticizer, the most similar curves are obtained with the number of hydroxyl groups contained in the plasticizer. But, the most influential factor in plasticization is the molecular weight of plasticizer: above 0.2 mol plasticizer 100 g^{-1} protein, glycerol and sorbitol both have plasticizer effect, as shown in Fig. 3. Glycerol and sorbitol have similar straight molecular structures. However, glycerol has smaller MW, so for the same plasticizer content, the number of moles of it will be higher than sorbitol. The plasticizing effect of glycerol could be attributed to its low molecular weight leading to the formation of protein–glycerol interaction to the detriment of protein–protein interaction. Sorbitol has higher MW than glycerol and water. Its accessibility to protein matrix could be limited compared to that of smaller molecules such as glycerol and water. This indicates that size and hygroscopicity were more important than the number of hydroxyl groups when comparing both plasticizers. Siew et al. (1999) concluded that the number of hydroxil groups (in the chemical structure) influences the plasticizer effectiveness. Our results are consistent with those of Osés et al. (2009), who proved that the most influential factor was the plasticizer molecular weight and hydrophilicity rather than the number of hydroxyl groups.

3.5. Puncture test

The results of puncture force (PF) on triticale films are shown in Table 1. Glycerol addition reduced the puncture force. The increase in plasticizer concentration reduces the puncture force due to a decrease in the intermolecular interactions (Sobral et al., 2001). The increasing of glycerol content from 20% to 33% decreased the puncture force from 1.71 to 0.52 N at 32% RH and 1.31 to 0.6 N at 52% RH. On the other hand, the behavior of films with sorbitol was different. The PF of sorbitol-based films at 32% relative humidity was so low that was nearly unaffected by sorbitol concentration. At 50% sorbitol PF was nearly unaffected by relative humidity.

3.6. Water vapor permeability (WVP)

WVP is a measure of the amount of water vapor passing through unit area of film per unit time. The effect of plasticizer and RH on WVP of triticale films is shown in Table 2. These WVP values were comparable to those reported in the literature for edible films (Denavi et al., 2009; Hernández-Muñoz et al., 2004; Liu et al., 2004; Pelissari et al., 2013). As expected with a hydrophilic film, WVP increased with increasing RH. At high RH extensive swelling of the protein network caused by sorbed water enhanced water molecule diffusion and the polymer free volume, thus substantially reducing the water vapor barrier ability of triticale films. As RH increases, the capacity of films to function as barrier to water decreases.

The addition of a plasticizer modifies the properties of the film by reducing the intermolecular bonds between the polymer chains, thus increasing the WVP of the film. The protein network becomes less dense because of the increase in the mobility of polymeric chains and in the free volume of the film. Also, the increase of WVP with plasticizer content is related to the hygroscopic plasticizer molecules, that increase the water content of the films (Sobral
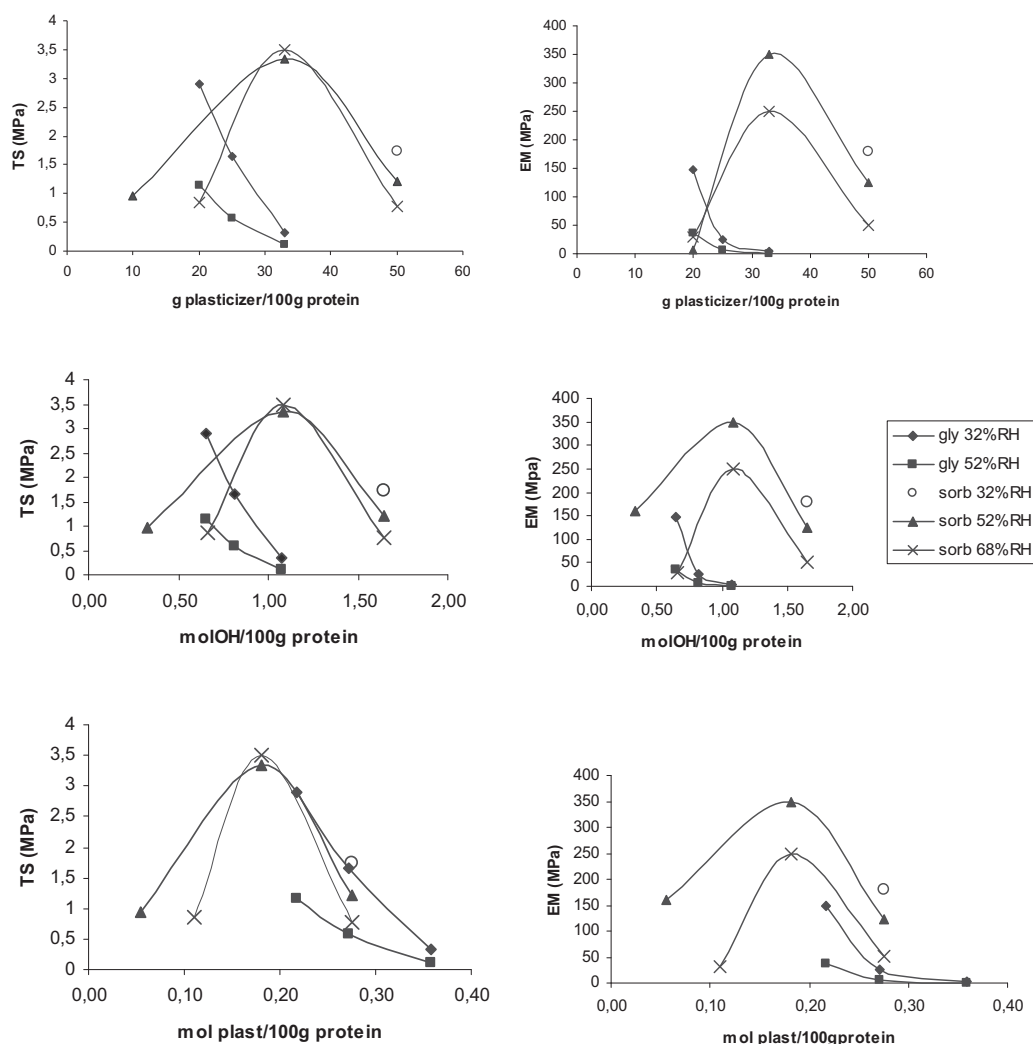
Fig. 3. Tensile strength (TS) and Young's modulus (EM) of triticale films conditionated at 32%, 52% and 68% RH as function of %, mole OH and glycerol or sorbitol mole fraction.

et al., 2005) and enhances the water holding capacity of the polymeric matrix (Hernández-Muñoz et al., 2004). This, produced an increase in the effective diffusion coefficient of water vapor in the films, due to WVTR is closely related to the mobility of the polymer chain and free volume of chain segments.

Permeability, defined as the product of the coefficients of solubility and diffusion, involves a thermodynamic factor (solubility) and a kinetic factor affected by molecular mobilities (diffusion). Permeability depends especially on the available free volume for diffusion of water vapour molecules within the polymer. The loss of local mobilities, attributable to the creation of bonds between proteins and sorbitol, decreased WVP in the film. Antiplasticization effect of sorbitol was also likely to be involved in transport mechanism, accompanied by reductions in permeability to water vapour, consistent with reduced mobility in the films. This phenomenon is comparable to that observed in antiplasticized starch–sorbitol films (Gaudin et al., 2000). Interactions between proteins matrix and water are modified by the presence of small molecules such as glycerol or sorbitol. Competition for water molecules can lead to redistribution of water in the film. At the same plasticizer concentration, films containing sorbitol showed lower WVP than those containing glycerol, probably due to sorbitol being less effective than glycerol in reducing intermolecular hydrogen bonding between molecules. As a result, spacing between macromolecules is decreased, resulting in a reduction in WVP but an increase in the

stiffness, a decrease in the strength, and a decrease in the elongation of the sorbitol films. An increase in rigidity results in a decrease in permeability. The higher WVP of glycerol-plasticized films as compared to sorbitol films also may be related to the hydration of the plasticizers since water is more closely associated with glycerol compared to sorbitol.

4. Discussion

The influence of selected platicizers (glycerol and sorbitol) on functional properties of triticale protein films was investigated. Glycerol plasticized triticale films showed higher capacity to absorb water at all concentrations and at all relative humidity conditions measured. At the same concentration, films containing sorbitol showed lower WVP than those containing glycerol. The results demonstrated that both tensile strength and Young's modulus of the films decreased as the glycerol content increased (plasticizing effect). The unexpected increase in the tensile strength and Young's modulus of the films containing low levels of sorbitol was ascribed to the antiplasticization of triticale films. The present study recognizes that the types of interactions in the triticale films containing two different diluents would be highly complex. Such contrasting behavior may be due to differences in molecular characteristics of glycerol and sorbitol and their possible dissimilar interactions with triticale protein. Our results indicate that size and hygroscopicity

Table 2
Water vapor permeability (WVP) values of triticale films at different relative humidity (RH) gradients.

Plasticizer	% Plasticizer	RH gradient (%)	WVP $\times 10^{10}$ (g m^{-1} s^{-1} Pa^{-1})
Glycerol[*]	20	0–32	0.33 ± 0.05[a]
	25	0–32	0.62 ± 0.04[b]
	33	0–32	1.61 ± 0.18[c]
	20	0–52	0.31 ± 0.02[a]
	25	0–52	0.59 ± 0.06[b]
	33	0–52	2.48 ± 0.17[d]
	20	0–68	0.30 ± 0.13[a]
	25	0–68	0.56 ± 0.04[b]
	33	0–68	1.57 ± 0.29[c]
Sorbitol[**]	20	0–32	0.37 ± 0.03[e]
	33	0–32	0.53 ± 0.09[f]
	50	0–32	1.06 ± 0.10[g]
	20	0–52	0.36 ± 0.11[e]
	33	0–52	1.02 ± 0.20[g]
	50	0–52	1.23 ± 0.29[g]
	20	0–68	0.35 ± 0.05[e]
	33	0–68	0.61 ± 0.08[f]
	50	0–68	1.28 ± 0.19[g]

Reported values are means ($n = 3$) $\pm$ standard deviation.

[*] Different letters as superscripts in a column (a,b,c,d) indicate significant differences ($p < 0.05$).

[**] Different letters as superscripts in a column (e,f,g) indicate significant differences ($p < 0.05$).

of molecules were more important than the number of hydroxyl groups when comparing both plasticizers. Mechanical antiplasticization by sorbitol has significant effects on physical properties of triticale films. The antiplasticization might provide an alternative for modifying the properties of triticale films.

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