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Effect of different combinations of glycerol and/or trehalose on physical and structural properties of whey protein concentrate-based edible films

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ABSTRACT

The aim of the present study was to develop and characterize edible films produced from whey protein concentrate (WPC) and plasticized with different contents of glycerol (Gly) and/or trehalose (Tre) in order to evaluate new edible film formulations for their potential use in food packaging applications. Additionally, potential changes in the film mechanical properties during storage at ambient and freezing conditions were considered. Moisture content, solubility, thickness, transparency, microstructure, colour parameters, and mechanical properties were assessed. The films incorporated with Tre were more insoluble in water than WPC/Gly films, being more suitable for food applications. WPC/Gly and WPC/Tre films were clear enough to be used as see-through packaging. However, when Tre was included into WPC/Gly film formulations, film opacity increased. Scanning electron microscope (SEM) images suggested that this phenomenon may be related to the growth of Tre crystals in the film matrix. Moreover, when Tre concentration increased in the WPC/Gly matrix, film surface was more heterogeneous. Interestingly, the presence of Tre in WPC-based films was effective in preventing Maillard reaction after heating. WPC/Tre films were the most rigid but the least stable for storage, resulting more susceptible to rupture and cracking. Only WPC/Gly and WPC/Gly-Tre 8% films were rather flexible, manageable, and stable up to 90 days of storage under ambient and freezing conditions. These findings can be used to better design applications of edible films containing plasticizers that may crystallize over time in order to optimize film formulation in a rational manner towards their eventual application as food packaging.

1. Introduction

Edible films have emerged as an alternative to synthetic plastics for food applications, receiving considerable attention from food researchers in the last decades. The main advantage of such films over traditional synthetics is that they can be consumed with the products (Bourtoom, 2008). Edible films based on natural polymers and food grade additives have been constantly developing (Tang, Kumar, Alavi, & Sandeep, 2012). The functionality and performance of edible films mainly depend on their barrier, mechanical and colour properties, which in turn depend on film composition and film development (Fadini et al., 2013).

The use of whey proteins for film formulation has been extensively reviewed, since these proteins are both edible and biodegradable, favour the reuse of cheese making effluents, and also have

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interesting mechanical and barrier properties (Javanmard, 2009; Ramos et al., 2013). Whey protein-based films mostly include food materials, which can change over time causing loss of protective functions and affecting the appearance of the covered food (Dangaran & Krochta, 2007). Therefore, the effect of storage on some film properties has been rather unexplored in most research papers.

In general, protein-based film formulations require the addition of a plasticizing agent above a minimum threshold to reduce film fragility and to confer certain plastic properties (Hernandez-Izquierdo & Krochta, 2008). The plasticizer molecules lead to decreasing intermolecular forces along the polymer chains, thus improving flexibility, extensibility, and toughness. However, plasticizers also decrease mechanical resistance and barrier properties of the films (Karbowiak et al., 2006). The most commonly used plasticizers are polyols, mono-, di- or oligosaccharides. Differences in composition, size, structure and shape of plasticizers directly influence their function in the film network (Orliac, Rouilly, Silvestre, & Rigal, 2003). Hydrophilic plasticizers, such as glycerol (Gly), polyethylene glycol, and sorbitol, are generally used for protein-based films; Gly being the one which produces the best plasticizing effects in whey protein films (Osés, Fernández-Pan, Mendoza, & Mate, 2009).

Several authors have studied the physicochemical characteristics of edible films made from whey proteins and plasticized with sucrose, showing that these films are flexible, tough, and highly glossy as well as having excellent oxygen barriers properties (Dangaran & Krochta, 2003; Sothornvit & Krochta, 2000). Although all these characteristics are desirable for wrapping applications, some deleterious changes have been observed in whey protein/sucrose films over time, especially those related to the growth of sucrose crystals resulting in gloss loss after storage (Dangaran & Krochta, 2007; Dangaran, Renner-Nantz, & Krochta, 2006). Additionally, the use of sucrose has some drawbacks such as a relatively low glass transition temperature, chemical instability under low pH conditions, and the ability to react with amino acids or proteins by Maillard browning reaction (Galmarini, Schebor, Zamora, & Chirife, 2009).

Trehalose (Tre) is a naturally occurring, non-reducing, bland, non-toxic, dietary disaccharide, with almost half the sweetness of sucrose and similar sweetness dynamic profiles. It is commonly found in nature including honey, mushrooms, and bakery yeasts, some of which are known to contain almost 20% of Tre on a dry solid basis (Elbein, Pan, Pastuszak, & Carroll, 2003). The use of Tre as a food ingredient has been approved as a GRAS additive (*i.e.*, generally recognized as safe) by the FDA. Its mild sweetness (45% of sucrose sweetness), moderate glycaemic index with low insulinemic response, low cariogenicity, low hygroscopicity, and protein protection properties are all of immense benefit to food technologists (O'Donnell & Kearsley, 2012). Tre may be a potential substitute for sucrose in food formulations because it is more stable at low pH and high temperatures, and it is not involved in caramelization and Maillard reaction. Moreover, glass transition temperature of Tre is higher than that of sucrose, thus Tre improves physical stability of dried products (Galmarini, Baeza, Sanchez, Zamora, & Chirife, 2011; Galmarini et al., 2009). Further, Tre can protect proteins from inactivation or denaturation caused by a variety of stress conditions, including desiccation, dehydration, heat, cold, and oxidation. In addition, Tre has also been suggested as a potential ingredient to protect foods against damage caused by dehydration during frozen storage or destructive chemical reactions (Jain & Roy, 2009; O'Donnell & Kearsley, 2012). Therefore, the aim of the present study was to develop and characterize edible films produced from whey protein concentrate (WPC) and plasticized with different contents of Gly and Tre in order to evaluate new edible film formulations for its potential use in food packaging applications. Additionally, potential changes in the film mechanical properties during storage at ambient and freezing conditions were considered.

2. Materials and methods

2.1. Materials

All reagents were of high-purity grade and used as received. Whey protein concentrate (WPC, 80%) was purchased from Arla Food Ingredients S.A. (Buenos Aires, Argentina). Glycerol (Gly, 87% purity) was obtained from Cicarelli (Buenos Aires, Argentina) and commercial food grade crystalline trehalose dihydrate (Tre) was provided by Hayashibara Co. Ltd. (TREHA™, Okayama, Japan).

2.2. Preparation of film-forming solutions

For each formulation, an 8% (w/w) solution of WPC was prepared by slowly dissolving the powder in deionized water. Gly and/or Tre were added at different proportions as plasticizers (Table 1). Film-forming solutions were heated at 90 °C for 30 min in a water bath to achieve whey protein denaturation, and were subsequently homogenized for 5 min at 20,000 rpm using an Omni GLH mixer (Omni International Inc., Kennesaw, GA, USA). After that, solutions were cooled to room temperature in an ice bath to prevent further protein denaturation. Finally, the film-forming solutions were degassed for 60 min at room temperature using a Cole–Parmer 8890E-MT sonicator (Cole–Parmer, Chicago, IL, USA).

2.3. Film formation

Teflon-coated plates of 18 cm diameter were employed as support to prepare the films. In all cases, 20 g of the degassed film-forming solutions (pH 6.4–6.5) were pipetted into the plates, which were placed on a levelled surface inside an environmental chamber (SCT Pharma, Buenos Aires, Argentina) setting at 25 °C and a constant relative humidity (RH) of 58%. Later films were manually removed from the Teflon-coated plates and were conditioned at 25 °C and 58% RH for 24 h prior to testing to ensure all films started at the same equilibrated state. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles and holes. Film failure was designated as loss of film cohesion resulting in cracking.

2.4. Film characterization

2.4.1. Film thickness

The amount of each film-forming solution dispensed was always the same (20 g). For each film sample nine thickness measurements

Table 1
Film-forming compositions.

Sample number	Composition (w/w %)			Sample name
	WPC	Glycerol	Trehalose	
1	8.0	8.0	0	*
2	8.0	8.0	4.0	*
3	8.0	8.0	8.0	*
4	8.0	4.0	0	WPC/Gly
5	8.0	4.0	4.0	WPC/Gly-Tre 4%
6	8.0	4.0	8.0	WPC/Gly-Tre 8%
7	8.0	0	0	*
8	8.0	0	4.0	*
9	8.0	0	8.0	WPC/Tre

WPC, whey protein concentrate; Gly, glycerol; Tre, trehalose.
*Film failure.

were taken with a manual digital micrometre (Schwyz®, Zhejiang, China). Determinations were performed in triplicate. Averaged values of thickness measurements were obtained and these values were used in all calculations.

2.4.2. Moisture content and solubility in water

The moisture content (MC) of the films was determined after drying in an oven at 105 °C for 24 h. Pieces of films (15 × 7.5 mm) were cut after adequate conditioning and placed on test tubes previously weighed before and after oven drying. MC values were determined as a fraction of the initial film weight lost during drying and reported on a wet basis, according to the ASTM D-644 (1994). Determinations were performed in quintuplicate.

Film solubility in water was determined according to Soazo, Pérez, Rubiolo, and Verdini (2013). Briefly, small pieces of films (15 × 7.5 mm) were dried in an oven (Dalvo Instruments, Santa Fe, Argentina) at 70 °C until constant weight to obtain the initial film dry weight. The piece of film was then placed into a test tube with 10 mL of distilled water. Then, the tubes were shaken slowly on a shaking platform (Viking, Buenos Aires, Argentina) for 24 h at 25 °C. After immersion, the remaining solids were dried in the oven at 70 °C until constant weight to determine the weight of dry matter not dissolved in water. Solubility, expressed as soluble solids (%), was obtained by subtracting the weight of the remaining dry matter (not dissolved) from the weight of the initial dry matter and reported on initial dry weight basis (Sothornvit & Krochta, 2000). Determinations were performed in quintuplicate.

2.4.3. Transparency

Film transparency was determined according to ASTM D-1746 (1997) with modifications of the method described by Ramos et al. (2013). In order to determine the barrier properties to visible light, transparency of dried films was measured at 600 nm using a spectrophotometer (Model V-530, Jasco International, Tokyo, Japan). Rectangular pieces of films (10 × 30 mm) were placed on the internal side of a spectrophotometer cell and the empty cell was used as a control. Five replicates of each film formulation were tested. Transparency (% Transparency) was calculated as the percentual relationship between the light intensity with the specimen in the beam and the light intensity control.

2.4.4. Scanning electron microscopy

A scanning electron microscope (SEM, AMR 1000, Leitz, Wetzlar, Germany) was used to study representative surface structure of WPC-based films and to assess their homogeneity. Film samples were cryo-fractured by immersion in liquid nitrogen and then mounted on bronze stubs perpendicularly to their surface. The film portions were coated with a fine gold layer for 15 min at 70–80 mTorr before obtaining the SEM micrographs. All samples were examined using an accelerating voltage of 20 kV and magnifications of 500x and 1000x.

2.4.5. Colour analysis employing digital images

2.4.5.1. Image acquisition. Digital images of films were taken following the procedure described in Soazo, Pérez, Rubiolo, and Verdini (2015). A Nikon P 7100 camera (Nikon, Jakarta, Indonesia) was employed to photograph the samples. The camera setting was defined as follows: manual mode with lens aperture at f = 8.0 and time of exposition 1/200, no flash, ISO sensibility of 400, maximum resolution, and storage in RAW format. Determinations were performed in triplicate.

2.4.5.2. Image processing. An International Colour Consortium (ICC) profile was applied to all images using Photoshop® (Adobe Systems Inc., San Jose, CA, USA). In order to obtain the ICC profile an IT8 calibration card (Wolf Faust, Frankfurt, Germany) was photographed under the same conditions. L*, a*, and b* were calculated according to Yam and Papadakis (2004), where L, a, and b average values (considering the whole sample) were obtained from the histogram window. Finally, total colour difference (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{(L^* - L')^2 + (a^* - a')^2 + (b^* - b')^2}$$

where L*, a* and b* are the colour parameter values of the sample and L', a' and b' are the colour parameter values of the standard white plate.

2.4.5.3. Heat treatment. To evaluate changes in colour parameters (L*, a* and b*) during film ageing, accelerated studies were performed for 72 h at 70 °C. Photographs were obtained and processed as described above. Determinations were performed in triplicate.

2.4.6. FTIR-ATR analysis

The spectra of the films were obtained using a Fourier Transform Infrared (FTIR) spectrophotometer Shimadzu IR Prestige-21 (Tokyo, Japan) with Attenuated Total Reflectance (ATR) attachment. Measurements were recorded in the scanning range of 650–4000 cm^{-1}. A total of 20 scans were performed at 4 cm^{-1} resolution and three replicates were collected for each film sample.

2.4.7. Mechanical properties

Tensile properties of films were evaluated using a motorized test frame Multitest 2.5d (Mecmesin, Sterling, VA, USA) equipped with a 100 N digital force gauge. Samples were prepared by cutting films into strips (60 × 7 mm) using a scalpel. The strip ends were fixed with double sided tape and squares of 30 mm of cardstock to prevent tearing and slippage in the testing device (Soazo et al., 2013). The exposed film strip length, as well as the initial grip distance, was 30 mm and the crosshead speed was set at 0.05 mm/s. For each mechanical test, samples were conditioned for 1 day at 25 °C and 58% RH, and ten replications were performed. All parameters were then obtained from the stress–strain curves. Tensile strength (TS) was calculated by dividing the maximum force by the cross sectional area (thickness of film × 7 mm) of the initial film, elongation at break (EAB) was calculated as the percentage of the film elongation at the point of rupture respect to the initial gauge length (30 mm) of the specimen, and Young's modulus (YM) was calculated from the initial slope of the stress–strain curve (Ozdemir & Floros, 2008).

2.4.7.1. Effect of storage temperature on mechanical properties of films. WPC-based films were stored in an environmental chamber at 25 °C and 58% RH or in a domestic freezer at −20 °C for 90 days. Films were observed periodically during the storage time or until film failure occurred. Film failure was designated as loss of film cohesion resulting in cracking. After the 90 days of storage, films were conditioned at 25 °C and 58% RH for 24 h prior to testing. Thickness, tensile strength (TS), Young's modulus (YM), elongation at break (EAB), and transparency ($\%T_{600nm}$) were performed as described above. Determinations were performed in quintuplicate.

2.5. Statistical analysis

A full factorial design was performed. Two factors (Gly and Tre) in three levels were studied (0%, 4% and 8% w/w). Statistical analyses were performed using the SigmaStat 3.5 software (Systat Software Inc., San Jose, CA, USA), via analysis of variance (ANOVA).

The difference of means between groups was resolved via confidence intervals using Tukey's test. The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Visual aspects and optical properties

Flexibility and strength of WPC-based edible films plasticized with different contents of Gly and Tre were observed during peeling off from the Teflon-coated plates. Observations are shown in Table 1, film failure was observed in samples 1, 2, 3, 7, and 8. Plasticizers are the main additives used in whey protein-based films because these molecules combine with the protein matrix, moving the component chains apart, and thus reducing the rigidity of the structure (Ramos et al., 2013). Without a sufficient threshold of plasticizer added to the formulation, most protein-based films are brittle (Hernandez-Izquierdo & Krochta, 2008). This may be the case for samples 7 and 8 (Table 1) where Tre content resulted insufficient to plasticize the films, resulting in their cracking. However, an excessive amount of plasticizers could reduce surface tension and the degree of protein interactions, decreasing the rate of film formation (Laohakunjit & Noomhorm, 2004; Sothornvit & Krochta, 2005). This could be the case for samples 1, 2, and 3 (Table 1) where Gly content was too high and films failed. As we can conclude from our experiences, Tre may be used alone as a plasticizer for WPC-based film in a relation WPC/Tre 1:1 (sample 9). When this sugar was incorporated into the formulation in a higher proportion (e.g., WPC/Tre 1:2), films could not be obtained (*data not shown*). Further, Tre proved to be effective to be combined with Gly to produce flexible films (samples 5 and 6, Table 1).

WPC films plasticized with both Gly and Tre (*i.e.*, WPC/Gly-Tre films) were initially bright and transparent but after a 24-h conditioning, they showed small white areas of sugar crystallization, which increased film opacity (Table 2). On the other hand, WPC/Gly and WPC/Tre films were clear enough to be used as see-through packaging (%T_{600nm}–57%), and transparency was maintained up to 90 days of storage at 25 °C and 58% RH (Piccirilli, Delorenzi, Verdini, & Pérez, 2014). Furthermore, negligible transmission of light in the range between 200 and 400 nm for all films was observed (*data not shown*). These results are in accordance with previous studies demonstrating excellent UV barrier properties for films made from whey products due to the high content of aromatic amino acids in the protein-based structure (Gounga, Xu, & Wang, 2007; Ramos et al., 2013).

The microstructure of WPC/Gly and WPC/Tre films (Fig. 1A and D) shows a smoother surface. When Tre was included into WPC/Gly-based formulations at 4% or 8% (w/w) film opacity increased due to the presence of large areas of crystallized sugar, which are clearly evident on the film surface (Fig 1B, C and E). This effect was more evident when Tre concentration was increased. Cross section of WPC/Gly-Tre 4% and WPC/Gly-Tre 8% edible films showed more structural heterogeneity and uneven side surfaces, whereas WPC/Gly and WPC/Tre films were smoother (Fig. 1). A different internal arrangement was observed when Tre was incorporated into the WPC/Gly network, which seems to be less organized when sugar concentration was increased. This could be due to the lack of miscibility of the components. As can be observed in Fig. 1C, WPC/Gly-Tre 8% showed the most granular structure; this heterogeneity probably constitutes different plasticization zones distributed within the film matrix.

The average thickness of the films was 0.132 ± 0.036 mm (Table 2), which is similar to those reported in previous works performed by our group using similar formulations (Pérez, Soazo, Balagué, Rubiolo, & Verdini, 2014; Soazo et al., 2013).

3.2. Moisture content and solubility in water

Moisture content (MC) and solubility of WPC-based edible films plasticized with different contents of Gly and Tre are presented in Table 2. WPC/Gly films exhibited significantly higher values of soluble solids (SS) as well as significantly higher values of MC than films incorporating Tre. The highest MC value observed for WPC/Gly films may be attributed to the hygroscopic nature of Gly, which attracts and holds water molecules, thus favouring the wetting of the film surface and consequent moisture absorption (Galus, Turska, & Lenart, 2012; Kokoszka, Debeaufort, Lenart, & Voilley, 2010). On the other hand, an increase in the sugar content from 4% to 8% (w/w) in WPC/Gly-Tre films produced a significant decrease in MC. This fact may be related to the low hygroscopicity of the dihydrate crystal of Tre that does not take up water from the atmosphere even at 90% RH (O'Donnell & Kearsley, 2012). According to this, WPC/Tre films showed the lowest MC values. Therefore, an appropriate selection of the plasticizer type and concentration will be helpful in controlling the MC and, as a consequence, the moisture adsorption rate of a film, thereby improving the film stability under varying RH conditions during storage (Mali, Sakanaka, Yamashita, & Grossmann, 2005; Osés et al., 2009).

In the case of solubility, our results showed that all WPC-based films did not completely lose their integrity after a 24-h immersion in water (Table 2). The partial insolubility of whey protein films may be attributed to strong intermolecular bonds (e.g. disulfide bonds) between protein molecules in the polymeric network (Ramos et al., 2013). Interestingly, the presence of Tre in WPC-based film formulations produced a significant decrease in SS due to Tre low hygroscopicity and the formation of stronger protein-sugar bonds. Solubility of whey-based edible films is a very important parameter as high solubility films have proved to have scarce applications. Therefore, achieving a reduction in edible film solubility is highly desirable for food applications.

3.3. Colour analysis

Colour is an attribute of fundamental importance for biopolymer films and is greatly affected by several factors including

Table 2
Values (average ± standard deviation) of thickness, moisture content (MC), soluble solids (SS), and transparency of WPC-based edible films plasticized with different contents of glycerol (Gly) and trehalose (Tre).

Sample	Thickness (mm)	MC (%)	SS (%)	Transparency (%)
WPC/Gly	0.126 ± 0.037^{a}	27.9 ± 2.8^{c}	41.1 ± 2.8^{d}	57.2 ± 0.8^{c}
WPC/Gly-Tre 4%	0.129 ± 0.034^{a}	20.2 ± 1.7^{b}	39.5 ± 1.7^{b}	50.5 ± 2.4^{b}
WPC/Gly-Tre 8%	0.138 ± 0.037^{a}	15.9 ± 1.4^{a}	23.5 ± 2.2^{a}	25.4 ± 0.6^{a}
WPC/Tre	0.133 ± 0.036^{a}	13.6 ± 1.5^{a}	30.4 ± 1.5^{c}	57.3 ± 2.5^{c}

Values with different letters in each column are significantly different ($p < 0.05$).
MC, moisture content; SS, soluble solids.

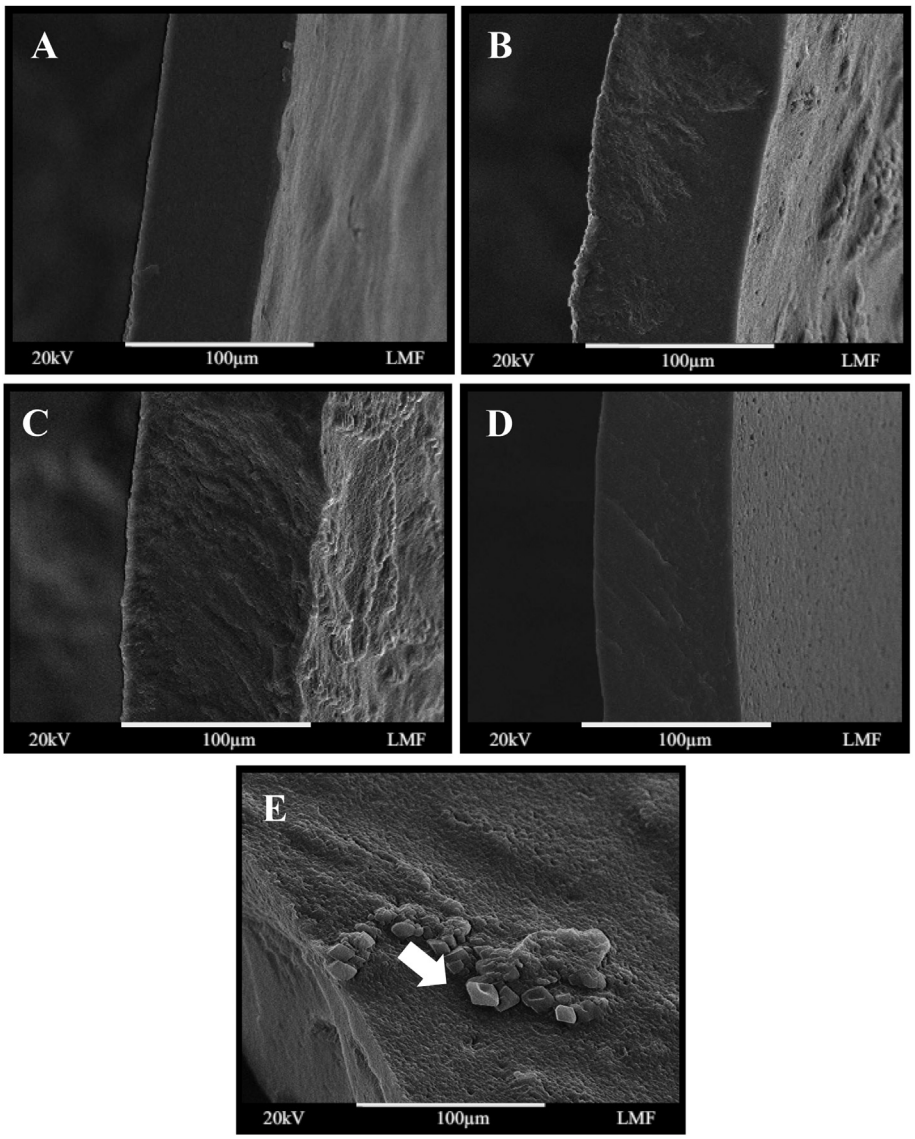

Fig. 1. Scanning electron micrographs (SEM) of WPC-based edible films plasticized with different contents of glycerol (Gly) and trehalose (Tre). WPC/Gly film (without Tre) is shown in **A)**; WPC/Gly films incorporated with Tre 4% or 8% are shown in **B)** and **C)**, respectively; WPC/Tre film is shown in **D)**. Comparison between micrographs **(A)** and **(D)** illustrates the greater homogeneity in the microstructure of WPC-based edible films plasticized with Gly or Tre alone. Note that WPC/Gly films containing Tre (B and C) shows rough surfaces. **E)** The white arrow shows the rhombohedral crystals of trehalose evident at WPC/Gly-Tre film surfaces.

plasticizer addition, thermal treatment, fabrication process, and storage conditions. Normally, for protein-based films, colour is most affected by protein concentration than by film treatments (Su, Yuan, Huang, Wang, Lu, et al., 2012). The total colour difference (ΔE) provides a better analysis of film colour attributes because this value includes the three colour parameters: lightness (L), red-green hue (a), and yellow-blue shade (b). As can be seen in Fig. 2, no statistically significant differences were observed for initial ΔE values between the WPC-based edible films plasticized with different contents of Gly and Tre. Moreover, all samples showed the characteristic yellowish colour of WPC-based films (Pérez et al., 2014; Ramos et al., 2013) which may be attributed to the presence of contaminants such as fat and phospholipids (Lorenzen & Schrader, 2006). For practical uses, this distinctive undesirable characteristic of WPC edible films may be overcome with the addition of colouring agents.

When films underwent ageing studies (70 °C for 72 h) ΔE values of WPC/Gly films increased significantly due to the Maillard reaction. It has been reported that the Maillard reaction that take place during heat treatment may involve the residual lactose in WPC and the −SH group in the cysteine residues and the ε-amino group in the lysine residues of the whey proteins (Gerrard, 2006; Loveday, Hindmarsh, Creamer, & Singh, 2010). Furthermore, non-enzymatic browning reactions between Gly and amino acids have been observed at 65 °C (Smarrito-Menozzi, Matthey-Doret, Devaud-Goumoens, & Viton, 2013). However, the presence of the non-reducing sugar in WPC/Gly-Tre films prevented the browning reaction and, therefore, an increase in ΔE value. In fact, WPC/Tre films did not exhibit any change in colour when heated (Fig. 2). These results strongly suggest that Tre may be interacting directly with the WPC network by hydrogen bonding between its hydroxyl groups and the polar residues of the proteins, interfering with the nucleophilic amino group of the amino acids, and thus preventing browning reaction to occur. These results may be promising since Maillard reaction occurrence may generate not only the production of potentially toxic by-products but also the loss of proteins and

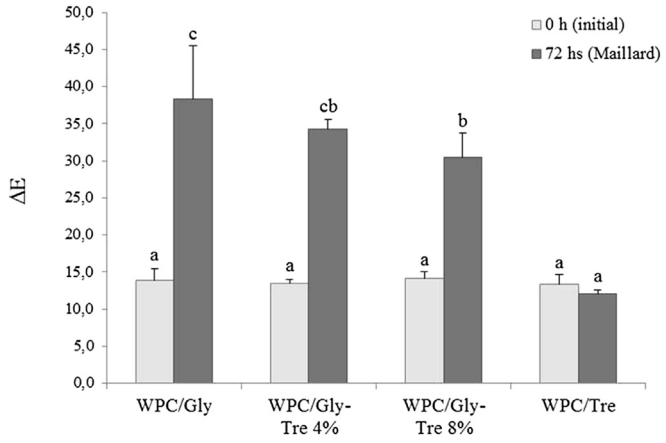

Fig. 2. Values (average ± standard deviation, $n = 3$) of ΔE for WPC-based edible films plasticized with different contents of glycerol (Gly) and trehalose (Tre). Different letters means significant differences ($p < 0.05$).

sugars with the concomitant reduction in the total nutritional value of the edible film (Hongsprabhas, Kerdchouay, & Sakulsom, 2011). Additional negative influences of Maillard reaction products on human health are the formation of mutagenic and carcinogenic compounds if consumed in the diet (Gerrard, 2006). Therefore, the complexity of the interactions among edible film constituents during food processing and storage may lead to difficulties in preserving the biological activities of whey proteins.

3.4. FTIR-ATR analysis

The FTIR spectra of WPC-based films plasticized with different contents of Gly and Tre are shown in Fig. 3. The absorption bands of Gly were located in the spectral range between 800 and 1150 cm^{-1} (Guerrero, Retegi, Gabilondo, & de la Caba, 2010); the absorption peaks corresponding to stretching vibrations of amide bonds associated with the protein network covers the range between 1200 and 1700 cm^{-1} (Karnnet, Potiyaraj, & Pimpan, 2005; Lodha & Netravali, 2005; Pereira, Souza, Cerqueira, Teixeira, & Vicente, 2010; Schmidt, Giacomelli, & Soldi, 2005); and the spectral range between 3000 and 3600 cm^{-1} corresponds to the free and bound O–H and N–H groups (le Tien et al., 2000).

It has been reported that Tre exhibits different polymorphism depending on given thermodynamic conditions (Sussich, Urbani, Princivalle, & Cesaro, 1998; Taylor & York, 1998). Akao, Okubo, Asakawa, Inoue, and Sakurai (2001) studied this behaviour from the observation of the symmetric and anti-symmetric stretch vibrations of the glycosidic linkage (α-(1 ↔ 1)-glycosidic bond) located between 914 cm^{-1} and 998 cm^{-1}. The positions of the bands in that region of the FTIR spectra sensitively reflect the difference in the conformation (or symmetry) around the glycosidic linkage.

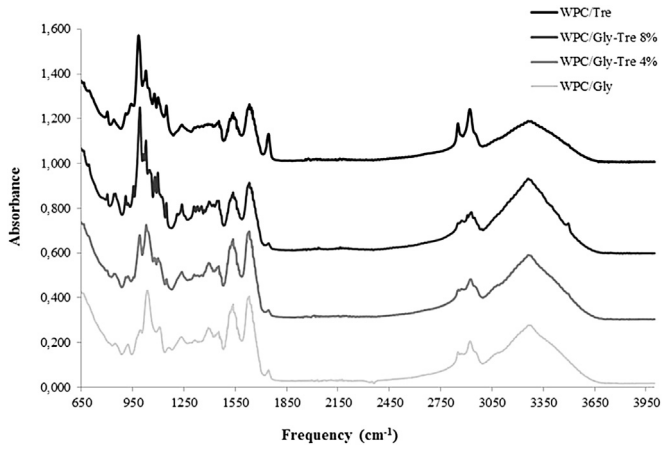

Fig. 3. FTIR absorbance spectra of WPC-based edible films plasticized with different contents of glycerol (Gly) and trehalose (Tre).

As can be seen in Fig. 3, the two peaks located at 956 cm^{-1} and 992 cm^{-1} observed for WPC/Gly-Tre 4% and WPC/Gly-Tre 8% edible films correspond well with the dihydrated form of Tre (Akao et al., 2001; Belton & Gil, 1994; Gil, Belton, & Felix, 1996). The small peak located at 3500 cm^{-1} is assigned to the stretch vibration of the two crystal water molecules corresponding to the dihydrated form of the sugar (Akao et al., 2001). On the other hand, the bending peak of the crystal water in Tre dihydrate appears at 1680 cm^{-1} (Akao, Okubo, Ikeda, Inoue, & Sakurai, 1998). This peak matches with other bands of the WPC/Gly matrix, and thus, it is not possible to be observed.

The FTIR spectrum obtained from WPC/Tre films showed two peaks located at lower frequency (942 cm^{-1} and 985 cm^{-1}, respectively) in contrast to that observed for WPC/Gly-Tre samples (Fig. 3). These slight shifts may be associated with a different conformation of the Tre molecule in WPC/Tre films compared with the dihydrated form present in WPC/Gly-Tre. In addition, the positions of the peaks corresponding to the stretch vibrations of the glycosidic bond in the WPC/Tre samples are in accordance with the anhydrous form of the sugar, as reported by Akao et al. (2001). In this state, water molecules cannot easily enter into the anhydrate crystal due to dense packing of the sugar. This observation may be related to the lowest humidity encountered for WPC/Tre edible films (Table 2).

3.5. Mechanical properties of films

Fig. 4 shows the tensile strength (TS), percentage of elongation at break (EAB), and Young's modulus (YM), of WPC-based films plasticized with different contents of Gly and Tre at initial storage time and after 90 days of preservation at ambient or freezing temperature. The measurement of the mechanical properties of edible films during storage is important because these parameters are related to film durability as well as to the ability to enhance the mechanical integrity of foods. At time zero, WPC/Tre films showed higher values of TS and YM than those incorporating Gly into the formulation. Hence, films only made from WPC and Tre are the strongest. When Tre was combined with Gly into WPC-based films (*i.e.*, WPC/Gly-Tre 4% and WPC/Gly-Tre 8% films) TS and EAB decreased, resulting in weaker and less elastic films. On the other hand, when Gly was incorporated into WPC/Tre films (WPC/Gly-Tre 8% vs. WPC/Tre at Fig. 4), TS and YM decreased leading to weaker films. In turn, no change in EAB was attained for those films (Fig. 4). According to these results, Gly and Tre seem to behave dissimilar by acting when alone or together as plasticizer agents in WPC-based edible films. This may be associated with certain incompatibility in the polymer network causing physical exclusion, and could be associated with the presence of sugar crystals on the surface of WPC/Gly-Tre films (mentioned before as well). The explanation for this phenomenon could be rationalized in the sense that Gly may be acting more efficiently than Tre as a plasticizer when both are present in the edible film formulation (Osés et al., 2009). Gly could intercalate more effectively between the proteinaceous structures of films due to its smaller size, thus interfering with the binding of Tre to proteins groups. Since amorphous Tre is in a non-equilibrium state, sugar crystals tend to grow and become more evident on the film surface over time (Hartel, 2001). During storage at ambient or

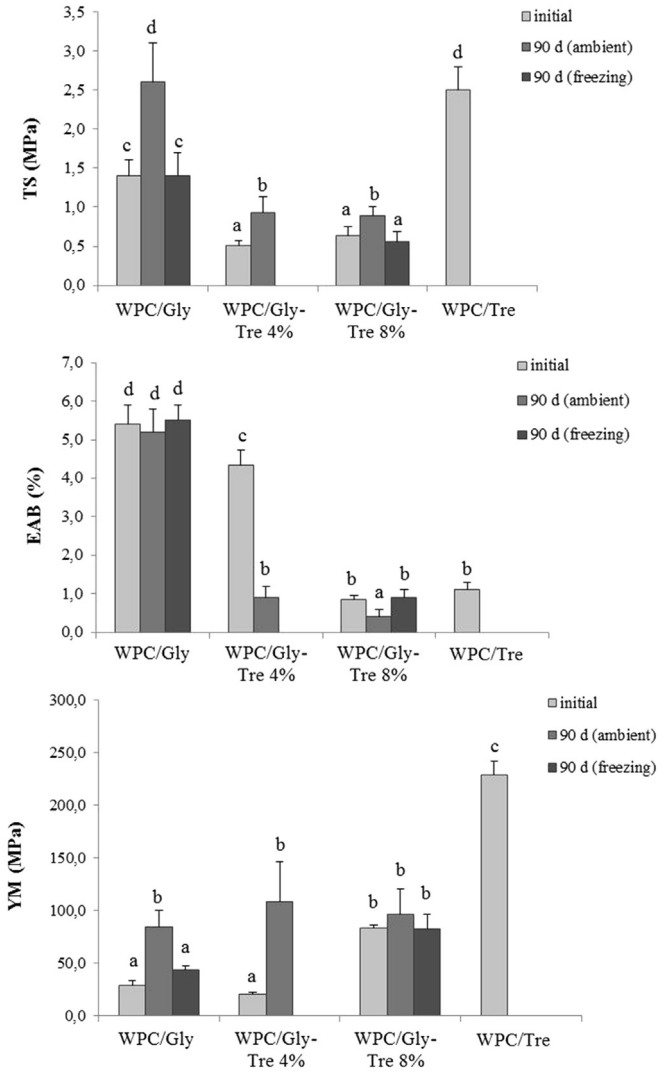

Fig. 4. Changes in mechanical properties of WPC-based edible films plasticized with different contents of glycerol (Gly) and trehalose (Tre) at initial storage time and after 90 days preservation at ambient or freezing temperature. Different letters means significant differences ($p < 0.05$).

freezing conditions WPC/Tre films (*i.e.*, without Gly) became brittle and failed (cracked) before the end of the 90-day period. The amorphous regions in the WPC/Tre films allowed for the initial flexibility of the films. However, conversion of Tre from amorphous to crystalline state over time produced brittle films that weakened and cracked. Similar results were observed by Dangaran and Krochta (2007) in whey protein films plasticized with sucrose. These authors also proved that the addition of sucrose crystallization inhibitors was effective to prevent the loss of the beneficial film properties. Only the films formulated with the addition of Gly were able to resist storage at ambient conditions, even though mechanical properties tended to decline. In general, mechanical properties of WPC/Gly and WPC/Gly-Tre 8% films remained unchanged after frozen storage, whereas WPC/Gly-Tre 4% films did not overcome this process.

4. Conclusions

WPC/Tre and WPC/Gly films obtained in the present work were clear enough to be used as see-through packaging. The addition of Tre into film formulation proved to be effective in preventing the non-enzymatic Maillard reaction that occurs upon heating and produces browning of WPC-based films. This result is promising since the Maillard reaction might generate potentially toxic by-products, and could cause the loss of the nutritive value of the whey constituents of the edible film.

WPC/Gly and WPC/Gly-Tre 8% films proved to be resistant to storage at ambient and freezing conditions (freezing, frozen storage, and thawing) maintaining their physical integrity and mechanical properties. However, the use of Tre did not significantly improve films physicochemical properties during long-term storage neither at 25 °C nor at −20 °C. In conclusion, additional research is necessary to better design edible films containing plasticizers like Tre, which may crystallize over time, in order to exploit their protein protective properties towards its potential use in the food packaging industry.

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