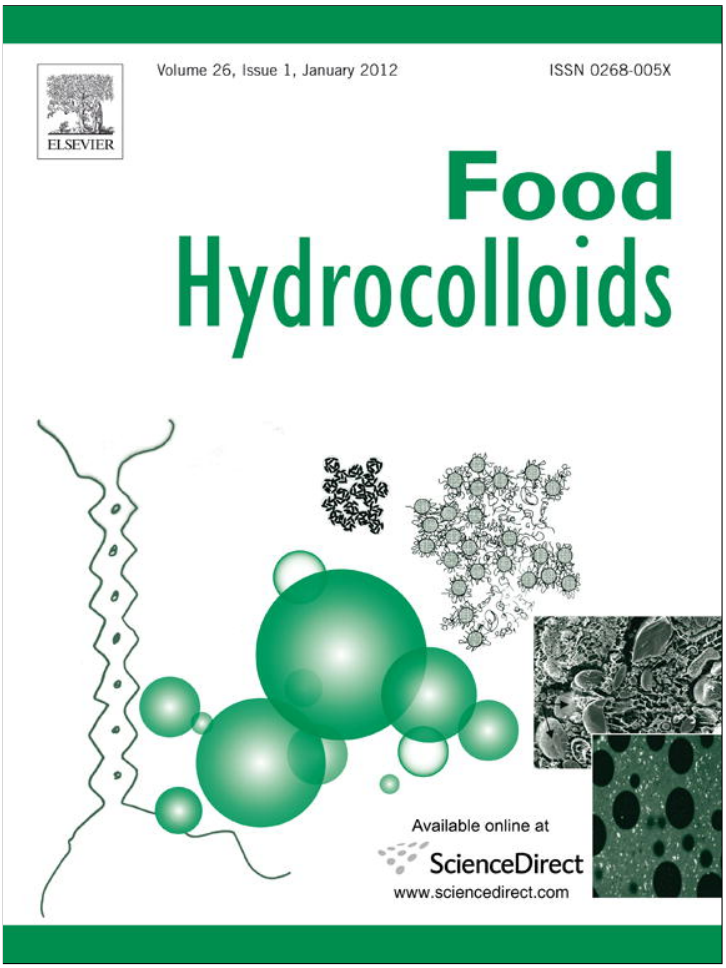

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Effect of freezing on physical properties of whey protein emulsion films

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ABSTRACT

The objective of this work was to study the effect of the freezing process on physical properties of whey protein emulsion films with different beeswax content dried at 5 °C. Thickness, microstructure, water vapour permeability, solubility in water, sorption isotherms and mechanical properties were measured in Control and Frozen films. The freezing process did not cause fractures or perforations in films, but films with beeswax showed a change in the appearance of the lipids after freezing. Only films with 40% of beeswax showed a significant increase in the water vapour permeability after freezing. The freezing process did not affect film solubility in water but produced small differences in the equilibrium moisture content values. In the puncture test, the freezing process increased puncture strength and deformation of films without beeswax but those parameters were not affected in films with beeswax. In tensile test, tensile strength and elastic modulus decreased, but elongation was not affected by freezing process. Principal component analysis accomplished an adequate condensation of the date grouping samples according to film formulation and treatment (Control and Frozen films). Indeed, the relationships of sample grouping and measured parameters were enlightened by principal component analysis. In conclusion, whey protein emulsion films were resistant to the freezing process (freezing, frozen storage and thawing) and could be a good alternative as a treatment to preserve the quality of frozen foods.

1. Introduction

Moisture loss in frozen foods has important economic consequences and is continuously receiving the attention of food scientists mainly due to the fact that drip loss during thawing, as a result of irreversible tissue damage during the freezing process (freezing, storage, and thawing), leads to reduced visual attraction and nutrient loss (Duan & Zhao, 2011). Weight loss is important not only for the economical impact of sealable weight reduction, but also because moisture loss is strongly related with the preservation of food structure and consequently food texture. In addition, moisture loss can produce freezer burn and a glassy appearance in some food products, caused by the presence of tiny cavities caused by sublimated ice (Pham & Mawson, 1997).

Edible films and coatings could constitute a good alternative for improving the quality of frozen foods, mainly because they can reduce the rate of moisture transfer between the food and the surrounding atmosphere, improve structural integrity of frozen foods during thawing, and slow down the occurrence of freezer burn (Duan & Zhao, 2011).

Edible coatings have been applied on frozen foods, mainly in fish, meat, and poultry. Several authors reported that the rate of moisture loss was reduced, and other quality parameters were either improved or maintained, in comparison with uncoated food samples, when coatings of different biopolymers (whey proteins, chitosan, alginate, collagen, cellulose, methylcellulose, etc.), with or without lipids, were applied (Duan, Cherian, & Zhao, 2010; Han, Zhao, Leonard, & Traber, 2004; Stuchell & Krochta, 1995; Yu, Li, Xu, & Zhou, 2008). Stuchell and Krochta (1995) described the application of an edible coating based on whey protein isolate and acetylated monoglycerides in salmon fillets that was effective in reducing the rate of moisture loss and to delay the onset of lipid oxidation after 3 weeks of storage at −23 °C. Another study reported that chitosan coatings incorporated with fish oil reduced the drip loss of frozen lingcod fillets (Duan et al., 2010). Moreover, coatings based on sodium alginate and calcium decreased thawing loss, shear force, and thiobarbituric acid reactive substances, and thus were effective to maintain the quality of frozen pork (Yu et al., 2008). Only one study analyzing the effect of edible coatings to

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maintain the quality of frozen fruits was found in literature (Han et al., 2004). These authors used edible coatings to improve storability of frozen strawberries and observed that chitosan based coatings were able to reduce drip loss and helped to maintain textural quality of frozen strawberries after thawing.

Edible films have been hardly applied in wrapped frozen foods. Only one study analyzing the effect of the freezing process on quality parameters of steak overwrapped with an edible film was reported (Farouk, Price, & Salih, 1990). These authors observed that round steak beef overwrapped with an edible collagen film (Coffi®) exhibited much less fluid exudate after a week of frozen storage at −25 °C, when compared with standard permeable film overwrap. However, the edible film had no significant effect on colour and lipid oxidation.

In the case of heterogeneous foods, edible films with good water barrier properties, placed between two food components with different water activity, can reduce moisture transfers between those components (Duan & Zhao, 2011). This area has also been scarcely studied and only three reports were found in literature in products like bread and tomato-based sauce, simulated sundae ice cream cone, and model system of tomato paste and ground crackers (Kamper & Fennema, 1985; Kester & Fennema, 1989; Rico-Peña & Torres, 1990). An edible lipid–cellulose composite film effectively limited internal transmission of water when situated at the interface between high and low moisture component of a model frozen-food system consisting of bread and a tomato-based sauce after 9 weeks of frozen storage (Kester & Fennema, 1989). Similarly, an edible bilayer film of methylcellulose and palmitic acid retarded moisture transfer from ice cream to the sugar cone keeping its crispness longer than the commercial storage-life of the uncoated product (Rico-Peña & Torres, 1990).

In addition to moisture migration, other physicochemical properties such as physical integrity, mechanical strength, and plasticity of free-standing films have an influence on the application, particularly in the case of overwrapped products. Thus, it is surprising that the effect of freezing on physical properties of free-standing films had been restricted to the study of moisture migration, and limited to certain film-forming materials like cellulose or hydroxypropyl methylcellulose and fatty acids (Kamper & Fennema, 1984, 1985; Kester & Fennema, 1989).

In a previous investigation we obtained whey protein emulsion films with good mechanical properties and improved water vapour permeability characteristics when films were dried at 5 °C compared with 25 °C (Soazo, Rubiolo, & Verdini, 2011a). Furthermore, we studied the sorption behaviour of whey protein emulsion films and showed the influence of both film formulation and drying temperature on the equilibrium moisture content of films (Soazo, Rubiolo, & Verdini, 2011b).

The objective of this investigation was to study the effect of freezing on microstructure, water vapour permeability, solubility in water, moisture sorption, and mechanical properties of whey protein emulsion films dried at 5 °C.

2. Materials and methods

2.1. Materials

Whey protein concentrate (WPC) 80% (Arla Food Ingredients S.A., Argentina), beeswax (BW) (yellow, refined, Sigma–Aldrich, USA), Glycerol (Gly) (Cicarelli, Argentina), potassium sorbate (Anedra, Argentina), and Tween 80 (Anedra, Argentina) were used.

2.2. Preparation of film-forming solutions

Aqueous solutions of 8% (w/w) WPC (pH = 6.2) were prepared as described in Soazo et al. (2011a). Glycerol (in proportion WPC/Gly 3:1 w/w dry solid basis) and potassium sorbate (to obtain a final concentration of 0.12% w/w dry solid basis) were added, and solutions were magnetically stirred during 15 min. Then, BW (at 0, 20 and 40% w/w dry solid basis in the mixture WPC/Gly) and Tween 80 (in proportion BW/Tween 4:1) were incorporated. Tween 80 was used as emulsifier in the solutions containing BW. The amount of distilled water was adjusted to obtain a total solid content of 11.5%. Film-forming solutions were heated at 90 °C for 30 min in a water bath (Dalvo Instruments, Argentina) to achieve whey proteins denaturation. Emulsions were obtained by homogenization in the water bath at 90 °C using a high-shear probe mixer Ultra-Turrax T25 (IKA Werke, Janke & Kunkel GmbH & Co KG, Staufen, Alemania) with a stator diameter of 10 mm, a beaker diameter of 70 mm, and a protein dispersion volume of 200 mL, during 5 min at 21,500 rpm. After homogenization, the emulsions were placed in an ice bath during 30 min to prevent further denaturation of the whey proteins and to crystallize the lipid particles. The emulsions were degassed at room temperature with a vacuum pump.

2.3. Film formation

Eight grams of the degassed emulsion were pipetted on 90 mm diameter disposable polyethylene Petri dishes. Films were dried at 5 °C and 58% relative humidity (RH) on a levelled surface in an environmental chamber Tabai Comstar PR 4GM (Tabai Espec. Corp., Japan) equipped with a fan that circulates interior air at approximately 60 m/min. Drying was completed after 20 ± 2 h. The films used in the different tests were selected based on the lack of physical defects such as cracks, bubbles, and holes. A group of films was conditioned at 25 °C and 58% RH for 3 days and subsequently employed for the determinations (Control Group). Another group of films was frozen in the environmental chamber at −30 °C, and then stored in plastic containers at −20 ± 2 °C for 30 days. Completed this period the films were thawed at 5 °C and conditioned at 25 °C and 58% RH for 3 days before analysis (Frozen Group).

2.4. Film thickness

Film thickness was measured with a digital micrometer (Schwyz, China). For each film, nine thickness measurements were taken. Films were obtained with an average thickness of 0.156 ± 0.011 mm.

2.5. Scanning electron microscopy

The film samples were cryo-fractured by immersion in liquid air and mounted on bronze stubs perpendicularly to their surface. The portions were coated with gold during 15 min at 70–80 mTorr. Micrographs of films cross-section were taken with a JEOL JSM-35C electron microscope (JEOL, Japan) using an accelerating voltage of 20 kV. Magnification of 400 was used.

2.6. Water vapour permeability

A modification of the (E96-95, ASTM, 2002) gravimetric method for measuring water vapour permeability (WVP) was used. Films were mounted on cups containing 10 mL of distilled water, with a permeation area of 19.6 cm^2, with the film surface which had been exposed to air during drying facing the external side of the cup, as described in Soazo et al. (2011a). Cups were placed in the environmental chamber at 25 °C and 58% RH and weighted every hour and a half to obtain four successive steady-state measurements. Weight loss–time curves were used to calculate the % RH at the film underside and the resulting WVP as described in McHugh,

Avena-Bustillos, and Krochta (1993). Determinations were performed in quintuplicate.

2.7. Solubility in water

Pieces of films of 15 × 7.5 mm were cut with a scalpel, dried in an oven (Dalvo Instruments, Argentina) at 70 °C for 24 h and weighed to obtain the initial film dry weight. Each piece of film was placed into a glass tube with 10 mL of distilled water and 0.01% potassium sorbate to prevent microbial growth. Capped tubes were placed on a shaking platform (Vicking, Argentina) for 24 h at 25 °C. Circular filter papers (qualitative grade, Boeco, Germany) were dried 24 h in the oven at 70 °C, cooled to room temperature in a desiccator, and weighed to obtain the initial dry filter weight. The solution containing the films was filtered by pouring the contents of the test tube onto a filter paper placed in a Buchner funnel attached to the neck of a 250 mL Erlenmeyer connected to a vacuum pump (Soazo et al., 2011a). The remaining solids on the filter were dried in the oven at 70 °C for 24 h to determine the final filter dry weight. The difference between the final dry filter weight and initial dry filter weight yielded the final dry film weight. Solubility in water, expressed as soluble solids (%), was obtained by subtracting the weight of dry matter not solubilised from the weight of initial dry matter and reported on initial dry weight basis (Sothornvit & Krochta, 2000). Determinations were performed in quintuplicate.

2.8. Moisture sorption isotherms

Films were cut in portions of 400 mg, placed in glass bottles previously weighed, and pre-dried in desiccators containing drierite ($a_w = 0$) during 10 days. Then, the bottles were placed in hermetically sealed glass jars containing different desiccants as described in Soazo et al. (2011b). Ten saturated salt solutions: LiCl, $KC_2H_3O_2$, $MgCl_2 \cdot 6H_2O$, K_2CO_3, $Mg(NO_3)_2 \cdot 6H_2O$, NaBr, $SrCl \cdot 6H_2O$, NaCl, KCl, and $BaCl_2 \cdot 2H_2O$ were used to obtained the respective RH/100 conditions: 0.11, 0.22, 0.33, 0.43, 0.53, 0.58, 0.71, 0.75, 0.84, and 0.90 (Jowitt & Wagstaffe, 1989). Film portions were equilibrated in contact with each salt solution during 10 days at 25 °C. After that, the bottles were weighed to obtain the sample weight at equilibrium and then dried in the oven at 105 °C during 4 h to obtain the weight of the dry sample. The analyses were made in quintuplicate. The equilibrium moisture content (EMC) was calculated using Equation (1):

$$\mathrm{EMC} = \frac{(w_2 - w_3)}{(w_3 - w_1)} 100 \quad (1)$$

where w_1 is the weight of the empty glass bottle, w_2 is the weight of the sample at equilibrium plus the weight of the empty glass bottle and w_3 is the weight of the dry sample plus the weight of the empty glass bottle.

In a previous work we studied the effect of drying temperature and beeswax content on moisture isotherms of whey protein emulsion films and observed that the Guggenheim–Anderson–De Boer (GAB) model was the most appropriate to describe the moisture sorption of the films (Soazo et al., 2011b). Consequently, the EMC values were fitted using the GAB model as followed:

$$\mathrm{EMC} = \frac{m_0 C k a_w}{(1 - k a_w)(1 - k a_w + C k a_w)} \quad (2)$$

where a_w is the water activity, m_0 is the monolayer moisture content (g H_2O/g solids), C is the surface heat constant and k is an additional constant.

Experimental data were fitted to Equation (2) using an algorithm written in Matlab 6.5.1 (MathWorks Inc, USA). The parameters were calculated minimizing the error function as described by Coupland, Shaw, Monahan, O'Riordan, and O'Sullivan (2000):

$$\mathrm{error} = \frac{1}{n} \sum_{a_w=0}^{n} \left| \frac{\mathrm{EMC}_{\mathrm{measured}(a_w)} - \mathrm{EMC}_{\mathrm{modeled}(a_w)}}{\mathrm{EMC}_{\mathrm{measured}(a_w)}} \right| \quad (3)$$

2.9. Mechanical properties

Tensile and puncture tests were performed to evaluate the mechanical strength of the films using a single column Universal Testing Machine Instron, Series 3340 (Instron, USA) with a 10 N load cell. Films strips of 7 mm wide and 60 mm length were used for the tensile tests, while discs of 90 mm of diameter were used for the puncture test. Samples were equilibrated to the testing environment for 2 h at 22 °C and 50% RH on average. For each mechanical test ten replications were performed.

Strips for the tensile test were cut using a scalpel. The ends were mounted with a double sided tape and 30 mm squares made of cardstock. These cardstock pads were placed on the film strips ends to prevent tearing and slippage in the testing device (Shellhammer & Krochta, 1997). The exposed film strip length between the cardstock was 30 mm. Crosshead speed was 0.05 mm/s. The parameters obtained from this test were tensile strength (TS), elongation (E), and elastic modulus (EM). TS was calculated dividing the peak load by the cross-sectional area of the initial film (thickness of film × 7 mm), E was obtained as the percentile of the change in the length of specimen to the original distance between the grips and EM was determined from the initial slope of the stress–strain curve (Han, Seo, Park, Kim, & Lee, 2006).

For the puncture test film discs were fixed to a support with a circular opening of 50 mm in diameter and 30 mm in depth. A cylindrical probe of 2 mm diameter was moved perpendicularly to the film surface at a constant speed of 0.8 mm/s until it passed through the film. Puncture strength (PS) and deformation (D) at the puncture point were obtained from force–distance curves (Chen & Lai, 2008).

2.10. Statistical analysis

A full factorial design was performed. Two factors (Freezing and BW content) in two and three levels, respectively, were studied (Frozen and Controls films, and 0, 20 and 40% of BW). Analysis of variance was used and when the effect of the factors was significant ($p < 0.05$), the Tukey multiple ranks honestly significant difference (HSD) test was applied (95% of confidence level).

Principal component analysis (PCA) was used to reduce the dimensionality of the data obtained in the determinations and to show the relationships between treatments (Johnson & Wichern, 1998). The principal components (PCs) are numbered in order of the amount of variation in the original data set. Consequently, the first principal component accounts for the most variation, and each subsequent principal component accounts for as much of the remaining variation. An adequate condensation of the information is achieved when no more than two or three PCs can explain at least 80–90% of the total variability (Verdini, Zorrila, Rubiolo, & Nakai, 2007).

Because the input variables differed in magnitude, all values of a given variable were scaled from 0 to 100 respect to the range between the smallest and the largest variable value before performing PCA analysis (Verdini et al., 2007). The statistical analysis was performed using Minitab 13.20 (Minitab Inc., USA).

3. Results and discussion

3.1. Scanning electron microscopy

Fig. 1 shows characteristic images of transversal sections of Frozen and Control emulsion films. The upper side (A) was exposed to air and the lower side (B) was in contact with the Petri dish surface during drying.

After the freezing process, no fractures or perforations were observed in Frozen films. Frozen and Control films without BW showed a continuous and homogeneous cross-section. The homogeneous cross-section was also observed by other authors in unfrozen films based on casein, amaranth flour and soy protein (Chick & Hernandez, 2002; Denavi et al., 2009; Tapia-Blácido, Sobral, & Menegalli; 2005).

Both Frozen and Control films with BW showed a preferential location of the wax on the film side exposed to air during drying. This

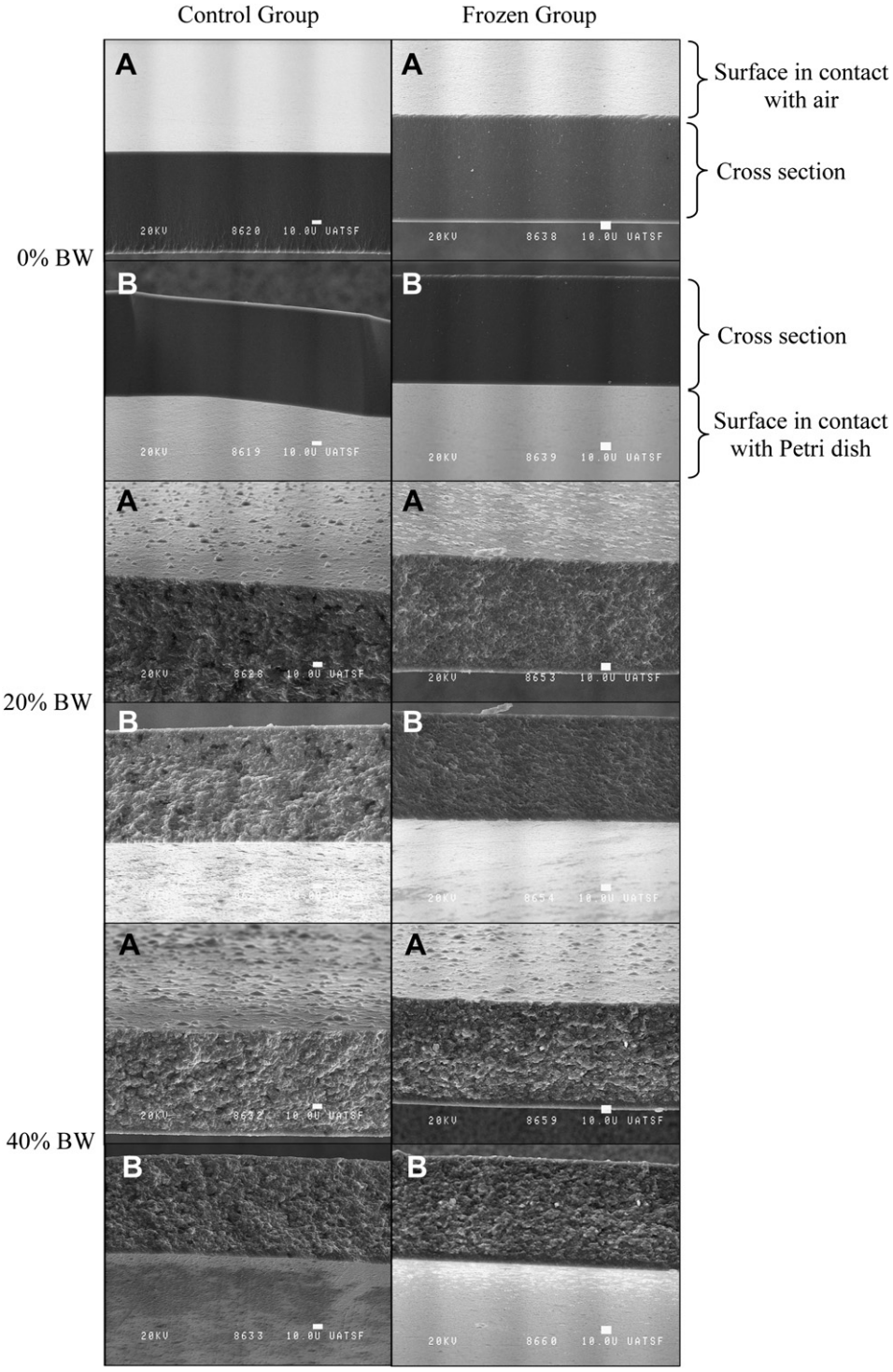

Fig. 1. Scanning electron micrographs of frozen and control WPC emulsion films: in all pictures the evaporation surface of the film is on the top. Micrographs labelled (A) showed the cross-section of the film and the surface exposed to air during drying. Micrographs labelled (B) showed the film cross-section and the surface in contact with the Petri dish during drying. The comparison between (A) and (B) illustrates the occurrence of the destabilization during drying of whey protein films containing BW.

phenomenon, may be related to the fact that film-forming solutions with BW suffered destabilization during drying, and was more evident as BW content increased. However, the destabilizing phenomenon taking place during the drying step is dependent on the interactions between the components of the film. Prodpran, Chinabhark, and Benjakul (2005) showed evidence of emulsion destabilization during drying in surimi protein based edible films. Atarés, Bonilla, and Chiralt (2010) reported that the final microstructure of unfrozen caseinate based films with ginger oil had more discontinuities as the ginger oil content increased. On the other hand, the authors found that unfrozen caseinate based films with cinnamon oil showed no apparent differences in their microstructural aspect, probably due to the fact that the small size of the particles in the emulsions was not modified during the drying process progress, and cinnamon oil stayed homogeneously distributed in the dry protein matrix.

Finally, lipids located on the surface of Frozen films looked flattened compared with those of Control films.

3.2. Water vapour permeability

Table 1 shows the WVP of Frozen and Control emulsion films with different BW contents. An increasing tendency in WVP values after 30 days of frozen storage was observed. However, this increase was statistically significant ($p < 0.05$) only in films with 40% of BW.

In agreement, Kester and Fennema (1989) who studied the effect of low temperature storage on barrier characteristics of wax-laminated films of lipids and cellulose ethers stored at −40 °C for 3 and 9 weeks, found a small increase in WVP possibly due to slight imperfections that could develop because of fluctuating storage temperatures and an accompanying contraction and expansion of the lipids. If major cracking or fracture had occurred, a dramatic elevation in permeability would be anticipated.

In the present work, cracking or fractures were not observed. But, the scanning electron microscopy analysis of Frozen films revealed that the morphology of lipids located on the surface showed a flattened appearance. This phenomenon may be related to the slight differences observed in the WVP between Frozen and Control films. In agreement, Kester and Fennema (1989) showed that the lipid morphology was related to moisture transfer resistance of the cellulose based edible films.

Analyzing the addition of BW in Frozen and Control films, the lipid addition decreased WVP in both groups, but the decrease was higher for Control films. In agreement, the micrographs showed a high proportion of the film surface exposed to air during drying covered by lipids; this bilayer like structure could explain the low WVP values.

3.3. Solubility in water

Table 1 shows the solubility in water of Frozen and Control WPC emulsion films with different BW contents. Both groups were partially soluble (solubility between 22.4 and 39.4%) maintaining their integrity during immersion in water.

Table 1
Effect of freezing on water vapour permeability (WVP) and solubility of whey protein concentrate emulsion films.

Group	Beeswax (%)	WVP (g mm/m^2 h kPa)	Solubility (%)
Frozen	0	3.76 ± 0.19^{a}	38.4 ± 5.1^{a}
Control	0	3.48 ± 0.17^{ab}	39.4 ± 5.0^{a}
Frozen	20	3.29 ± 0.15^{bc}	34.8 ± 6.3^{ab}
Control	20	3.07 ± 0.14^{c}	27.1 ± 3.4^{bc}
Frozen	40	3.38 ± 0.15^{b}	31.1 ± 4.1^{abc}
Control	40	2.56 ± 0.08^{d}	22.4 ± 1.7^{c}

Data correspond to mean values and standard deviations of five samples.
Values with different letters in each column are significantly different ($p < 0.05$), according to Tukey's test.

Solubility of whey protein films without BW was not affected by the freezing process. Films with BW showed an increasing tendency in solubility that was not statistically significant ($p > 0.05$). These results are in accordance with the microscopic images that showed no signs of altered matrix porosity that could modify the solubility of the hydrophilic compounds included in the film formulation.

In reference to the addition of BW, Frozen and Control films showed a tendency to decrease solubility with the corresponding BW addition, although this effect was statistically significant ($p < 0.05$) only in the Control films group. Similar measurements were reported in unfrozen whey protein based edible films (Kim & Ustunol, 2001; Ozdemir & Floros, 2008). These authors considered that, because the total solids level remained constant in the formulation, the incorporation of BW reduced the soluble matter present in the films, and consequently the solubility.

3.4. Moisture sorption isotherms

Experimental data of EMC for Frozen and Control emulsion films with different BW contents are shown in Fig. 2. A slow increase in the EMC until 0.58 a_w, followed by an abrupt increase in the EMC was observed. Such sigmoidal water sorption isotherms are characteristic of materials rich in hydrophilic polymers (Zinoviadou, Koutsoumanis, & Biliaderis, 2009). The effect of the freezing process on the EMC was dependant on the a_w region. Although the freezing process produced slight differences in the EMC values, those differences were more notorious at higher a_w values.

Analyzing the parameters of the GAB model (m_0, k and C), parameter C was more sensible to freezing, showing an increase in films without BW and with 40% of BW; and a decrease in films with 20% of BW (Table 2). Although parameter m_0 slightly changed, a clear tendency was not found. On the other hand, parameter k remained almost invariable.

In reference to the effect of the addition of BW, a decreasing tendency in parameter m_0 was observed. The incorporation of lipids reduces the water sorption capacity of the film due to the fact that lipids correspond to a fraction of solids with a low water uptake capacity, especially beeswax, which is very hydrophobic (Fabra, Talens, Gavara, & Chiralt, 2002). Thus, the decrease in the monolayer moisture content was also reported by Kim and Ustunol (2001) in the case of WPI/candelilla wax and WPI/butterfat. These authors also found a decrease in C and k parameters when lipids were present in the formulations. In our investigation, a clear effect of the addition of BW on parameter C was not found, and lipid addition did not affect parameter k.

3.5. Mechanical properties

Tables 3 and 4 show the puncture strength (PS) and deformation (D) calculated from force–distance and the tensile strength (TS), elongation (E) and elastic modulus (EM) derived from stress–strain curves, respectively.

The freezing process increased PS and D of films without BW, but did not affect the response to puncture of films with BW. The addition of BW decreased the PS and D of Control and Frozen films. This could be explained because of the expected weakening and lubricating effect of lipids on the whey protein films (Banerjee & Chen, 1995).

In tensile test, the freezing process decreased both TS and EM, in films without BW as well as in films with BW. In general E was not affected by freezing. The addition of BW decreased all parameters of Control and Frozen films. These results may be related to the fact

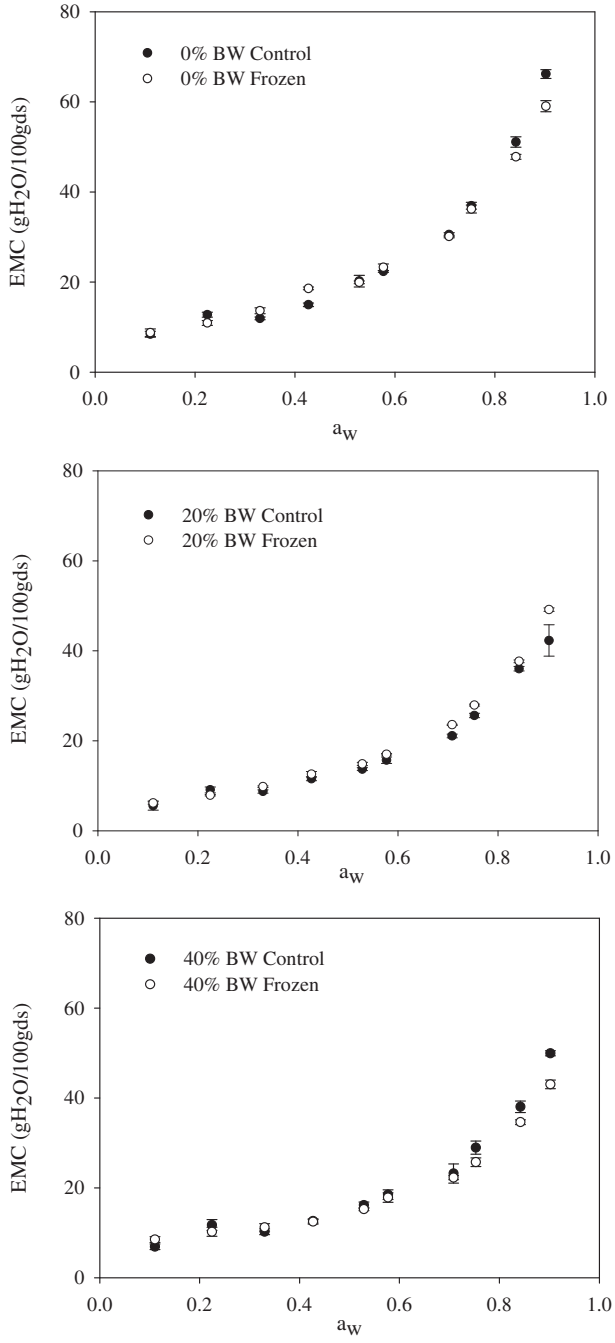

Fig. 2. Moisture sorption isotherms of frozen and control WPC emulsion films. Bars are based on standard deviations ($n = 5$).

that BW causes the disruption of the continuous matrix and induces the development of a heterogeneous film structure (Navarro-Tarazaga, Sothornvit, & Pérez-Gago, 2008). Our results were similar to the reports of other authors in unfrozen whey protein emulsion films (Shellhammer & Krochta, 1997; Talens & Krochta, 2005).

3.6. Principal component analysis

PCA was applied to visualize the distribution of film samples according to film formulation and treatment (Control and Frozen films) and to achieve an adequate condensation of the information.

Table 2
Parameters obtained by fitting of equilibrium moisture content of whey protein concentrate emulsion films to the GAB model: m_0 (monolayer moisture content), C (surface heat constant) and k (additional constant).

Group	Beeswax (%)	m_0	C	k	R^2	Error[a] (%)
Frozen	0	10.54	25.56	0.93	0.99	3.98
Control	0	10.78	22.37	0.93	1.00	5.16
Frozen	20	8.34	15.27	0.93	1.00	2.81
Control	20	7.40	25.52	0.93	0.99	5.54
Frozen	40	8.18	98.75	0.90	1.00	2.44
Control	40	8.27	25.61	0.93	0.99	5.43

[a] Calculated according to Coupland et al. (2000).

PC1 and PC2 explained together the 82.2% of the variance, with PC1 and PC2 explaining the 72.2% and 10% of the variance, respectively.

Fig. 3a shows PC1 versus PC2 score plot displaying the relationship between the samples in the new coordinate (PC defined) space and assembling film samples according to their composition and treatment (Control or Frozen). The score plot illustrated the separation of the samples in four groups: Control 0% BW, Frozen 0% BW, Control 20 and 40% BW and Frozen 20 and 40% BW. PC1 separated the samples according to the presence of BW in the film formulation, and PC2 spreaded the samples from down to up according to treatment.

Fig. 3b shows the PC1 and PC2 loadings plot illustrating the relationship between the original variables and the principal components and helping to identify the most important parameters of the discrimination observed in the PC score plot (Henrique, Teófilo, Sabino, Ferreira, & Cereda, 2007). Variables with higher absolute values of PC1 loadings (PS and D) explained the sample separation according to the presence of BW in the film formulation. On the other hand, variables with higher absolute values of PC2 loadings (WVP, Solubility and EM) explained the sample arrangement according to treatment (Frozen and Control films). The EMC at different a_w did not show any clear effect on the sample distribution.

PCA results allowed to summarize the information showing that freezing increased WVP and solubility but decreased the elastic modulus of whey protein films, both in films with and without BW. On the other hand, freezing increased puncture parameters only in formulations without BW, being this property the most affected by the film formulation.

4. Conclusions

Whey protein emulsion films probed to be resistant to the freezing process (freezing, frozen storage and thawing) maintaining their physical integrity. Frozen films were as efficient as Control films with regards to moisture sorption. In reference to the effect of the freezing process on the mechanical properties, puncture resistance was maintained and in some formulations was even

Table 3
Effect of freezing on parameters derived from the puncture test of whey protein concentrate emulsion films: PS (puncture strength) and D (deformation).

Group	Beeswax (%)	PS (N)	D (mm)
Frozen	0	2.20 ± 0.28^{a}	1.92 ± 0.19^{a}
Control	0	1.86 ± 0.34^{b}	1.75 ± 0.14^{b}
Frozen	20	0.25 ± 0.07^{c}	0.55 ± 0.11^{c}
Control	20	0.21 ± 0.03^{c}	0.50 ± 0.09^{c}
Frozen	40	0.15 ± 0.03^{c}	0.45 ± 0.06^{c}
Control	40	0.19 ± 0.04^{c}	0.44 ± 0.07^{c}

Data correspond to mean values and standard deviations of ten samples.
Values with different letters in each column are significantly different ($p < 0.05$), according to Tukey's test.

Table 4
Effect of freezing on parameters derived from the tensile test of whey protein concentrate emulsion films: TS (tensile strength), *E* (elongation) and EM (elastic modulus).

Group	Beeswax (%)	TS (MPa)	*E* (%)	EM (MPa)
Frozen	0	3.69 ± 0.43^{b}	4.99 ± 0.59^{a}	154 ± 9^{c}
Control	0	4.92 ± 0.78^{a}	3.50 ± 0.98^{b}	229 ± 22^{a}
Frozen	20	1.40 ± 0.10^{d}	2.30 ± 0.23^{c}	109 ± 9^{d}
Control	20	2.63 ± 0.36^{c}	2.38 ± 0.36^{c}	185 ± 12^{b}
Frozen	40	1.09 ± 0.22^{d}	1.89 ± 0.35^{c}	103 ± 9^{d}
Control	40	1.64 ± 0.33^{d}	1.80 ± 0.42^{c}	149 ± 12^{c}

Data correspond to mean values and standard deviations of ten samples.
Values with different letters in each column are significantly different ($p < 0.05$), according to Tukey's test.

improved while tensile resistance was negatively affected. In summary, whey protein emulsion films could constitute a good alternative as a treatment to preserve the quality of frozen foods; however optimization of the formulations should be performed.

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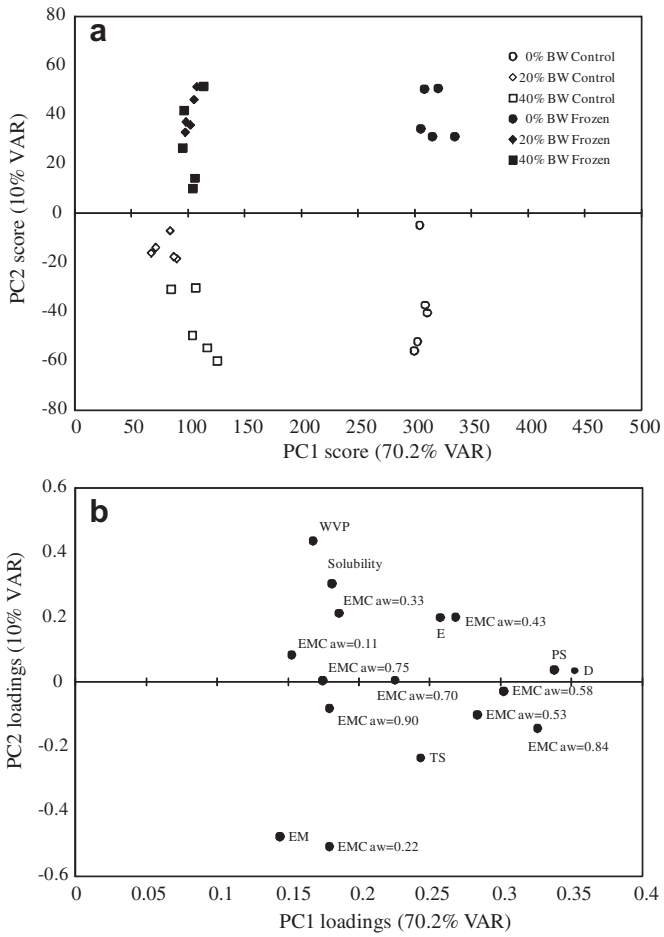

Fig. 3. Plots of the first two principal components (PC1 versus PC2): (a) PC scores plot of control and frozen WPC emulsion films. (b) PC loadings plot. WVP: water vapour permeability; PS: puncture strength; *D*: deformation; TS: tensile strength; *E*: elongation; EM: elastic modulus. EMC: equilibrium moisture content (at each a_w value).

References

ASTM. (2002). Standard test methods for water vapor transmission of materials. In *Annual book of ASTM standards* (pp. 697–704). Philadelphia, PA: ASTM E96-95.
Atarés, L., Bonilla, J., & Chiralt, A. (2010). Characterization of sodium caseinate-based edible films incorporated with cinnamon or ginger essential oils. *Journal of Food Engineering, 100*, 678–687.
Banerjee, R., & Chen, H. (1995). Functional properties of edible films using whey protein concentrate. *Journal of Dairy Science, 78*, 1673–1683.
Chen, C. H., & Lai, L. S. (2008). Mechanical and water vapor barrier properties of tapioca starch decolorized hsian-tsao leaf gum films in the presence of plasticizer. *Food Hydrocolloids, 22*, 1584–1595.
Chick, J., & Hernandez, R. J. (2002). Physical, thermal, and barrier characterization of casein–wax-based edible films. *Journal of Food Science, 67*(3), 1073–1079.
Coupland, J. N., Shaw, N. B., Monahan, F. J., O'Riordan, E. D., & O'Sullivan, M. (2000). Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. *Journal of Food Engineering, 43*, 25–30.
Denavi, G., Tapia-Blácido, D. R., Añón, M. C., Sobral, P. J. A., Mauri, A. N., & Menegalli, F. C. (2009). Effects of drying conditions on some physical properties of soy protein films. *Journal of Food Engineering, 90*, 341–349.
Duan, J., Cherian, G., & Zhao, Y. (2010). Quality enhancement in fresh and frozen lingcod (*Ophiodon elongates*) fillets by employment of fish oil incorporated chitosan coatings. *Food Chemistry, 119*, 524–532.
Duan, J., & Zhao, Y. (2011). Edible coatings and films and their applications on frozen foods. In D.-W. Sun (Ed.), *Handbook of frozen food processing and packaging* (pp. 875–892). CRC Taylor and Francis.
Fabra, M. J., Talens, P., Gavara, R., & Chiralt, A. (2012). Barrier properties of sodium caseinate films as affected by lipid composition and moisture content. *Journal of Food Engineering, 109*(3), 372–379.
Farouk, M. M., Price, J. F., & Salih, A. M. (1990). Effect of an edible collagen film overwrap on exudation and lipid oxidation in beef round steak. *Journal of Food Science, 55*(6), 1510–1513.
Han, J. H., Seo, G. H., Park, I. M., Kim, G. N., & Lee, D. S. (2006). Physical and mechanical properties of pea starch edible films containing beeswax emulsions. *Journal of Food Science, 71*(6), 290–296.
Han, C., Zhao, Y., Leonard, S. W., & Traber, M. G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (*Fragaria × ananassa*) and raspberries (*Rubus ideaus*). *Postharvest Biology and Technology, 33*, 67–78.
Henrique, C. M., Teófilo, R. F., Sabino, L., Ferreira, M. M. C., & Cereda, M. P. (2007). Classification of cassava starch films by physicochemical properties and water vapor permeability quantification by FTIR and PLS. *Journal of Food Science, 72*(4), 184–189.
Johnson, R. A., & Wichern, D. W. (1998). *Applied multivariate statistical analysis*. London: Prentice Hall International.
Jowitt, R., & Wagstaffe, J. P. (1989). *The certification of the water content of microcrystalline cellulose (MCC) at 10 water activities*. CRM 302. Commission of the European Communities. Community Bureau of Reference.
Kamper, S. L., & Fennema, O. (1984). Water vapor permeability of and edible, fatty acid, bilayer film. *Journal of Food Science, 49*, 1482–1485.
Kamper, S. L., & Fennema, O. (1985). Use of an edible film to maintain water vapor gradients in foods. *Journal of Food Science, 50*, 382–384.
Kester, J. J., & Fennema, O. (1989). An edible film of lipids and cellulose ethers: barrier properties to moisture vapor transmission and structural evaluation. *Journal of Food Science, 54*(6), 1383–1389.
Kim, J. S., & Ustunol, Z. (2001). Solubility and moisture sorption isotherms of whey-protein-based edible films as influenced by lipid and plasticizer incorporation. *Journal of Agricultural and Food Chemistry, 49*, 4388–4391.
McHugh, T. H., Avena-Bustillos, R., & Krochta, J. M. (1993). Hydrophilic edible films: modified procedure for water vapor permeability and explanation of thickness effects. *Journal of Food Science, 58*(4), 899–903.
Navarro-Tarazaga, M. L., Sothornvit, R., & Pérez-Gago, M. B. (2008). Effect of plasticizer type and amount on hydroxypropil methylcellulose–beeswax edible film properties and postharvest quality of coated plums (*cv. Angeleno*). *Journal of Agricultural and Food Chemistry, 56*, 9502–9509.
Ozdemir, M., & Floros, J. D. (2008). Optimization of edible whey protein containing preservatives for water vapor permeability, water solubility and sensory characteristics. *Journal of Food Engineering, 86*, 215–224.
Pham, Q. T., & Mawson, R. F. (1997). Moisture migration and ice recrystallization in frozen foods. In M. C. Erickson, & Y. C. Hung (Eds.), *Quality in frozen food* (pp. 67–91). New York: Chapman & Hall.
Prodpran, T., Chinabhark, K., & Benjakul, S. (2005). Properties of composite film based on bigeye snapper surimi protein and lipids. *Songklanakarin Journal of Science and Technology, 27*(3), 775–788.
Rico-Peña, D. C., & Torres, J. A. (1990). Edible methylcellulose-based films as moisture-impermeable barriers in sundae ice cream cones. *Journal of Food Science, 55*(5), 1468–1469.
Shellhammer, T. H., & Krochta, J. M. (1997). Whey protein emulsion film performance as affected by lipid type and amount. *Journal of Food Science, 62*(2), 390–394.

Soazo, M., Rubiolo, A. C., & Verdini, R. A. (2011a). Effect of drying temperature and beeswax content on physical properties of whey protein emulsion films. *Food Hydrocolloids, 25*(5), 1251–1255.

Soazo, M., Rubiolo, A. C., & Verdini, R. A. (2011b). Effect of drying temperature and beeswax content on moisture isotherms of whey protein emulsion film. *Procedia Food Science, 1*, 210–215.

Sothornvit, R., & Krochta, J. M. (2000). Water vapor permeability and solubility of films from hydrolyzed whey protein. *Journal of Food Science, 65*(4), 700–703.

Stuchell, Y. M., & Krochta, J. M. (1995). Edible coatings on frozen king salmon: effect of whey protein isolate and acetylated monoglycerides on moisture loss and lipid oxidation. *Journal of Food Science, 60*(1), 28–31.

Talens, P., & Krochta, J. M. (2005). Plasticizing effects of beeswax and carnauba wax on tensile and water vapor permeability properties of whey protein films. *Journal of Food Science, 70*(3), 239–243.

Tapia-Blácido, D., Sobral, P., & Menegalli, F. (2005). Effect of drying temperature and relative humidity on the mechanical properties of amaranth flour films plasticized with glycerol. *Brazilian Journal of Chemical Engineering, 212*, 249–256.

Verdini, R. A., Zorrila, S. E., Rubiolo, A. C., & Nakai, S. (2007). Multivariate statistical methods for Port Salut Argentino cheese analysis based on ripening time, storage conditions, and sampling sites. *Chemometrics and Intelligent Laboratory Systems, 86*, 60–67.

Yu, X. L., Li, X. B., Xu, X. L., & Zhou, G. H. (2008). Coating with sodium alginate and its effects on the functional properties and structure of frozen pork. *Journal of Muscle Foods, 19*, 333–351.

Zinoviadou, K. G., Koutsoumanis, K. P., & Biliaderis, C. G. (2009). Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. *Meat Science, 82*(3), 338–345.