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Prefreezing application of whey protein-based edible coating to maintain quality attributes of strawberries

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Summary Prefreezing application of whey protein concentrate (WPC)-based edible coating to maintain quality attributes of strawberries was studied. Beeswax (BW) was added to the solutions (0%, 20% and 40% with respect to the solids contained in the mixture WPC/glycerol). Coated and control fruits were frozen, stored at −20 °C and thawed. After thawing, weight loss, firmness, microstructure and colour parameters were measured. Coating with 20% BW reduced strawberries weight loss after thawing (55%). Strawberries firmness was maintained equally in all groups analysed although a slight improvement at the cell microstructure alterations caused by the freezing process was observed in coated fruits. Strawberries brightness was similar in all groups. Colour parameter a^* showed a tendency to decrease with the increasing BW concentration, and only b^* of coated fruits was lower than controls. The application of whey protein coating could be an attractive treatment to maintain quality attributes of strawberries undergoing the freezing process.

Keywords Edible coatings, freezing, strawberries, whey proteins.

Introduction

Strawberry fruits have a very short shelf life and senescent period due to their susceptibility to mechanical injury, water loss, bruising, excessive texture softening, physiological disorders and infection caused by several pathogens that can rapidly reduce the quality of fruit and make marketing a challenge. These characteristics of strawberries highlight the need to implement technologies that extend the postharvest life of the fruit.

Freezing of fruits and vegetables is one of the most common ways for maintaining the quality of these products and can potentially deliver a high degree of safety, nutritional value, sensory quality and convenience, and can supply pleasurable eating experiences (Berry *et al.*, 2008). Moreover, this is an efficient preservation process due to the transformation of liquid water into ice that significantly reduces microbial and enzymatic activities. However, frozen fruits undergo quality deterioration, not only during the freezing stage but also during frozen storage and thawing because of structural collapse that produces both texture and drip loss (Li & Sun, 2002; Galetto *et al.*, 2010). Nevertheless, it is accepted that high freezing rates produce minor quality loss than slow ones because the production of a large number of small ice crystals (Delgado & Rubiolo, 2005).

Consumer's continuous demands for nonseasonal fruits have contributed to an increase in the frozen food industry. This fact has forced food technologists to work on the improvement of the existing preservation methods and the development of new ones intended to maintain the quality standards of fresh fruits (colour, flavour, texture and nutritive value). Different authors have focused their investigations on increasing the quality of frozen strawberries through the application of various prefreezing treatments such as the addition of different sugars or the incorporation of calcium or enzymes acting specifically on the cell

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wall, and more recently, ultrasound irradiation (Suutarinen *et al*., 2002; Galetto *et al*., 2010; Cheng *et al*., 2014).

Among its many applications, edible films and coatings have been proposed as barriers to minimise moisture loss in frozen foods reducing the rate of moisture transfer between the food and the surrounding environment, thus retarding the rate of package ice formation and dehydration of the product surface (George, 2006).

Edible coatings have been successfully applied to improve the quality of strawberries during refrigerated storage (Perdones *et al*., 2012; Wang & Gao, 2013). However, only one publication was found about the effect of edible coating application as a pretreatment of fruit submitted to freezing. Han *et al*. (2004) showed that the application of chitosan-based coatings to *Totem* strawberries reduced drip loss and helped to maintain their textural quality after thawing. Thus, the use of edible coatings as a prefreezing treatment could be an interesting strategy to preserve the quality of frozen fruits.

In a previous work, we studied the effect of the freezing process on physical properties of whey protein emulsion films with different beeswax content. The freezing process did not cause fractures or perforations in films. We also demonstrated that freezing did not affect the puncture strength and deformation/elongation of films with beeswax, concluding that whey protein emulsion films may constitute a good alternative to be applied in frozen foods (Soazo *et al*., 2013). To advance with our previous investigation, the aim of this study was to evaluate the prefreezing application of whey protein-based edible coating to maintain quality attributes of strawberries.

Materials and methods

Materials

Whey protein concentrate (WPC) 80% protein content was used as the main component (Arla Food Ingredients S.A., Martínez, Argentina), beeswax (BW) refined, yellow was added as lipid component (Sigma-Aldrich, Martínez, AR, USA), glycerol (Gly) was employed as plasticiser (Cicarelli, San Fernando, Argentina), Tween-80 was used as emulsifier (Anedra, San Fernando, Argentina), and potassium sorbate was added to prevent microbial growth (Anedra).

Strawberries of the cultivar *Winter Dawn* obtained from a local producer were selected according to colour and size. Damaged and nonuniform fruits were discarded, and selected fruits were washed, drained and dried with tissue paper. After removing the calyx and peduncle, the strawberries were randomly assigned for the studies. Each group of strawberries consisted of thirty fruits.

Coating solutions

Coating solutions were prepared as described in Soazo *et al*. (2011). Briefly, 2 L of aqueous solutions of WPC was prepared; Gly (WPC/Gly 3:1) and potassium sorbate (final concentration of 0.1% w/w) were added. The mixture was magnetically stirred for 15 min for complete dissolution. BW was added to the solutions (0%, 20% and 40% with respect to the solids contained in the mixture WPC/Gly). Tween-80 was used as emulsifier only in the solutions containing BW (BW/Tween 4:1). The amount of distilled water was adjusted to obtain a total solid content of 11.5% (w/w), and thus, the final concentration of WPC ranged between 6.6% (w/w), for coating solutions containing 40% BW, and 9.9% (w/w), for coating solutions without BW. Immediately, film-forming solutions were heated at 90 °C for 30 min in a water bath (Dalvo Instruments, Santa Fe, Argentina) to achieve BW melting and whey proteins denaturation. Emulsions were prepared employing an Ultra-Turrax T25 (IKA® Werke GmbH & Co. KG, Staufen, Germany) with a stator diameter of 10 mm, a beaker diameter of 70 mm, during 5 min at 21 500 rpm. After homogenisation, the emulsions were placed in an ice bath for 30 min to crystallise the lipid particles. The emulsions were degassed at room temperature with a vacuum pump.

Coating application

Strawberries were coated with the different suspensions employing a vacuum infusion device. Fruits were placed in a basket and dipped in the coating-forming suspensions. The system was covered with a lid, and a light weight was put over the lid to ensure that the strawberries were completely covered by the solutions. A vacuum pulse of 5 kPa was applied for 4 min and until the atmospheric pressure was restored, the strawberries remained immersed for 2 min more (Vargas *et al*., 2009). Then, the strawberries were allowed to drip off for 10 min in the basket and, after that, were dried at 5 °C and 58% relative humidity (RH) for 90 min in an environmental chamber Tabai Comstar PR 4 GM (Tabai Espec. Corp., Osaka, Japan). A group of strawberries dipped in distilled water were used as control of the entire vacuum infusion process. After drying, strawberries were placed at 5 °C in the refrigerator until they were frozen.

Freezing process

Strawberries were frozen under a rapid freezing process. Briefly, fruits were placed in a basket, immersed in liquid nitrogen for 10 s and, subsequently, remained in contact with nitrogen vapours until the centre of

the fruit reached −18 °C. During the freezing process, the temperature of the strawberries was monitored using an acquisition data system (Omega Engineering, Inc., Stamford, CT, USA) with T thermocouples. The time of immersion was selected to avoid the cracking of the fruit, which produces an irreversible damage. After freezing, strawberries were placed in plastic trays inside of freezer bags, were stored in a domestic freezer at −20 °C for 30 days and, finally, were thawed in a refrigerator at 5 °C in the remaining 12 h.

Analyses

Weight loss

Weight loss was evaluated by weighting fifteen strawberries, which were divided into subgroups of three, before and after the freezing process. The result was calculated as the percentage of loss with respect to the initial weight.

Textural analysis

Penetration test was carried out in a room with controlled temperature and relative humidity (20 °C and 50% RH) where fruits were equilibrated to ambient conditions for 2.5 h. Then, strawberries were cut longitudinally, and each half of the fruit was penetrated in the equatorial zone according to Galetto *et al.* (2010). A single-column Universal Testing Machine Instron, Series 3340 (Instron, Norwood, MA, USA) with a 10N load cell and a cylindrical probe of 3 mm diameter were used. Penetration speed of 100 mm min^{-1} and penetration distance of 8 mm were used. Force-deformation curves were registered and analysed to obtain two textural parameters: *firmness*, as the maximum puncture force expressed in N (F_{max}), and *deformation*, as the distance to reach the maximum deformation force expressed in mm (D_{max}) (Galetto *et al.*, 2010).

Microscopic analysis

After thawing, strawberries were cut longitudinally and then transversally to obtain slices of 5 mm of thickness from the equatorial zone. The slices were fixed in formaldehyde, ethylic alcohol and acetic acid solution (10 mL of formaldehyde 40% v/v, 50 mL of ethyl alcohol 96% v/v, 2 mL of glacial acetic acid 99.5% v/v and 38 mL of distilled water) at 4 °C in a refrigerator for 24 h. Then, the slices were washed and dehydrated in ethanol solutions series (50%, 70%, 80% and 96%) for 12 h and, finally, in 100% ethanol for 24 h. Next, fixed slices were cleaned by immersion in ethanol/xylene mixtures (3/1, 1/1 and 1/3) for 12 h and xylene for 24 h. After that, the slices were transferred to paraffin/xylene mixtures (1/1 and 3/1) for 12 h and then were infiltrated with paraffin for 24 h. Finally, sections of 8 μm were obtained and were stained with toluidine blue (Van Buggenhout *et al.*, 2008). Micrographs were obtained under 20× magnification with an Olympus E-420 digital camera (Olympus, Tokyo, Japan) adapted to and Olympus BH2 microscope (Olympus).

Colour analysis employing digital images

Image acquisition. A wooden box according to the design described in Mendoza & Aguilera (2004), with some modifications, was used to obtain the digital images of strawberries. Samples were illuminated using four fluorescent lamps (Osram, Biolux, Natural Daylight, 18W/965, München, Germany) with a colour temperature of 6500 K (*D65*, standard light source commonly used in food research) and a colour-rendering index Ra of 95%. The four lamps (60-cm long) were arranged as a square, 30 cm above the sample forming with it an angle of 45°. Additionally, electronic ballast and an acrylic light diffuser were used to ensure a uniform illumination system. Strawberries were cut transversally and photographed on a matte black background using the following camera settings: manual mode with lens aperture at $f = 8$ and time of exposition 1/80, maximum zoom, no flash, ISO sensibility 400, maximum resolution (3648 × 2736 pixels) and storage in JPEG and RAW formats. The camera was connected to the serial port of a personal computer provided with a remote-control driver (Olympus Studio 2) to visualise and acquire the images directly from the computer.

Image processing. An IT8 calibration card (Wolf Faust, Frankfurt, Germany) was photographed under the same conditions than strawberries and was used to obtain the International Colour Consortium (ICC) profile employing the CoCa 1.6 software (Andrew Stawowczyk Long, Australia). This profile was applied to strawberries images using Photoshop (Adobe Systems, Inc., San Jose, CA, USA). L, a and b average values (considering the whole sample) were obtained from histogram window and then were converted to L^*, a^* and b^* values as follows (Yam & Papadakis, 2004):

$$L* = \frac{L}{255}100 \quad (1)$$

$$a* = \frac{240a}{255} - 120 \quad (2)$$

$$b* = \frac{240b}{255} - 120 \quad (3)$$

Statistical analysis

Analysis of variance (ANOVA) was used, and when the effect of the factor was significant ($P < 0.075$), a multiple comparison of means was performed using the least significant differences (LSD) test. The statistical analysis was performed using Minitab 13.20 (Minitab Inc., State College, PA, USA).

Results and discussion

Weight loss

It is well known that quality losses of strawberries are related to the percentage of fruit weight loss (Galetto, 2006). Our results show that pretreatment with whey protein edible coatings without BW showed a tendency to decrease strawberries weight loss after thawing, and when 20% BW was included in the coating formulation, a significant reduction was observed. On the other hand, coating solution with 40% BW did not improve weight loss of strawberries (Fig. 1). The observed effect of formulation without BW and 20% BW might be related to the formation of a uniform coating on the strawberries, which could prevent fruit moisture loss and, as a consequence, weight losing due to water exudation. Furthermore, the presence of a lipid component, such as the BW, contributed to improve the water transfer resistance of the coating. Accordingly, Kester & Fennema (1989) showed that lipid presence was related to moisture transfer resistance of cellulose-based edible films after thawing. Interestingly, our results show that increasing BW concentration did not positively affect weight loss. These results are in congruence with our previous observations demonstrating that WPC-based edible films with 40% of BW showed a significant increase in the water vapour permeability after the freezing process possibly because of slight imperfections developed in the films due to contraction and expansion of the lipids in relation to slight fluctuations of storage temperatures (Soazo *et al.*, 2013).

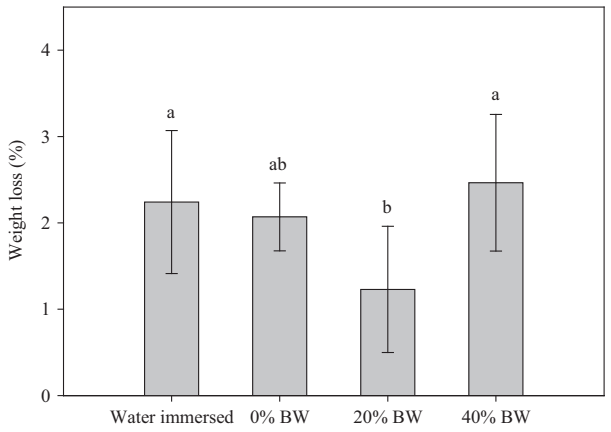

Figure 1 Weight loss of control and coated strawberries after thawing. Bars are based on standard deviations. Different letters show significant differences ($P < 0.075$).

Textural analysis

Cell lysis due to ice crystals formation during freezing produces an irreversible loss of turgor and firmness especially in fruits with delicate texture such as strawberries (Galetto, 2006). Parameters derived from force-deformation curves of controls and coated strawberries are shown in Fig. 2. After thawing, strawberries showed similar values of parameter F_{max} for all groups indicating that, apparently, whey protein-based coatings did not provide additional benefit for maintaining firmness. A possible explanation to these observations

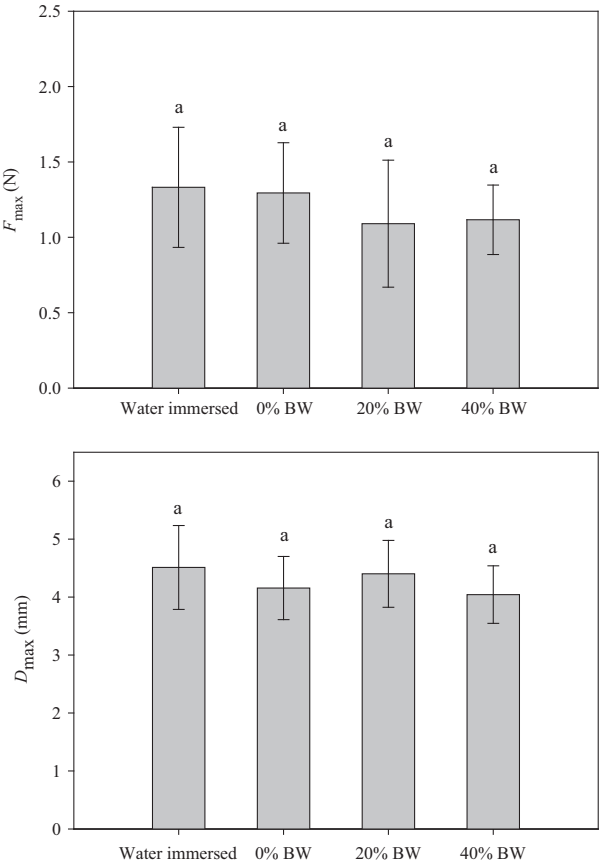

Figure 2 Parameters obtained from force/deformation curves of control and coated strawberries. Bars are based on standard deviations. Same letters are representative of no significant difference ($P < 0.075$).

has been already suggested by Bourne (2002). Penetration test evaluates the local fracture behaviour of a product. Thus, the application of a surface treatment such as an edible coating possibly had no significant effect on firmness because the edible coating formed on strawberries samples was of a thickness that does not affect the local response of the fruit to penetration.

According to Han *et al.* (2004), chitosan-based coatings helped to maintain textural quality of frozen *Totem* strawberries after thawing. However, among the three coatings studied by the authors, chitosan-containing calcium demonstrated the best result, probably because calcium may interact with pectic acid in cell walls to form calcium pectate, a compound helpful for maintaining fruit structure.

Microscopic analysis

Micrographs of fresh, control (water immersed) and coated strawberries stained with toluidine blue are shown in Fig. 3. Comparison with fresh strawberries indicated that cellular structure of frozen samples was somewhat damaged as a result of the freezing process. Van Buggenhout *et al.* (2008) showed that the structural damage of untreated strawberry tissue caused by freezing was large for all freezing methods applied but rapid and cryogenic freezing conditions were least harmful. Cells showed an alteration in both size and shape, and also a certain degree of cellular breakdown. As can be seen in Fig. 3, water-immersed control fruits exhibited little contact between cells and a collapsed appearance due to the low resistance of the tissue to the freezing process. In contrast, the histological sections of the coated fruits displayed more cellular adhesion zones and were densely stained indicative of more conserved membranes. Additionally, coated tissues appeared more organised ('honeycomb-like' structure) with some cells even maintaining their volume. Therefore, our results show that coated strawberries were lesser influenced by the freezing damage than water-immersed controls.

Suutarinen *et al.* (2002) found that the application of prefreezing treatments using calcium chloride and pectin methylesterase under vacuum, together with the quick freezing method used, presumably stabilised the original structure of the strawberries during freezing, jam making and storage. Van Buggenhout *et al.* (2008) also showed that vacuum infusion with pectin methylesterase and calcium seemed to stabilise the cell walls and the cell–cell contact maintained the cell wall integrity. Reno *et al.* (2011) submitted strawberries to freezing after pretreatments with high pectin concentrations and calcium chloride and showed that loss of cellular fluid occurred during the growth of ice in the intercellular spaces was retarded. To the best of our knowledge, in the literature, there is only one published report demonstrating the application of coatings to strawberries to maintain their textural quality after thawing (Han *et al.*, 2004).

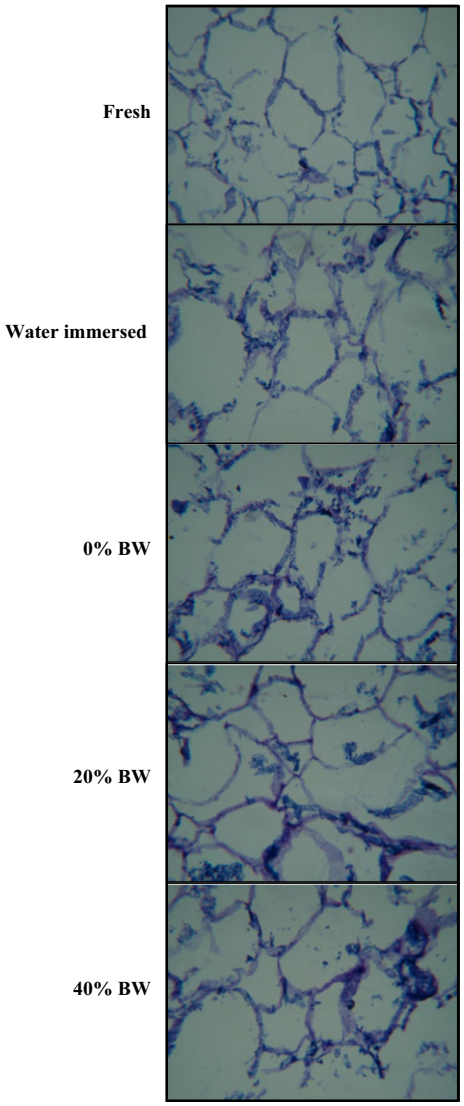

Figure 3 Micrographs of fresh, control and coated strawberries stained with toluidine blue. Magnification of 20× was used.

Colour analysis

Colour is one of the most important attributes of food, both for its aesthetic value and for quality judgement (Torreggiani *et al.*, 1999). Colour parameters L^*, a^* and b^* of control and coated strawberries are shown in Fig. 4. L^* measures the lightness or

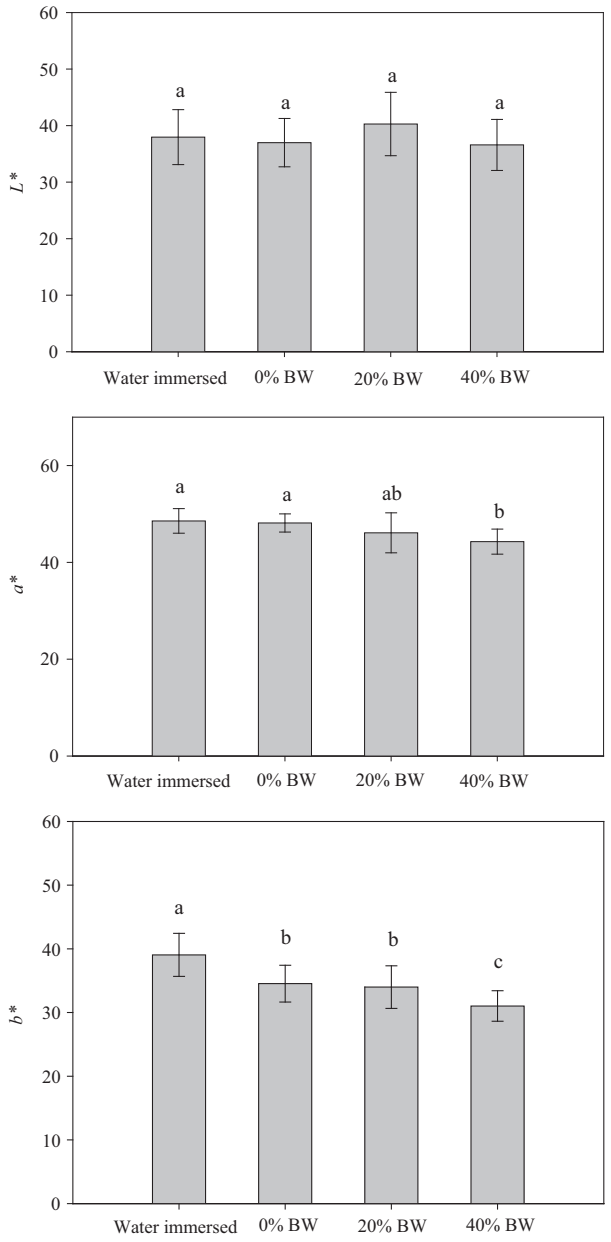

Figure 4 Colour parameters of control and coated strawberries. Bars are based on standard deviations. Different letters show significant differences ($P < 0.075$).

brightness of the sample, a^* hue from green to red and b^* shades of blue to yellow. As can be seen in Fig. 4, coated strawberries were as bright as control (similar values of lightness component, L^*). Only strawberries coated with solutions containing 40% BW were less red in comparison with controls (water immersed strawberries). On the other hand, WPC-coated strawberries presented lower b^* values and were less yellow than controls. In strawberries, the red colour is mainly determined by two anthocyanin pigments; these pigments are not very chemically stable and may change easily if not properly protected (Torreggiani *et al.*, 1999). Han *et al.* (2004) reported that different reactions may occur between anthocyanin and coating components that could justify the change of colour in maturation/ripening of raspberries. Our results suggested that there could be some interaction between the anthocyanin pigments and coating components that produced a decrease in red colour only in formulations containing 40% BW.

Conclusions

The obtained results in weight loss determination and penetration together with the structural changes evidenced by optical microscopy revealed the structural and textural deterioration due to loss of turgor by dehydration of strawberries. The application of whey protein-based coating with 20% BW was successful in preventing weight loss after thawing. The observed damage at the level of the shape and size of the cells was also partially attenuated by applying WPC-based edible coating. Only colour parameter b^* showed a slight tendency to decrease in all coated strawberries. The application of whey protein coating-forming solutions could be an alternative treatment attempting to maintain the quality attributes of strawberries submitted to rapid freezing.

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