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

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Running title: Pre-freezing coatings for strawberries

ABSTRACT

Pre-freezing application of whey protein concentrate (WPC) based edible coating to maintain quality attributes of strawberries was studied. BW was added to the solutions (0, 20 and 40% respect to the solids contained in the mixture WPC/Gly). 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 were 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: freezing; strawberries; edible coatings; whey proteins.

1. Introduction

Strawberry fruit 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 that 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 vegetable 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 non-seasonal fruits have contributed to an increase of the frozen food industry. This fact, has forced food technologists to work on the improvement of 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 pre-freezing treatments such as the addition of different sugars or the incorporation of calcium or enzymes acting specifically on the cell 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 minimize 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 pre-treatment 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 pre-freezing 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.,20*13). In order to advance with our previous investigation, the aim of the present study was to evaluate the pre-freezing application of whey protein based edible coating to maintain quality attributes of strawberries.

Materials and Methods

1.1. Materials

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

Strawberries of the cultivar *Winter Dawn* obtained from a local producer were selected according to colour and size. Damaged and non-uniform 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 30 fruits.

2.2. Coating solutions

Coating solutions were prepared as described in Soazo, Rubiolo and Verdini (2011). Briefly, 2 L of aqueous solutions of WPC were prepared; Gly (in proportion WPC/Gly 3:1) and potassium sorbate (final concentration of 0.1% w/w) were added. The mixture was magnetically stirred during 15 min for complete dissolution. BW was added to the solutions (0, 20 and 40% respect to the solids contained in the mixture WPC/Gly). Tween 80 was used as emulsifier only in the solutions containing BW (in relation 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 ºC for 30 min in a water bath (Dalvo Instruments, Argentina) to achieve BW melting and whey proteins denaturation. Emulsions were prepared employing an Ultra-Turrax T25 (IKA Werke, Germany) at 21500 rpm during 5 min. After homogenization, the emulsions were placed in an ice bath during 30 min to crystallize the lipid particles. The emulsions were degassed at room temperature with a vacuum pump.

2.3. 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 during 10 min in the basket and, after that, were dried at 5 ºC and 58% relative humidity (RH) during 90 min in an environmental chamber Tabai Comstar PR 4 GM (Tabai Espec. Corp., 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 ºC in the refrigerator until they were frozen.

2.4. Freezing process

Strawberries were frozen under a rapid freezing process. Briefly, fruits were placed in a basket, immersed in liquid nitrogen during 10 seconds and, subsequently, remained in contact with nitrogen vapours until the centre of the fruit reached -18 ºC. During the freezing process the temperature of the strawberries was monitored using an acquisition data system (Omega Engineering, Inc., USA) with T thermocouples. The time of immersion was selected in order to avoid the cracking of the fruit which produce an irreversible damage. After freezing, strawberries were placed in plastic trays inside of freezer bags, were stored in a domestic freezer at -20 ºC during 30 days and finally were thawed remaining 12 h in a refrigerator at 5 ºC.

2.5. Analyses

2.5.1. Weight loss

Weight loss was evaluated by weighting 15 strawberries, divided in subgroups of three, before and after the freezing process. The result was calculated as the percentage of loss respect to the initial weight.

2.5.2. Textural analysis

Penetration test was carried out in a room with controlled temperature and relative humidity (20 ºC and 50% RH) where fruits were equilibrated to ambient conditions during 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, USA) with a 10 N load cell and a cylindrical probe of 3 mm diameter were used. Penetration speed of 100 mm/min and penetration distance of 8 mm were used. Force-deformation curves were registered and analyzed 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}) (Galleto *et al.*, 2010).

2.5.3. 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 ºC in a refrigerator during 24 h. Then, were washed and dehydrated in ethanol solutions series (50, 70, 80 and 96%) during 12 h and, finally, in 100% ethanol for 24 h. Next, fixed-slices were clarified by immersion in ethanol/xylene mixtures (3/1, 1/1 and 1/3) during 12 h and xylene during 24 h. After that, the slices were transferred to paraffin/xylene mixtures (1/1 and 3/1) during 12 h and next were infiltrated with paraffin during 24 h. Finally, sections of 8 µm were obtained and were stained with toluidine blue (Van Buggenhout *et al.,* 2008). Micrographs were obtained under 20X magnification with an Olympus e420 digital camera (Olympus, Japan) adapted to and Olympus BH2 microscope (Olympus, Japan).

2.5.4. Colour analysis employing digital images

Image acquisition

A wooden box according to the design described in Mendoza and Aguilera (2004), with some modifications, was used to obtain the digital images of strawberries. Samples were illuminated using 4 fluorescent lamps (Osram, Biolux, Natural Daylight, 18W/965, 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 4 lamps (60 cm long) were arranged as a square, 30 cm above the sample forming with it an angle of 45º. 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 x 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 visualize and acquire the images directly from the computer.

Image processing

An IT8 calibration card (Wolf Faust, 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., 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):

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

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

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

2.5.5. 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., USA).

Results and Discussion

3.1. Weight loss

It is well known that quality losses of strawberries are related with the percentage of fruit weight loss (Galetto, 2006). Our results show that pre-treatment 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 (Figure 1). The observed effect of formulation without BW and 20% BW might be related with 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. In accordance, Kester and 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).

3.2. 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 Figure 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 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.

2.6. Microscopic analysis

Micrographs of fresh, control (water-immersed) and coated strawberries stained with toluidine blue are shown in Figure 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 is 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 Figure 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 organized ("honeycomb-like" structure) with some cells even maintaining their volume. Therefore, our results shows that coated strawberries were a little less influenced by the freezing damage than water-immersed controls.

Suutarinen *et al.* (2002) found that the application of pre-freezing treatments using calcium chloride and pectin methylesterase under vacuum, together with the quick freezing method used, presumably stabilized 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 stabilize the cell walls and the cell–cell contact maintained the cell wall integrity. Reno, Prado and Resende (2011) submitted strawberries to freezing after pre-treatments 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. Until we know, in the literature there is only one published report demonstrating the application of coatings to strawberries in order to maintain their textural quality after thawing (Han *et al.*, 2004).

3.4. Colour analysis

Colour is one of the most important attribute 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 Figure 4. L* measures the lightness or brightness of the sample, a* hue from green to red, and b* shades of blue to yellow. As can be seen in Figure 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 control (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.

3.5. 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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Figure captions

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

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 ($p < 0.075$).

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

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

Figure 1

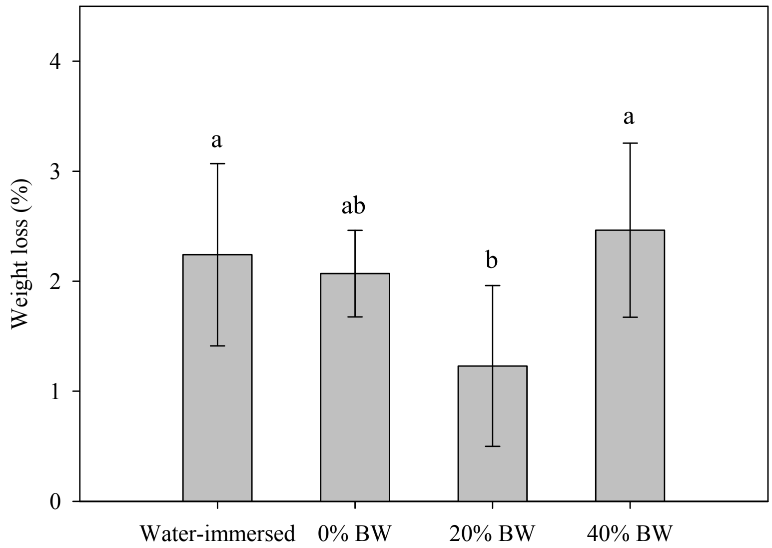

Figure 2

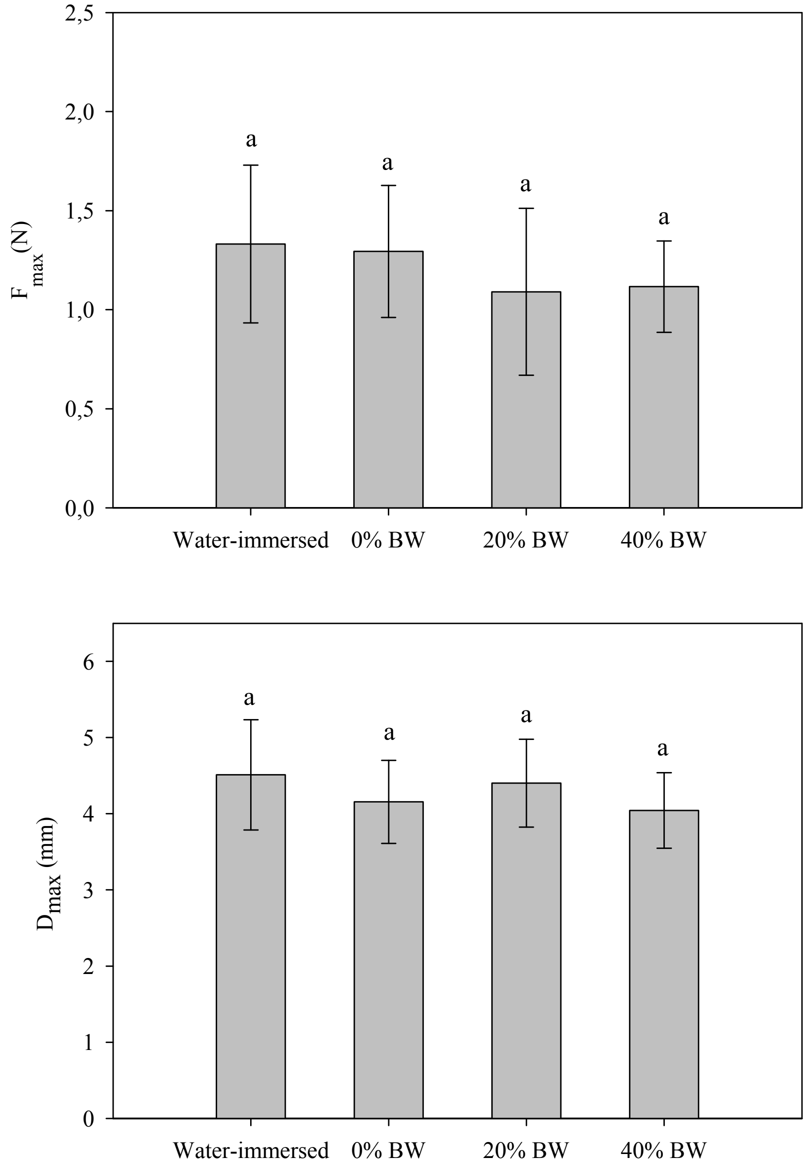
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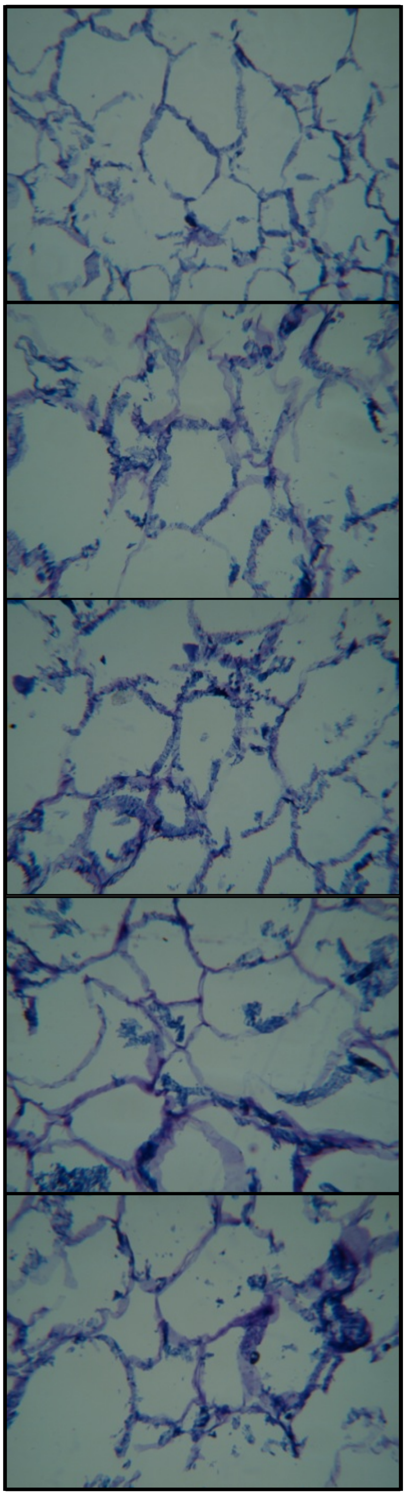
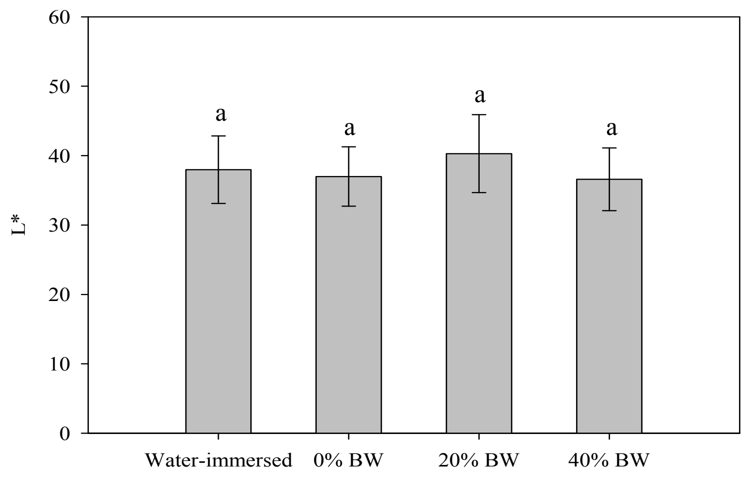
Figure 3

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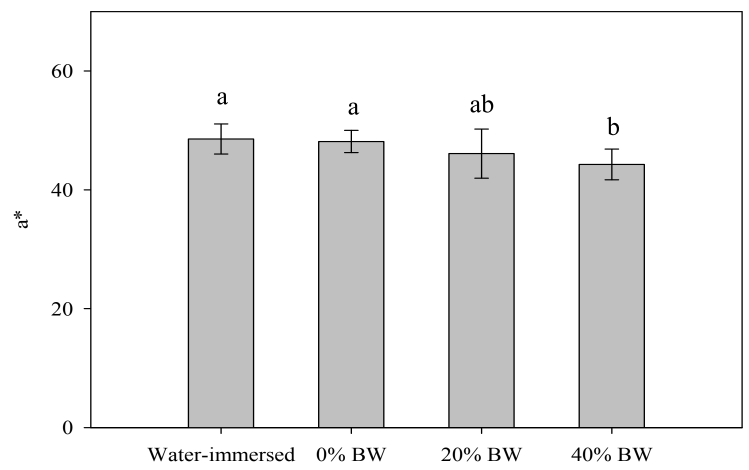
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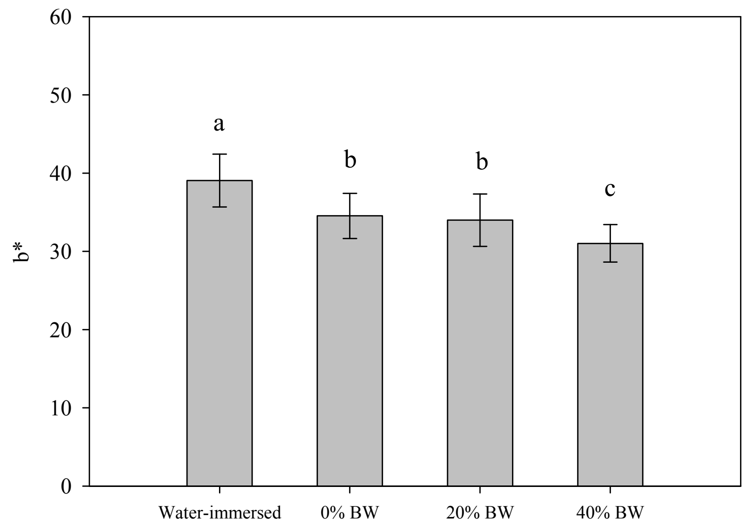
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