



Original Contribution

EDIBLE COATING BASED ON WHEY PROTEIN CONCENTRATE-RICE BRAN OIL TO MAINTAIN THE PHYSICAL AND CHEMICAL PROPERTIES OF THE KIWIFRUIT (*ACTINIDIA DELICIOSA*)

F. Hassani^{1*}, F. Garousi¹, M. Javanmard²

¹ Department of Food Science and Technology, Science and Technology Branch, Islamic Azad University, Tehran-Iran.

² Department of Food Science, Institute of Chemical Technologies, Iranian Research Organization for Science & Technology (IROST), Tehran-Iran.

ABSTRACT

Edible coating can provide effective protection for fresh fruits. In the present paper we use an edible coating based on whey protein concentrate (WPC) and four different levels of rice bran oil (0, 0.2, 0.4, and 0.6 g/100ml) to maintain the quality of kiwifruit (*A. deliciosa*). We have tested and compared the following postharvest storage quality conditions of coated groups and a control group in four subsequent weeks: weight loss, color parameters (L*, a*, b*), texture changes, titratable acidity, soluble solids content, and sensory attributes.

Coating application reduced the weight loss in kiwifruits. Our results indicate also a significant difference in the soluble solids content, and a non-significant difference in titratable acidity between the control and coated kiwifruits after 4 weeks of storage at 4°C. Moreover, our results show that kiwifruits coated with whey protein concentrate-based coatings had higher L*, and lower a* than the uncoated kiwifruits. Our coated kiwifruits received higher scores than the control samples in sensory evaluations. Coating reduced texture loss up to 5% with respect to the control samples depending on the rice bran oil contents.

Key words: Kiwifruit; Edible coating; Whey protein concentrate; Rice bran oil; Shelf-life.

INTRODUCTION

Botanically, the kiwifruit is a berry with numerous locules filled with many small, soft black seeds. Its green-colored flesh has three regions: the outer pericarp, the inner pericarp, and the columella (core). The columella is lighter than the outer and inner pericarps. The relatively thin brown skin includes aperioderm and hypodermal cells (1). Kiwifruit has good flavor and high vitamin C content (2). Currently about 1.5-1.6 million MT of kiwifruit are produced each year. About two thirds of current kiwifruit plantings are in the northern hemisphere and one third in the southern hemisphere. The most widely planted kiwifruit cultivar is *A. deliciosa* 'Hayward'. 'Hayward' and its associated pollenizer males

account for about half of kiwifruit plantings throughout the world. 'Hayward' fruit represents about 90-95% of the kiwifruit traded internationally (3).

Kiwifruit rots more rapidly than other fruits due to its high ethylene concentration in atmosphere and its sensitivity to ethylene (4). Many years of research are conducted to develop a material that would coat fruit so that an internal modified atmosphere would develop (5-6). Studies have shown that using a simple and environmentally friendly technology such as edible coating can retard the ripening of the fruit, can delay the change of its color, can reduce the loss of its water, and can improve its appearance (6-7). The concept of edible films as protection has been used since the 1800s (8). The first edible coating, which was used in China, was wax (9). For an extensive review on the historical and current uses of edible coatings see for

*Correspondence to: F. Hassani, Department of Food Science and Technology, Science and Technology Branch, Islamic Azad University, Tehran-Iran, E-mail: bhasani258@yahoo.com

example Han, 2005; Park, 2003 and Reinoso, et. al., 2008 (10-12). Extensive research in the area of edible coating has paved the way for different effective edible films and coatings. The purpose of the application of edible films or coatings is to inhibit migration of moisture, oxygen, carbon dioxide, or any other solute materials, to serve as a carrier for food additives like antioxidants or antimicrobials, and to reduce the decay of the food without affecting the quality of it. Edible coatings might offer a potential solution to form a barrier for the absorption of the fat during the frying process (13). Edible coatings can reduce moisture loss, can restrict oxygen uptake, can lower respiration, can retard ethylene production, can seal in flavor volatiles, and can carry additional functional ingredients (such as antioxidants and antimicrobial agents) that retard discoloration and microbial growth (14).

Edible coatings may be composed of polysaccharides, proteins, lipids, or a blend of these compounds (15). Some of the proteins that are used in coating formulations for fruits and vegetables are soy protein, whey protein, casein and corn-zein, maize, egg albumen, collagen and wheat (14). Edible films based on proteins were found to possess satisfactory mechanical properties (16). Polysaccharide and protein-based coatings have suitable gas barrier properties but show poor water vapor properties; while the lipid-based coatings help controlling the moisture loss but tend to be brittle and prone to oxidation (17). When used as coatings on food products or as films for separating the layers of heterogeneous foods, the whey protein films are mechanically adequate to provide the needed durability for the food (6). However, little research has been carried out for preserving kiwifruit with edible coatings. But the present study intends to provide a composite edible coating for the preservation of kiwifruit. The objectives of this investigation are 1) to develop methodologies for the formation of simple protein and composite protein-oil films based on WPC on kiwifruit, 2) to extend the postharvest life of Kiwifruit and to improve its sensory attributes.

MATERIALS AND METHODS

Materials

Using the hexane solvent, (some) rice bran oil was extracted in laboratory from the rice bran powder produced in Astaneh Asrafiyeh, Iran. WPC with 85% purity in protein was supplied from Arla Foods in Denmark. The glycerin

from Merck in Germany was added as a plasticizer to the film-forming solutions. Distilled water was used in all stages of our experiments.

Fruit selection and preparation

The kiwifruit that was used in this experiment was the *A. deliciosa* in variety. The fruits were provided from a local garden in Ramsar, Iran, and were soon transported to a laboratory for conducting the needed experiments on them. In shape, size, and coloration, the selected fruits were almost the same. Before conducting the experiment, and to eliminate any probable deflection, the fruits were washed in distilled water and were dried.

Rice bran oil extraction

Firstly, some rice bran was powdered and placed in a Soxhlet extractor. In the extractor, the oil of the powder was extracted using hexane solvent. Then, the oil was separated from the Hexane solvent, and the solvent was completely removed.

Edible coating application

For 30 seconds the kiwifruits were placed in a coating solution. Then, the coating was dried on them in room conditions. All the while, and in similar conditions, the control fruits (uncoated kiwifruits) were placed in distilled water. The coated fruits were maintained for 28 days in a place where the temperature was 5 degrees centigrade and the average humidity was 80 percent. The results of the experiment were regularly analyzed once a week.

Experimental design

Initially, all the planned tests were conducted on 10 kiwifruits the results of which were considered as the zero instant data. The rest of the fruits were divided into 5 groups: 1 group was the control group, and 4 other groups included the fruits coated in rice bran oil with different concentrations (0/0, 0/2, 0/4, and 0/6). The tests were conducted on the 7th, 14th, 21st, and 28th days. These weekly tests would determine the total soluble solids content, the titratable acidity, the firmness, the color, and the weight loss of the fruits. At the end of the 28-day testing period, the sensory attributes of taste and overall acceptability were also measured.

Coating formulations

In the present study four types of coating treatment were applied to the kiwifruits. In one type, the coating was without any rice bran oil,

while in the three other types it contained different percents of oil; that is, 0.2, 0.4, and 0.6. Here comes the coating formula which consists of 4 emulsions:

(Emulsion 1) 10 grams WPC + 0 g rice bran oil+ 100ml distilled water, (Emulsion 2) 10g WPC+ 3 g Gly+0.2 g rice bran oil + 100ml distilled water, (Emulsion 3) 10g WPC+ 3 g Gly +0.4 g rice bran oil +100 ml distilled water, (Emulsion 4) 10g WPC + 3 g Gly +0.6 g rice bran oil +100 ml distilled water. To provide these coatings, the WPC was firstly mixed with water. Then, to achieve total solution, an electronic mixer was used to stir the mixture for 15 minutes. The 10% (wt/wt) aqueous solution of WPC was held for 30 minutes in a 90 °C water bath in order to denaturize the whey protein (18). Afterwards, the solutions were cooled to the room temperature in an ice bath. Deionized water was used for all solutions. After the obtained solution was cooled in room temperature, the glycerine and rice bran oil were added to it. 0, 0.2, 0.4, and 0.6 of rice bran oil was added to the solution and the solution was finally homogenized. This film was obtained according to the formula of Shaw and his colleagues (19).

Film formation

The heat-denatured WPC films were prepared according to the method of Shaw (19). The films were cast by pipetting a 12-g whey protein solution onto rimmed smooth plates (100-mm i.d.) resting on a leveled granite slab. For each studied property, different numbers of replicates were prepared. The films were allowed to dry at room temperature from 24 to 48 hours. Then the films were peeled from the plates and were stored for 24 h at 50 ± 5% RH and 23 ± 2°C.

Water vapor permeability (WVP)

In this research, McHugh's method has been used to measure the water vapor permeability (WVP). 12 ml. of deionized water was poured in each of the test cells the openings of them were then covered by the film samples. After 2 hours, when the conditions became stable enough, to measure their weights, they were tested 12 times in a 24-hour period in time intervals of longer than 1/5-hour. At least three replicates of the film samples were tested. Water vapor transmission rate was measured, and WVP were calculated using the ASTM-E 96 method (ASTM 1995) as well as the

corrected WVP method proposed by McHugh, et. al.(18).

Determining the film thickness

The thickness of the film was measured with a caliper micrometer (Feinmesszeug SUHL-DDR, Tokyo, Japan) at five random positions. The WVP and mechanical properties were calculated based on the average thickness of the film.

Tensile test conditions

All (mechanical) tests were performed at 50 ± 5% RH and in a temperature of 23 ± 2°C using an Instron Universal Testing Instrument (model 2712-002, Instron Corp., Canton, MA) fitted with a 5-N static load cell. The tensile strength at maximum (TS), the percentage elongation at break (%E) and the elastic modulus (EM) were calculated as advised by ASTM D882 E 95 (1995).

Soluble solids content

The soluble solids content was determined every 7 days for 28 days. The total soluble solids in the juice were measured with a refractometer KrussD-22976 (Hamburg, Germany) using a sucrose scale calibrated at 20°C. Therefore, the amount of the total soluble solids obtained was compensated for weight loss as follows:

$$V = X (100 - \%WL_t) 100,$$

where X is the value for the total soluble solids obtained from the kiwifruit juice before the weight loss compensation, $\%WL_t$ is the percentage of weight loss at the time t , and V is the corresponding true value for the total soluble solids after the weight loss compensation.

Titrateable acidity

The titrateable acidity was measured according to the method of Lees (20). Ten milliliters of pressed kiwifruits juice was diluted with distilled water to 100 ml. Then, the diluted juice was titrated with 0.1mol/L sodium hydroxide to a pH of 8.1. The results were expressed as percent citric acid.

Determining the weight loss

Ten fruits for each specific condition were randomly selected and the fruits were weighed during the study with a laboratory weight balance Mettler AE 200-S (Greifensee, Switzerland) per replication. The results were expressed as percentage weight loss.

Fruit color measurement

The external fruit color was measured by a CIE $L^*a^*b^*$ system using a chromameter Minolta Model CR-300 (Minolta Co. Ltd., Japan). Their coloration was determined one time in each 7-day interval, and for 28 days. In this process, a white tile (L^* : 97.46; a^* : -0.02; b^* : 1.72) was used as reference.

Fruit firmness measurement

The firmness of the fruit was measured by a firmness-tester (Chatillon DFI-50) with a 6-mm point. Values were taken at four points on the circumference of each fruit. The results were expressed in Newton (N).

Sensory evaluations

A 9-point hedonic scale (on which 1 would mean "excellent" and 9 would mean "unacceptable"), was used to evaluate both the control and the treated kiwifruits for their taste. To take this step, 100 panelists (untrained consumers) were used for performing a consumer test. They were employees, students, and institute members who were recruited from the Iranian Research Organization for Science & Technology (IROST). In this test, the evaluators were provided with 1 sample of coded control (C) kiwifruits and 5 samples of coded treated (T) kiwifruits. The T group consisted of 4 samples: T1 was without any rice bran oil, while T2 had 0/2 gram, T3 had 0/4 gram, and T4 had 0/6 gram of rice bran oil. Panelists had to evaluate and rank the samples for their taste. An overall acceptability test was also done by a 5-point hedonic scale. In this test too, the panelists were provided with 1 sample of coded control (C) and 5 samples of coded treated (T) kiwifruits in the same containers. This time again the T group of fruits consisted of 4 samples: T1 was without any rice bran oil, while T2 had 0/2 gram, T3 had 0/4 gram, and T4 had 0/6 gram of rice bran oil. But this time the panelists had to evaluate and rank the fruits for their flavor, color, external shape, and also for their overall acceptability. In this test, rank 5 was considered to imply "the most acceptable" and rank 1 to imply "unacceptable."

Statistical analysis

The statistical plan of this research was prepared by complete randomized design. For each treatment, the plan consisted of 3 replicates. The data obtained from it were analyzed using MINITAB and SPSS softwares. The significant differences between samples

were determined using the analysis of variance (ANOVA), and the mean comparison was measured by Duncan's test method in which the maximum acceptable error is 5 percent. Also, the Matlab software (R2007a) was used to plotting the changes in different indicators and figures.

RESULTS AND DISCUSSION

Total soluble solids content (TSS) and titratable acidity (TA)

Figure 1.c shows the soluble solids content, usually called Brix, in both coated and uncoated (control) fruits that were maintained for 4 weeks in a certain temperature and humidity ($4\pm 1^\circ\text{C}$ where the average humidity was 80-85 percent).

When the primary soluble solids content of the fruit ($13/40\pm 0/66$) is compared to the Brix of the end of the storage period of kiwifruits, all of them show an increase in their Brix which in different treated samples is surely a little different. At the end of storage period, the lowest degree of Brix is found in the fruits which were coated with a coating of WPC and the 0.6 rice bran oil, that is with the T4 sample of treated fruits. On the other side, the highest degree of Brix or overall content of the soluble solid materials was found in uncoated (control) fruits. This might happened because of the higher amount of water-loss and the increase in concentration of the soluble solids.

The amount of titratable acids depends on the ripeness of the fruits, and causes a sour taste in them. As the fruit ripens the amount of organic acids decrease. In the harvesting period, the amount of organic acids depends on the soluble solid materials and also on the speed of acid degradation in them. As the fruit ripens, the organic acid dissolution depends on the fruit respiration rate. In the present research it has been shown that at the beginning of storage period, the titratable acidity ($3/90\pm 0/018$) in all kiwifruits is more stable than their acidity at the end of storage period. With the exception of those fruits coated with 0.2 of rice bran oil (T2 sample), the use of coatings on kiwifruits delayed the decrease in titratable acidity on their pulp (**Fig. 1.b**).

Coating with the whey protein at the different concentrations of rice bran oil especially in T3 formulation was found to be effective in the retention of TA compared to the other treatments and control samples.

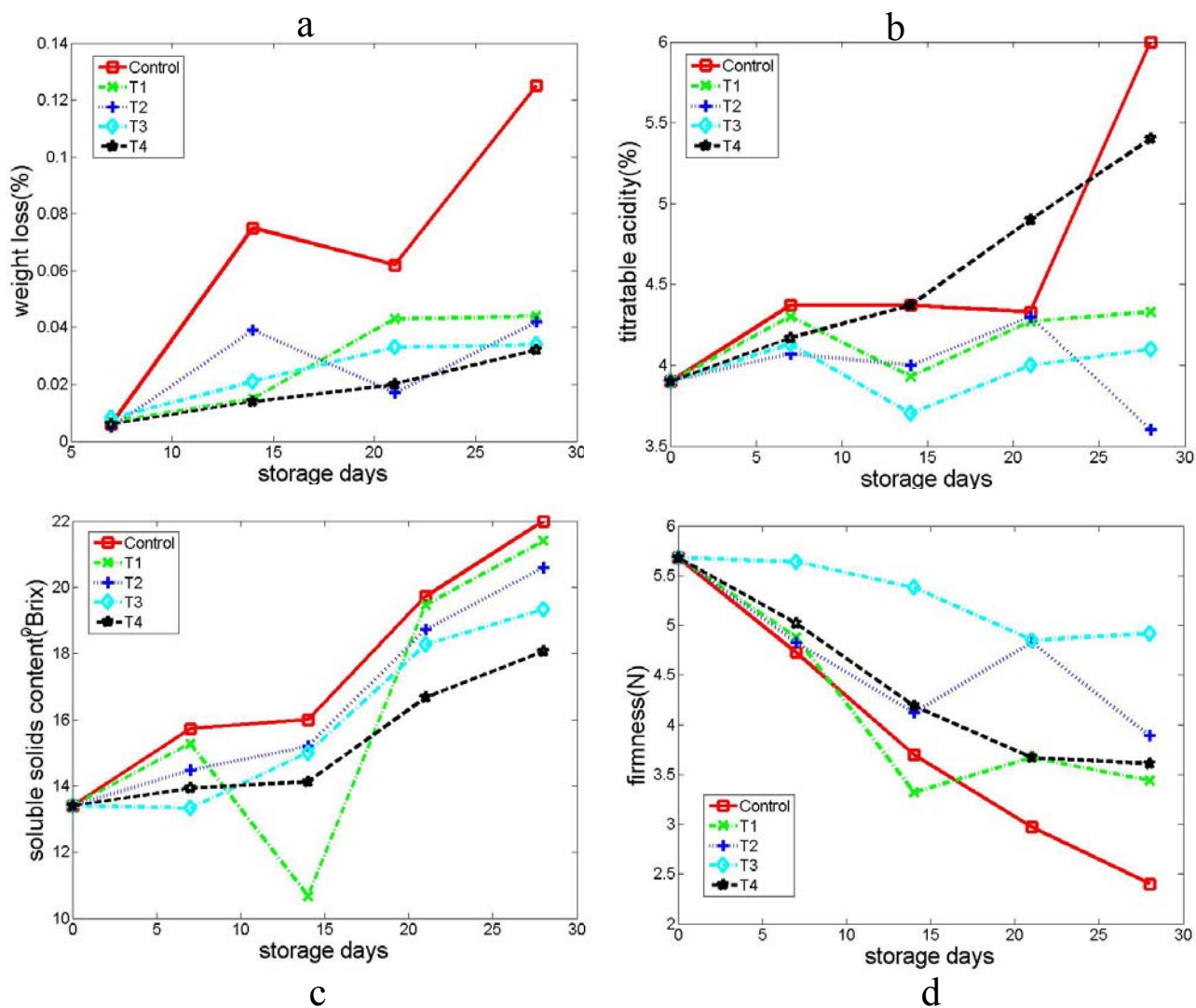


Figure 1. Weight loss (a), titratable acidity (b), soluble solids (c), and firmness (d) in whey protein concentrate-rice bran oil coated kiwifruits and uncoated samples during the cold storage period (control: uncoated, T1:10g whey protein concentrate (wpc) + 3 g gly + 100 ml distilled water+ 0 g rice bran oil, T2:10g WPC + 3 g gly +0.2 g rice bran oil + 100 ml distilled water, T3:10g WPC+3 g gly+ 0.4 g rice bran oil + 100 g distilled water, T4:10g WPC + 3 g gly + 0.6 g rice bran oil+ 100 ml distilled water).

Weight loss

We obtained the least amount of weight loss (0.032 %) for edible coatings containing the highest level of rice bran oil (T4). The fruits coated with T1, T2 and T3 solutions presented lower weight loss than uncoated samples. After 28 days of storage, the weight loss in fruits without any coating was highest (0.125%) (Fig. 1.a). The postharvest weight changes in fruits and vegetables are usually due to the loss of water through transpiration. This loss of water can lead to wilting and shriveling both of which reduce the commodity's marketability. Edible films and coatings can also offer a possibility to extend the shelf life of fresh-cut productions by providing a semi-permeable barrier to gases and water vapor; and therefore, reducing respiration, enzymatic browning, and

water loss (14,21). No shrinkage was detected in WPC-rice bran oil and rice bran oil (T3 and T4) coated fruits (Fig.3). Many experts (22-23) reported that even as little as 3.5-5 percent weight loss can lead to shrivel in kiwi. Only the T3 and T4 kiwis lost lower than 1.5 percent of initial weight, which is considered not enough to induce shriveling. When the coating application effect on weight loss was compared, a consistent effect was observed due to rice bran oil content. The increase in rice bran oil levels caused the kiwi weight loss to decrease. This result is in agreement with the behavior of stand-alone formed films, where an increase in rice bran oil decreased the WVP of the films.

Firmness

Figure 1.d shows the flesh firmness of coated and uncoated kiwifruits. Coatings reduced the texture loss up to 5 % with respect to the control samples depending on the rice bran oil contents. Firmness of coated kiwifruits remained practically constant, with significant difference in coated kiwi (the coated kiwis) with 0.4 rice bran oil content, whereas that of control kiwis decreased considerably after 4 weeks storage ($p < 0.05$). The use of edible coating with 0.4 rice bran oil content showed a significant ($p < 0.05$) effect on keeping texture. Texture loss is the most noticeable change occurring in fruits and vegetables during prolonged storage, and it is related to metabolic changes and water content (24).

Color changes

Lightness (L^*) values of coated kiwifruits were higher than those of uncoated kiwifruits. After 4 weeks storage, T2 samples had the highest

L^* value (**Fig. 2.c**). As was shown by changes in the L^* , a^* , and b^* values (**Fig. 2**), all when protein concentrate with and without rice bran oil coatings were effective in the retention of kiwi flesh color. Values of a^* (indicating changes of green to red colors) decreased and were higher in the control and coating with no rice bran content (T1) than in other coated fruit after 1 week of storage. The level of a^* value was high in control and coated samples without significant difference in second and third weeks of storage. After 4 weeks of storage the lowest a^* value was found in T2 samples (**Fig. 2.a**). In both control and treated samples, the b^* value increased after storage. T4 and the control samples had the lowest b^* value at the end of storage period and there was a non-significant difference between control and other treatments (**Fig. 2.b**). The coatings also were not effective in maintaining primary b^* value.

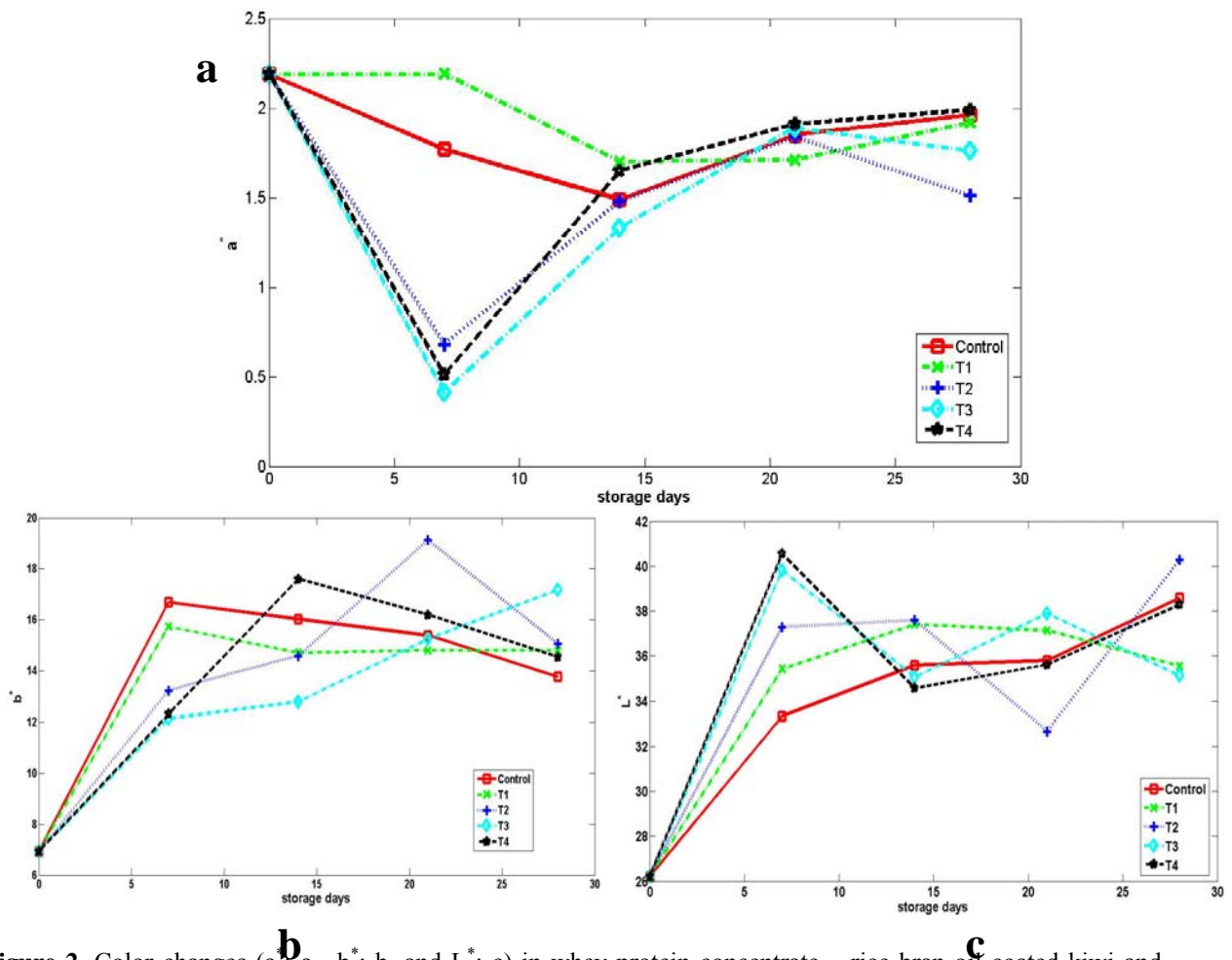


Figure 2. Color changes (a^* : a, b^* : b, and L^* : c) in whey protein concentrate – rice bran oil coated kiwi and uncoated samples during cold storage period (control: uncoated, T1:10g whey protein concentrate (WPC) + 3 g gly + 100 ml distilled water+ 0 g rice bran oil, T2:10g WPC + 3 g gly +0.2 g rice bran oil + 100 ml distilled water, T3:10g WPC+3 g gly+ 0.4 g rice bran oil + 100 g distilled water, T4:10g WPC + 3 g gly + 0.6 g rice bran oil+ 100 ml distilled water).

Sensory evaluation

Table 1 shows the sensory evaluations in different coated and uncoated kiwifruits. Coated and uncoated fruits were firstly stored for 28 days. Then, a ranking system was used

to compare them for their taste. According to our panelists, the treated samples with 0.4 (T3) and with 0.6 rice bran oil (T4) respectively received the best ranks. See also **Figure 3**.

Table 1. Sensory attributes of kiwies coated with WPC-rice bran oil edible coatings and uncoated (control) kiwies after 28 days at 4°C.

Sensory attribute	Control**	T1**	T2**	T3**	T4**
Taste	6.16±0.06 ^{a*}	4.33±0.23 ^b	4.27±0.37 ^b	7.50±0.56 ^c	7.00±0.06 ^c
Firmness	6.33±0.40 ^a	4.88±0.12 ^b	4.48±0.62 ^b	7.14±0.42 ^c	6.58±0.26 ^c
Overall acceptability	5.04±0.08 ^a	5.32±0.14 ^a	5.45±0.12 ^b	8.02±0.54 ^b	6.86±0.30 ^a

* Means with same superscript are not significantly different ($P > 0.05$)

** Control (uncoated), (T1) 10 g WPC+ 3 g Gly + 0 g rice bran oil, (T2) 10g WPC+ 3 g Gly+0.2 g rice bran oil + 100ml distilled water, (T3) 10g WPC+ 3 g Gly +0.4 g rice bran oil +100 ml distilled water (4) 10g WPC + 3 g Gly +0.6 g rice bran oil +100 ml distilled water.



Figure 3. Coated kiwifruits (left) and uncoated samples (right) after 4 weeks

Mechanical tests and physical properties of WPC-rice bran oil films

The increase in the levels of rice bran oil in films led to a decrease in Modulus (EM) and Tensile Strength (TS) and an increase in the elongation (E). The tensile tests showed that

the lipid functioned as an apparent plasticizer by enhancing the fracture properties of the emulsion films. As it can be seen in **Table 2**, an increase in the amount of rice bran oil causes a decrease in the film tensile strength.

Table 2. Physico-chemical properties of whey protein concentrate (WPC) - rice bran oil composites films

Film (WPC- Rice bran oil)	WVP* (g/mm/m ² h kPa.)	Tensile strength (Mpa)	Module (Mpa)	Elongation (%)	Extension (mm)
(10 /0.0)	7.55±0.18 ^{d**}	10.52±0.08 ^a	13.27±1.76 ^a	59.00±3.59 ^{ab}	39.73±4.12 ^a
(10 / 0.2)	7.32±0.19 ^d	7.74±0.28 ^b	14.66±0.86 ^a	65.17±2.28 ^{ab}	34.23±8.28 ^a
(10 / 0.4)	6.31±0.22 ^e	7.71±0.19 ^b	13.06±0.75 ^a	81.6±2.26 ^{ab}	37.07±0.21 ^a
(10 / 0.6)	6.24±0.81 ^e	5.19±0.68 ^c	10.74±0.18 ^b	96.92±4.32 ^c	38.77±0.78 ^a

*Water vapor permeability

**Values quoted are mean values ± standard deviations of results for six experiments. Means with the same letter are not significantly different. ($P < 0.001$)

Water vapor permeability measurement of films

At low and intermediate relative humidity (RH), protein and polysaccharide films are generally good barriers against oxygen, and have good mechanical properties. It can generally be suggested that with the increase of oil percentage, the WVP tends to decrease (See **Table 2**). In contrast with protein and polysaccharide films which provide rather penetrable barriers against water vapor (WVP), the lipid-based films are better barriers. As the present study shows, we have used this property of lipids against water vapor in our protein-based films.

CONCLUSION

The present research intends to analyze the effects of dipping kiwifruits in innovative coating solutions in cold storage. A composite coating of WPC protein and rice bran oil with added glycerol as plasticizer effectively preserved the color, firmness, taste, and the overall acceptability of the fruits during storage. The collected data in this study showed that the sensory attributes in uncoated samples were lower than in coated samples. During storage, the coated and uncoated samples showed considerable differences in the changes of their chemical values (their weight loss, soluble solids, and their titratable acidity). Overall, the most weight loss and color development was found in T1 samples which were coated in a composite film of WPC and 0.0 rice bran oil, whereas the least weight loss and color development was found in T4 samples which were coated in a composite film of WPC and 0.6 rice bran oil. The present research also shows that such coatings are most effective for delaying ripening the fruits. During the 28 days of storage, a composite coating of whey protein and rice bran oil can slow down the increase of acidity and weight loss in coated kiwifruits, can preserve their total soluble solid materials, and can maintain their firmness, color, and their sensory attributes.

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