



## RESEARCH PAPER

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## Shelf life extension of fresh-cut apples using whey protein concentrate, soy protein isolate, carrageenan and alginate films

Sharare Mohseni<sup>2</sup>, Reihaneh Ahmadzadeh Ghavidel<sup>1\*</sup>, Ahmad Fahim Adib Asl<sup>1</sup>

<sup>1</sup>*Department of Food Science and Technology, Islamic Azad University, Quchan Branch, Quchan, Iran*

<sup>2</sup>*Department of Applied Chemistry, Quchan Branch, Islamic Azad University, Quchan, Iran*

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### Abstract

Edible films and coatings have received considerable attention in recent years because of their advantages including use as edible packaging materials over synthetic films. This could contribute to the reduction of environmental pollution. By functioning as barriers, such edible films and coatings can feasibly reduce the complexity and thus improve the recyclability of packaging materials, compared to the more traditional non-environmental friendly packaging materials, and may be able to substitute such synthetic polymer films. The main objective of this work was to determine the optimal composition of edible coatings in view of their application to extend the shelf life fresh-cut apple. Coatings constituted by WPC (Whey Protein Concentrate), Soy Protein Isolate (SPI), carrageenan and alginates were characterized as coatings. Apples pieces were coated with the emulsion coatings. Color (CIE L\*, a\*, b\*, and browning index (BI)) and texture analysis and sensory evaluation were done during storage. Results show that edible coatings extend shelf-life of fresh-cut apples and keep the quality of the apples during storage.

\* **Corresponding Author:** Reihaneh Ahmadzadeh Ghavidel ✉ [reahmadzadeh2@yahoo.com](mailto:reahmadzadeh2@yahoo.com)

## Introduction

Fresh-cut fruit and vegetables offer to consumers highly nutritious, convenient and healthful commodities while maintaining the freshness of the non-processed products. Diets rich in fruit and vegetables can help in the prevention of diseases, including cancer and cardio- and cerebro-vascular diseases; due to the antioxidants they contain (Oliu *et al.*, 2008). Recently, there has been an increasing market demand for minimally processed fruits and vegetables due to their fresh-like character, convenience, and human health benefits. Minimal processing includes grading, washing, sorting, peeling, slicing, chopping, and then packaging (Lee *et al.*, 2003; Grau *et al.*, 2007; Belloso *et al.*, 2007; Carvajal *et al.*, 2009). Since minimal processing results in quality deterioration associated with water loss, softening, microbial contamination, increased respiration and ethylene, and cut-surface browning, minimally processed products become more perishable (Lee *et al.*, 2003). Consumers usually judge the quality of fresh-cut fruit on the basis of appearance and freshness at the time of purchase (Kader, 2002; Grau *et al.*, 2009).

The color of products, such as apple slices, is an important quality index. Brown apple slices are aesthetically unattractive (Lee and Lee *et al.*, 2003). Enzymatic browning is a major problem reducing shelf-life of fresh-cut fruit and vegetables due to the reaction of phenolic compounds with atmospheric oxygen diffusing into the tissue. This reaction is catalyzed by endogenous polyphenol oxidase, which is released through cutting injury of the fresh-cut products (Perez-Gago *et al.*, 2005). Several preservation technologies, including cold storage, UV irradiation, modified atmosphere packaging and ozonation, have been used to reduce deterioration, prolong shelf life, and retain the nutritional value of fresh fruits (Duan *et al.*, 2011). In addition, edible coatings have been studied for extending shelf life of some fresh fruits (Duan *et al.*, 2011). In recent years researcher has focused more on biodegradable films, including films made from plant and animal edible protein sources such as corn zein, wheat gluten

(Palmu and Grosso., 2005), soy (Denavi *et al.*, 2009) and peanut protein, cottonseed, albumin, gelatin (Andreuccetti *et al.*, 2011), collagen, casein and whey proteins (Seydim, 2006). Edible coatings may control the internal gas atmosphere of the fruit, minimizing fruit respiration rate and may serve as a barrier to water vapor, reducing moisture loss and delaying fruit dehydration (Duan *et al.*, 2011). The main advantage of edible films over traditional synthetics is that they can be consumed with the packaged products. There is no package to dispose even if the films are not consumed they could still contribute to the reduction of environmental pollution. The films are produced exclusively from renewable, edible ingredients and therefore are anticipated to degrade more readily than polymeric materials (Bourtoom., 2008; Guerrero *et al.*, 2010). The films can enhance the organoleptic properties of packaged foods provided they contain various components (flavorings, colorings, sweeteners) (Bourtoom., 2008). Several researchers have studied the application of coatings to vegetables such as carrot (Carvajal *et al.*, 2009), melon (Oliu 2008) and fruits like blueberries (Duan *et al.*, 2011), bananas (Sothornvit *et al.*, 2007), avocado (Pompa *et al.*, 2009), strawberries (Patricia and Grosso, 2005; Vargas, 2006; Ribeiro, 2007; Munz *et al.*, 2008). In this study we compare the effectiveness of four edible coating (alginate, carrageenan, WPC (Whey Protein Concentrate) and (SPI) Soy Protein Isolate) to prolong shelf-life of fresh-cut fruits.

## Materials and methods

### Materials

Whey protein concentrate (WPC) (Multi,Iran) had a protein content of 65% (dry basis), soy protein isolate (SPI) (90% protein content on dry basis) (Maxsoy,Iran), alginate (Food grade sodium alginate, Danisco, Denmark), carrageenan (Robertet, France), glycerol (99% reagent grade). Apples were harvested at a commercial orchard in Mashhad (Iran) and placed in cold storage at 4 °C until used. The apple was chosen for this experiment because of its wide use and the rapid browning of slices after cutting. Selected apples of uniform size and color were

washed in distilled water. Apple slices were treated in all test solutions for 2 min and drained for 5 min at room temperature (25 °C).

#### *Film perpetration*

##### *Whey protein films*

The films were prepared by slowly dissolving of whey protein 5 % (w/w) in distilled water on an Erlenmeyer flask shaker (Edmund Buhler, Germany) at 180 rpm for 1 h until the WPI was completely dissolved. Solutions were placed in a water bath (Schwabachw, Germany) at 90 °C and kept there for 30 min to denature the protein. Solutions were then cooled to room temperature, and Glycerol (50%, w/w) were added to plasticize the films. Solutions were stirred again on the shaker at 140 rpm for 10 min to reach to steady state solutions (ozdemir, 2008).

##### *Soy protein isolates films*

Film-forming solutions were prepared by slowly dissolving 5 g of SPI (minimum 90% protein content on dry basis) in a constantly stirred mixture of distilled water (100 ml) and glycerol (2.5 g). Glycerol was added as a plasticizer to overcome film brittleness and to obtain freestanding films. The solution pH was adjusted to  $10 \pm 0.1$  with 1 M sodium hydroxide. Alkaline conditions favor SPI film formation, presumably by aiding protein dispersion in film forming solutions. After heating the solutions for 20 min at 70 °C in constant temperature water bath (Rhim *et al.*, 2002).

##### *Carrageenan*

Carrageenan-based coating solutions (0.5 g/100mL) were prepared by dissolving carrageenan in distilled water. A 50:50 (w/w) mixture of glycerol and PEG 200 was used as a plasticizer (0.75 g/g carrageenan). The solutions were equilibrated at 70 °C and stirred vigorously with a magnetic stirrer bar for 40 min on a hotplate. The solutions were cooled to room temperature (Cho *et al.*, 2005).

##### *Alginate*

Edible coating solutions were elaborated from a base solution of alginate (Food grade sodium alginate) at

2% (w/v) and glycerol at 1.5% (v/v) which were prepared into sterile distilled water heated previously at 70 °C and stirred until total dissolution of the components. The solutions were cooled to room temperature (Oliu, 2008).

##### *Water content*

Water content (WC) was determined measuring weight loss of fresh-cut fruit, upon drying in an oven (Memmert600, Germany) at 105 °C until constant weight. The relative variation of water retention % WR in the coated product relative to the uncoated one was calculated as follows: (Garcia, 2002)

$$WR = \left[ \frac{WC_{coated}}{WC_{uncoated}} - 1 \right] \times 100$$

For each condition, results were obtained using all the samples from at least two different batches. The equilibrium water content was defined as the humidity reached at storage times.

##### *Colorimetric measurements*

Color measurements of different coated and uncoated samples were made periodically with image processing (Color characterization was carried out by using a flatbed scanner (HP, USA). Images were saved as a JPG format for further applications. Pieces with 400\*200 pixels Dimension separated from the center of each strips by using the Image J software). On apple slices per treatment using the CIELAB color parameters,  $L^*$ ,  $a^*$ , and  $b^*$ . Results were also reported as browning index (BI), defined as brown color purity, which is usually used as an indicator of browning in sugar-containing food products The following equation was used to determine BI (Gago,2006).

$$BI = \frac{(x-0.31)}{0.172} * 100$$

Where, x is the chromaticity coordinates calculated from the XYZ tristimulus values according to the following formula

$$x = X/(X + Y + Z)$$

##### *Texture analysis*

Firmness evaluation was performed using a Texture

Analyzer (CNS Farnell, USA) by measuring the maximum penetration force. Fruit wedges were cut in rectangular shaped 2.0cm high samples and were penetrated by a 4mm diameter rod. The downward distance was set at 10mm at a rate of 5mm/s and automatic return. Samples were placed so that the rod penetrated their geometric centre (Oliu, 2008).

#### Sensory analysis

Sensory properties were evaluated at room temperature (25 °C) by 10 inexperienced panelists. Panelists were served apple slices in randomly coded containers. Each panelist was asked to rate three apple quality attributes, color, firmness, and flavor and also overall preference.

#### Statistical analysis

All data were analyzed using the Analysis of variance (ANOVA) was performed to compare mean values of

different coatings and control samples. The Duncan multiple range test was applied to determinate differences among means at a 5% significance level.

## Results and discussion

### Water content and water retention

The water content and water retention of uncoated and coated fresh cut apple slices with different coatings are presented in Table 1. The analysis showed significant ( $p < 0.05$ ) differences of water content between coatings with SPI having the lowest, which agrees with the findings of Freitas *et al.* (2009) for a product from potato. The percent water retention of samples with different coatings ranged from 0.7 to 4.9 %. Samples with coating of alginate had the highest water retention and SPI had the lowest, which the differences were statistically significant ( $p < 0.05$ ).

**Table 1.** Water content (g per 100 g of fresh sample) and water retention (%) of uncoated and coated fresh-cut apple slices with different coatings.

Coatings	Water content (g/100g)	Water retention (%)
Carrageenan	$83.4 \pm 2.1^a$	$3.6 \pm 0.2^a$
Alginate	$84.5 \pm 3.2^b$	$4.9 \pm 0.3^b$
WPC <sup>2</sup>	$84.4 \pm 2.6^b$	$4.2 \pm 0.3^a$
SPI <sup>3</sup>	$82.5 \pm 3.5^c$	$0.7 \pm 0.1^c$
Control	$80.1 \pm 2.4^d$	-

All mean scores bearing different superscripts in columns are significantly different on application of Duncan's New Multiple Range Test ( $P < 0.05$ ).

**Table 2.** Water content (g per 100 g of fresh sample) and water retention (%) of uncoated and coated fresh-cut apple slices during storage.

Time	Water content (g/100g)	Water retention (%)
T1 <sup>2</sup>	$83.7 \pm 2.3^a$	$2.9 \pm 0.2^a$
T2 <sup>3</sup>	$85.0 \pm 1.8^b$	$4.4 \pm 0.3^b$
T3 <sup>4</sup>	$82.5 \pm 1.5^c$	$2.0 \pm 0.1^c$
control	$80.2 \pm 2.1^d$	-

All mean scores bearing different superscripts in columns are significantly different on application of Duncan's New Multiple Range Test ( $P < 0.05$ ).

Table 2 shows the percentage of water retention of coated and uncoated apple pieces with different coatings measured at the end of the storage periods 15 days at 4 °C. Analysis of variance showed that water content and water retention decreased at 5th and 15th day of storage, but in the 10th day of storage water

content had the maximum value between all days of processing.

### Color and browning index

Changes in the external color of fresh-cut fruits were monitored by measuring lightness ( $L^*$ ) (lightness),

(b\*) (yellowness), (a\*) (redness), and browning index (BI) during 15 days of storage at 4 °C.

An increase in enzymatic browning in apple pieces during storage was accompanied by an increase in

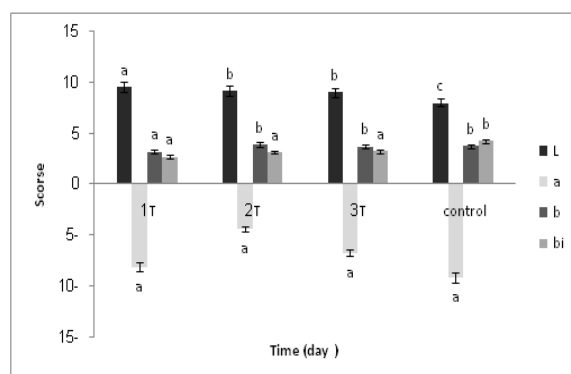
colorimetric a\* and b\* values, and a decrease in lightness (L\*). Browning index was calculated and used as a good indicator of color change during storage. Application of all coatings significantly reduced BI compared to control samples (Table 3).

**Table 3.** Color and browning index of uncoated and coated fresh-cut apple slices with different coatings.

Coatings	BI	b*	a*	L*
Carrageenan	3.18±0.3 <sup>b</sup>	3.88±0.7 <sup>a</sup>	-7.26 ±0.2 <sup>b</sup>	94.26 ±1.2 <sup>ab</sup>
Alginate	4.27±0.5 <sup>b</sup>	4.04±0.4 <sup>b</sup>	-4.36 ±0.4 <sup>c</sup>	81.61±2.5 <sup>c</sup>
WPC <sup>2</sup>	2.35±0.1 <sup>a</sup>	3.23±0.3 <sup>a</sup>	-8.6 ±0.6 <sup>a</sup>	96.96±1.6 <sup>a</sup>
SPI <sup>3</sup>	2.56±0.2 <sup>a</sup>	3.44±0.1 <sup>a</sup>	-7.47 ±0.3 <sup>b</sup>	91.85±2 <sup>b</sup>
Control	5.65±0.4 <sup>c</sup>	5.38±0.6 <sup>c</sup>	-2.63 ±0.7 <sup>d</sup>	78.39±1.4 <sup>d</sup>

All mean scores bearing different superscripts in columns are significantly different on application of Duncan's New Multiple Range Test (P< 0.05).

Lightness of fresh cut apple slices was improved in samples coated with whey protein concentrate and carrageenan. Samples coated with whey protein based coatings had significantly higher L\* values than other coatings and control apples.

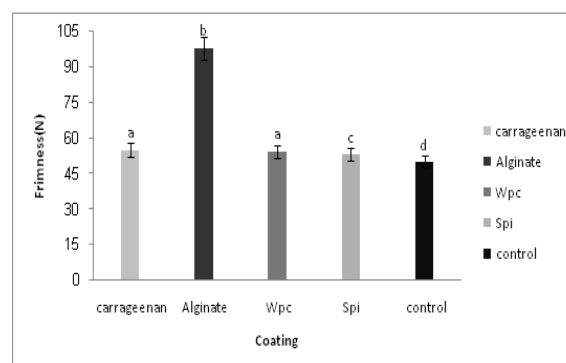


**Fig. 1.** Effect of coatings on color and browning index of uncoated and coated fresh-cut apple slices during storage.

Fig 1 shows the effect of coatings type on BI, L\*, a\* and b\* of fresh cut apples stored 15 days at 4 °C. The results shows that BI didn't increase during storage, but L\* and b\* increased after 5th day.

In the previous work, Gago P (2006) found that apple pieces coated with whey protein-based coatings had higher L\*, and lower b\*, a\*, and BI-values than HPMC-based coated and uncoated apple pieces, which indicate that whey proteins exert an antibrowning effect. Coatings containing BW were

more effective in decreasing enzymatic browning than coatings containing Carrageenan. The sensory panel differentiated samples coated with whey protein-based coatings from samples coated with HPMC based coatings.

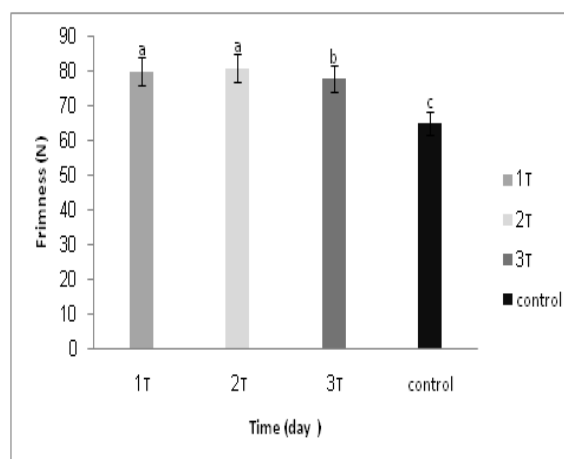


**Fig. 2.** Effect of coatings on firmness of uncoated and coated fresh-cut apple slices with different coatings.

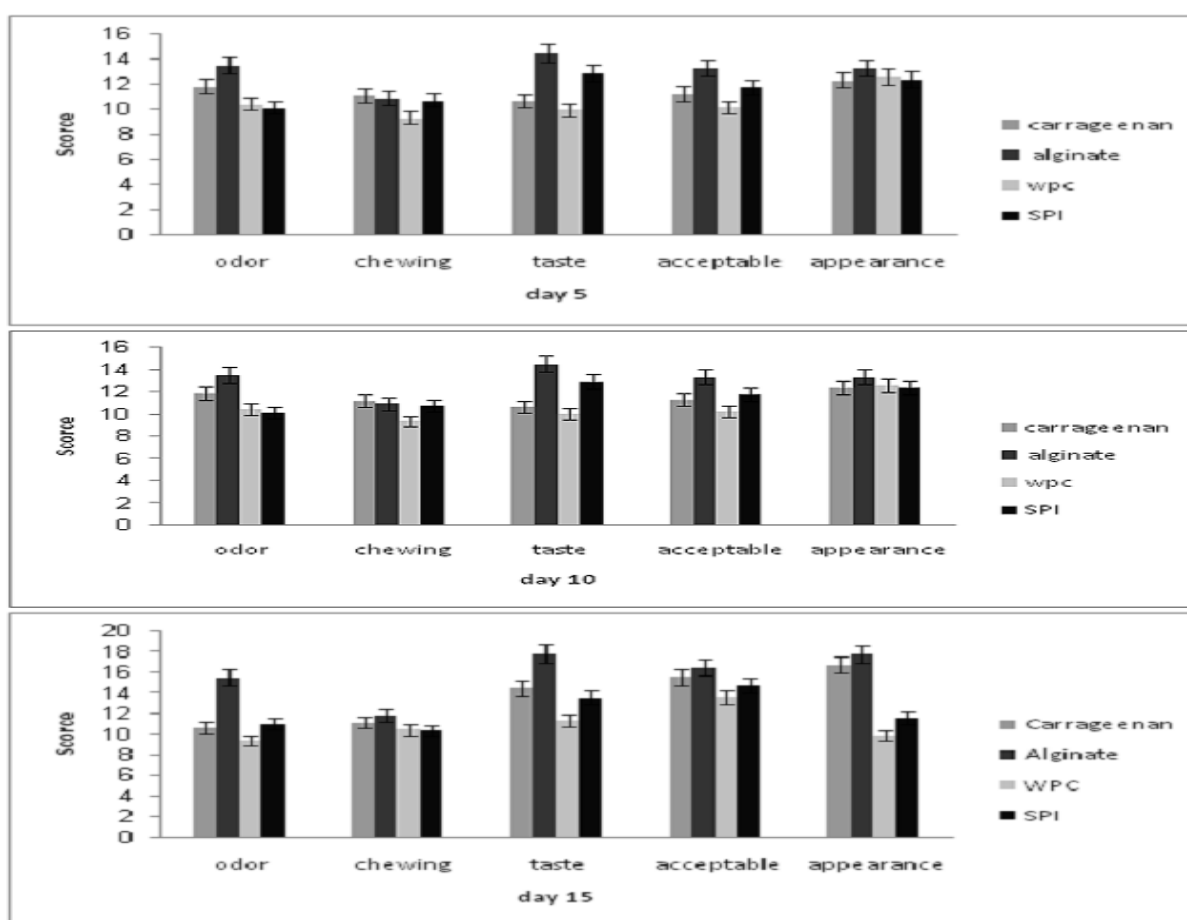
Grau M, *et al.* (2008) found that The effect of alginate and gellan-based edible coatings on the shelf-life of fresh-cut Fuji apples packed in trays with a plastic film to measure changes in color during 23 days of storage at 4 °C, Alginate and gellan edible coatings effectively prolonged the shelf-life of Fuji apple wedges by 2 weeks of storage compared with the control apple slices which showed a considerable cut surface browning and tissue softening from the very early days of storage, limiting their shelf-life to less than 4 days.

### 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. Softening observed in fresh-cut apples may be due to the pectic acid undergoing acid hydrolysis. Changes in flesh firmness between control and treated fruit during 15 days of storage at 4 °C are shown in (Fig: 2, 3). The firmness of uncoated apples pieces decreased during 15 days of storage, showing a substantial softening of tissues. By contrast, the use of edible coating applied on the pieces of cut apple showed a significant ( $p < 0.05$ ) effect on keeping texture.



**Fig. 3.** Effect of coatings on firmness of uncoated and coated fresh-cut apple slices during storage.



**Fig. 4.** Sensory characteristics of fresh cut apple slices coated and uncoated with different coatings during 15 day at 4 °C.

Alginate coating showed a beneficial result on firmness retention of apple wedges during the entire storage period. In a found that alginate edible coatings applied to fresh-cut apples were effective in controlling moisture loss when the formulation contained 0.025 ml sunflower oil/100 ml film

forming solution. Thus, the use of sunflower oil could maintain texture due to the oil-mediated moisture retention of the coated fruit. Whey protein concentrate showed constant firmness throughout two weeks of storage.

Munz (2006, 2008) reported that Chitosan coatings delayed changes in firmness compared to untreated strawberries.

#### *Sensory analysis*

Changes in sensory attributes including odor, color, taste, firmness and overall preference of coated and uncoated fresh-cut fruits during 15 days of storage with different kind of packaging are shown in (Fig: 4,5). All edible coating treatments resulted in higher sensory scores than uncoated fruits for all quality factors tested. Carrageenan coated apples showed the best overall preference up to 1 week, but their fresh colors were not maintained for the 2 week test period. But the odor was kept during storage. WPC coating resulted the highest overall preference after 2 week storage was due to good color, appearance and odor of fruits slices. Fresh-cut fruits coated with alginate had lower scores for odor and taste attributes than carrageenan and WPC coatings. Alginate had a salty taste, but highest firmness. SPI coating wasn't keeping the appearance during storage and became yellow after 2 week, but the taste was acceptable.

Grua (2008) worked on Alginate-based edible coating containing malic acid to improve shelf-life and safety of fresh-cut melon and found that fresh-cut melon, since has a good acceptance by panelists, maintains the quality parameters of the fruit. Oms.G (2008) found alginate or pectin-based coatings containing the antibrowning agents were effective in keeping sensory quality for 2 weeks.

#### **Conclusions**

The result showed that the edible coatings proved to extend the shelf-life of fresh cut apple slices by decreasing delaying changes in color during storage. Among the films tested, whey protein and soy protein isolate showed the most effective properties. Addition of sunflower oil to alginate coating improved the texture quality and kept water retention of fresh-cut fruits. Also sensory evaluation on quality of the products showed that using edible films influence sensory characteristics of the fruit maintained visual quality during shelf life and the consumers approved

the taste and flavor of the edible coatings fruits.

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