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Full Length Research Paper

An application of methylcellulose-whey protein films on Japanese persimmon

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Enzymatic and non-enzymatic browning reactions, water loss, oxidation and microbial growth can cause decreases in the quality of fresh fruits. Packaging can be an effective way to control and prevent these undesirable changes. Edible films and coatings having low water vapor permeability (WVP) are used to extend the shelf life of these perishable foods. Use of the methylcellulose-whey protein (MC-WP) based edible films which have low WVP may retard ripening and softening of fruits. In this study, Japanese persimmons (Diospyros kaki L.) were wrapped with MC-WP films and stored at 10 and 25°C. Changes in the weight and firmness of the fruits during storage were investigated. Wrapping had no effect on weight losses and firmness of the fruits stored at 10°C. On the other hand, wrapping had no significant effect on firmness but caused an increase in weight loss of the fruits stored at 25ºC. The wrapped fruits showed higher weight loss than non-wrapped ones at 25°C after the 10 days. The appearance of wrapped fruits was better than non-wrapped ones at the end of storage, since mold growth on non-wrapped fruits was observed.

Key words: Edible film, packaging, *Diospyros kaki* L., shelf-life, storage.

INTRODUCTION

Deterioration of food quality due to physicochemical changes or chemical reactions is often caused by mass transfer between food and the surrounding medium. Since water, oxygen, and some water soluble compounds are responsible for such deteriorations, transfer of these compounds must be controlled (Morillon et al., 2002). For this purpose, food products are packed in different packaging materials such as plastics, papers etc. On the other hand, some of these packaging materials such as plastics cause considerable waste problems due to their low biodegradability. Therefore, new biodegradable packaging materials have been developed to alleviate this problem (Kranz et al., 2000; Chen and Wang, 2002; Turhan and Sahbaz, 2004; Martino et al., 2006). One of the most conspicuous biodegradable packaging materials is edible films/coatings.

Edible films can improve food quality, extend shelf-life, add some beneficial functional properties to foods and reduce the use of synthetic packaging materials. Edible films are typically prepared from various combinations of proteins, polysaccharides and/or lipids (Erdohan and Turhan, 2005). Although, use of edible films and coatings to preserve food quality and to extend shelf life is not a novel concept, research in this field has been intensifying since 1970 (Miller, 1970; Fennema et al., 1990; Gennadios et al., 1997). While many researches have been focused on composition, preparation and storage conditions which affect the film properties (Ayrancı and Çetin, 1995; Famá et al., 2005; Bourtoom and Chinnan, 2008), only some are focused on the application of these edible materials for food packaging (Lerdthanangkul and Krochta, 1996; Cisneros-Zevallos and Krochta, 2003; Artharn et al., 2009).

The polymeric materials for edible packaging are so varied and their combinations are so diverse that an appropriate film or laminate can always be found for a specific application. One criterion to consider in choosing

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Abbreviations: **WVP,** Water vapor permeability; **MC,** methylcellulose; **WP,** whey protein; **MC-WP,** methylcellulose-whey protein; **Gly,** glycerol; **LSD,** least significance difference; **RH,** relative humidity.

a specific edible film or coating for a given application is their cost. By-products of food and other industries, like whey and cellulose derivatives, can be used to reduce the cost of these materials (Morillon et al., 2002).

Whey is a by-product of the dairy industry in which the principal components are lactose, proteins and mineral salts. Worldwide production of whey is about 115 to 125 million tons annually (Fernandez et al., 2007; Saddoud et al., 2007). Approximately 47% of the whey is disposed to the environment which represents a significant loss of resources and creates serious pollution problems (Fernandez et al., 2007). To alleviate this disposal problem, whey proteins are isolated and used as a food ingredient, especially in bakery products or nutrient supplement for athletes and infant formulas. As an alternative way to increase whey protein usage, researches on the production of edible film and coating have been accelerated for the last two-decades (Banerjee and Chen, 1995).

Cellulose is one of the other most abundant natural biopolymers. It is composed of unbranched, linear chains of D-glucose molecules. It is obtained from renewable sources such as agricultural wastes. Cellulose and its derivatives are capable of creating tough and flexible edible films. Methylcellulose (MC) which is the least hydrophilic among the cellulose derivatives has been used to produce edible films and coatings (Turhan and Şahbaz, 2004).

The application of edible film or coating having selective permeability characteristics, especially to water vapor, O_2, and CO_2, can help control overall quality of fruits and vegetables. The quality parameters of these perishable products are weight loss, enzymatic browning, texture deterioration, and microbial growth (Cisneros-Zevallos and Krochta, 2003; Lin and Zhao, 2007). Weight loss can accelerate ripening and increase incidences of physiological disorders and diseases. Along with other standard postharvest technologies (refrigeration, high relative humidity storage and tray liners), edible film and coating applications can also reduce water loss (Banks et al., 1997). Lipid based edible coatings are usually preferred for fruits and vegetables, since the permeability of them is much lower than polysaccharide or protein based ones (Gennadios et al., 1997; Morillon et al., 2002). On the other hand, most lipid based films or coatings are brittle when used alone, so they are often combined with polysaccharides or proteins. However, combining lipids with a hydrocolloid usually causes an increase in permeability of the film (Hernandez, 1994; Morillon et al., 2002). Erdohan and Turhan (2005) showed MC-WP films have lower water vapor permeability (WVP) than many MC-lipid and WP-lipid films.

Japanese persimmon (*Diospyros kaki* L.) is a subtropic, climacteric fruit. The fruit is harvested when it has attained a yellow to reddish color while still firm, and harvesting period is short (2 to 4 weeks) (Harima et al., 2003). The most limiting factor for distribution and storage of this fruit is rapid softening occurring during the postharvest period (Ramin, 2008). Cold storage, chemical treatment, or modified atmosphere packaging with polymer bags/films have been used to extend the shelf life of Japanese persimmon. Depending on the variation of the fruit and storage conditions, shelf life of the fruit changes between 1 to 6 months (Harima et al., 2003; Fernandez et al., 2007; Ramin, 2008). Application of the MC-WP based films on perishable fruits like Japanese persimmon may be an alternative way to prevent quality losses in them. The objective of this study is to investigate the potential of a MC-WP film to extend the shelf life of Japanese persimmon.

MATERIALS AND METHODS

Methylcellulose (MC, MW 41 000) was purchased from Sigma Aldrich Ltd (Poole, Dorset, UK; Cat. No.274429). Whey protein (WP, 91 % protein) was provided by Euro Proteins (Wapakoneta, OH, USA). Japanese persimmons were obtained from a local farmer near Yaprakli village, Mersin, Turkey, in November. The fruits were harvested by hand when they reached commercial index, transferred in cases to the laboratory. Any previous treatments were not applied to the fruits. The mean weight value of the fruits was 107±16 g.

Film formation

The films were prepared with MC, WP and glycerol (Gly) according to Erdohan and Turhan (2005). The mass ratios of MC/WP and Gly/total polymer in the film solutions were adjusted to 0.3 to 0.8 (w/w) and 0.5 (w/w), respectively.

Film Thickness

Thickness was measured at five random points for each film, using a digital micrometer (Mitutoyo Co. Ltd, Japan; ± 0.001 mm). The average film thickness was 0.041±0.004 mm.

Film properties

The water vapor permeability of the films was determined gravimetrically according to ASTM E96–80 (1983), at 25±1°C and 0 to 53% relative humidity (RH) difference. WVP tests were repeated in triplicate for each film.

A texture analyzer (Model TA-XT2; Stable Micro Systems, Surrey, UK) was used to determine the mechanical properties of the films, according to ASTM D638M (1993). Film specimens were cut into strips of 40×6 mm. The film strips were equilibrated in a desiccator, containing saturated $MgNO_3$ solution (52±2% RH) at 25±1°C for 48 h. Mechanical tests were repeated five times for each film.

Film application

Japanese persimmons were tightly wrapped with the MC-WP films and sealed with adhesive tape. Wrapped fruits and non-wrapped fruits were stored at 10 and 25°C in separate incubators. Relative humidity in the incubator was measured 45±5% during storage.

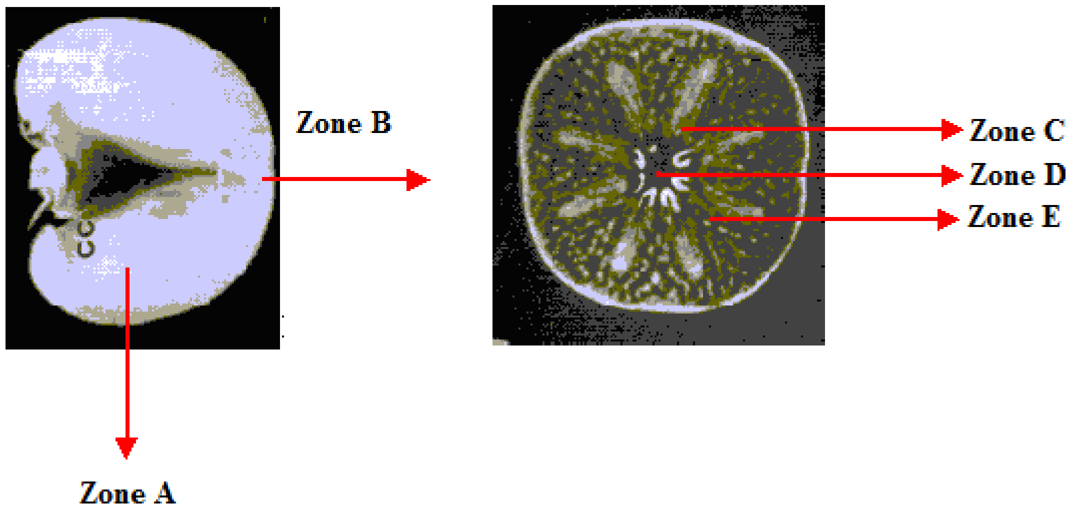

Figure 1. Firmness was measured in five designated zones of Japanese persimmon.

Table 1. The effect of MC content on WVP and mechanical properties of MC-WP films.

MC:WP (w/w)	WVP (g mm/ m^2 kPa)	Tensile strength (N/mm^2)	Elongation (%)
0.3	0.214±0.017^a	1.2±0.4^c	10.0±2.1^d
0.4	0.103±0.008^b	2.4±0.2^b	19.6±1.4^c
0.6	0.086±0.009^c	4.0±0.1^a	33.3±2.0^b
0.7	0.060±0.004^d	4.0±0.2^a	37.0±2.7^a
0.8	0.030±0.004^e	4.2±0.5^a	40.0±1.7^a

Weight loss

Weight loss of the fruit was measured gravimetrically (± 0.0001 g, Sartorius, BP221 S, Germany) and expressed as a percentage of initial fruit weight. Three fruit samples were chosen randomly to measure the fruit weight loss.

Fruit firmness

Firmness was measured at five different zones (A, B, C, D and E) of the fruit due to textural heterogeneity of persimmon. A texture analyzer (Model TA-XT2; Stable Micro Systems, Surrey, UK) having a needle plunger (SMS P-2N) was used to measure firmness of the fruits at different intervals during storage. A resistance force (N), penetrating 1 cm of the fruit, was used to measure each fruit at the defined zones (Figure 1). Measurements at zones A and B were taken without peeling fruit. Measurements belonging to C, D and E zones were taken on horizontal cross sections. Total firmness was calculated the sum of A, B, C, D and E. The experiments were carried out in triplicate and the results averaged.

Statistical analysis

Statistica® 6.0 (StatSoft Inc, 1984 to 1985) was used to perform analysis of variance (ANOVA) test, and the significance ($p<0.05$) of the differences was determined using a Least Significance Difference (LSD) test.

RESULTS AND DISCUSSION

Film properties

The effect of MC concentration (MC/WP ratio = 0.3 to 0.8, w/w) on the film properties was investigated. Increasing the MC concentration in the film solution caused a significant decrease on WVP ($p<0.05$) (Table 1).

Polymer structures significantly affect the WVP of packaging films. Simple linear polymer chains, as in WP, are loosely packed and have high permeability (Erdohan and Turhan, 2005). Moreover, whey proteins are incompatible with MC when the solution is heated to a temperature that allows WP denaturation and gelation. At this temperature, polymers form phase-separated gels with two phases forming at equilibrium: one rich in WP and the other rich in MC. In this study, MC was added after protein denaturation. The mixture was then homogenized with one phase probably dispersing in the continuous phase. Transport properties of the films were strongly affected by the addition of MC, which does not bind to whey protein. MC decreased WVP of the films by increasing the solid content and producing a smaller pore size (Erdohan and Turhan, 2005). The WVPs of MC–WP films determined in this work were significantly lower than

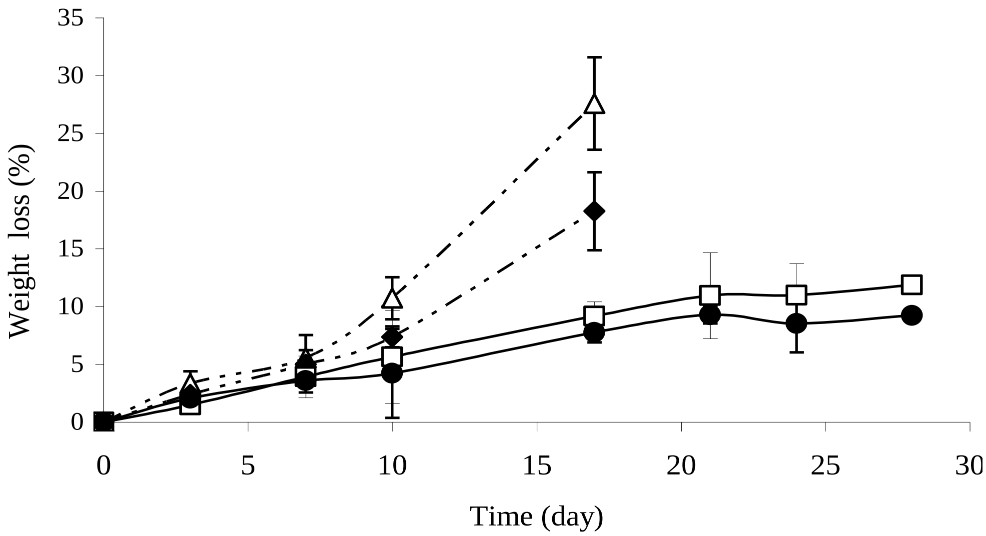

Figure 2. Weight losses of wrapped and non-wrapped Japanese persimmon during storage at different temperatures. Error bars indicate standard deviation (n=3). Δ→ wrapped fruit stored at 25°C, ◆→ non-wrapped fruit stored at 25°C, □→ wrapped fruit stored at 10°C, ●→ non-wrapped fruit stored at 10°C.

that of WP–lipid and MC–lipid films in literature (Debeaufort et al., 1998; Pérez-Gago et al., 2003; Fernandez et al., 2007; Ozdemir and Floros, 2008). Based on these results, the use of MC–WP films is preferred over lipid based edible films for reducing WVP.

The increase in MC/WP ratio in the solution from 0.3 to 0.8 (w/w) caused an increase in tensile strength (TS, 1.2±0.4 to 4.2±0.5 N/mm^2) and elongation (E, 10.0±2.1 to 40.0±1.7%). Significant increases in TS and E of the films were observed with increasing MC concentration ($p<0.05$) (Table 1). According to Banerjee and Chen (1995), cellulose films have comparatively high TS, since cellulose and its derivatives generally consist of long straight chains that impart greater molecular order, thereby resulting in strong and rigid films. Moreover, the linear structure of the polymer backbone gives flexibility to these films (Hernandez, 1994). The elongation of WP films (10 g WP and 0.3 g Gly /100 ml solution) was reported to be 16% (Pérez-Gago and Krochta, 2001). Compared to the results obtained by Pérez-Gago and Krochta (2001), although the WP amount in film forming solution was lower than the ones used by these authors, E percentages of the MC-WP films were higher. These results were considered that MC improved the mechanical properties of the MC-WP films in comparison to WP films.

Although the film with the highest MC content (MC/WP ratio = 0.8, w/w) had the lowest WVP and the highest TS and E among the prepared films ($p<0.05$) (Table 1), handling of this film was more difficult because of the high viscosity of the solution. Wrapping of the fruits with this film was another complication. Therefore, the film with a WVP 0.060±0.004 g mm/m^2 kPa (MC/WP ratio = 0.7, w/w) was used to wrap Japanese persimmon. These films showed similar TS and E with the films in which the MC/WP ratio was 0.8 (w/w).

Weight loss during storage

Weight losses of wrapped and non-wrapped fruits were measured for 17 days at 25°C and for 28 days at 10°C (Figure 2). The fruit could not be stored at 25°C after 17 days and 10°C after 28 days during storage, because the fruit lost its physical integrity. It was observed that non-wrapped fruits showed lower weight losses at 10°C compared to that at 25°C ($p<0.05$) (Figure 2). Increasing storage temperature causes an increase on both respiration and transmission rates (González-Aguilar et al., 2004) which explains why weight loss was higher at 25°C.

There was no significant weight loss between 3 and 10 days of storage for both wrapped and non-wrapped fruits ($p>0.05$), but a slight increase was observed after 10 days of storage at 10°C ($p<0.05$). Moreover, weight losses were constant after 10 days of storage for both wrapped and non-wrapped fruits and wrapping of fruits with MC-WP films had no effect on the weight loss of fruits during storage at 10°C ($p>0.05$). However, in contrast with expected results, wrapped fruits stored at 25°C showed higher weight loss than non-wrapped fruits after 10 days of storage ($p<0.05$).

Some studies have reported MC and WP films/coatings that have low WVP, reduced weight loss of fresh fruits significantly (McHugh and Senesi, 2000; Pérez-Gago et al., 2002; Conforti and Zinck, 2002). Although, Erdohan

Table 2. Firmness of wrapped and non-wrapped Japanese persimmon during storage.

Time (day)	*Firmness (N) at 10°C		*Firmness (N) at 25°C	
	Non-wrapped	Wrapped	Non-wrapped	Wrapped
0	53.7±14.0[a]	53.7±14.0[a]	53.7±14.0[a]	53.7±14.0[a]
3	55.0±8.9[a]	52.1±7.2[a]	53.7±12.9[a]	47.0±12.5[a]
7	49.1±9.4[a]	49.8±22.3[a]	36.9±4.5[b]	55.5±6.7[a]
10	15.7±2.0[b]	13.9±6.2[b]	32.9±1.3[b]	60.5±25.5[a]
17	14.8±11.5[b]	17.5±17.5[b]	46.6±18.4[a,b]	54.8±8.9[a]
21	9.9±4.5[b]	8.8±4.2[b]		
24	16.7±11.7[b]	9.6±4.4[b]		
28	7.7±6.4[b]	6.6±3.4[b]		

*Average firmness values with different letters (a and b) in a column indicate a significant difference at α=0.05.

and Turhan (2005) showed MC-WP films had lower WVP than single MC or WP containing films (Erdohan and Turhan, 2005), they did not reduce weight loss of wrapped persimmons. These hydrophilic films might adsorb water produced during respiration and transpiration and the water might have a plasticizer effect on the film structure. The most effective plasticizer in hydrophilic films is water and the amount of moisture in the films is related to the RH percent of the environment through their moisture sorption isotherm behavior (Conforti and Zinck, 2002). Thus, permeability of hydrophilic films increases with increasing moisture content and RH percentage during storage.

The results of weight losses in this study were similar to those found in literature, reinforcing the idea that some stand-alone films or coatings are not effective in reducing the weight loss of some fruits (Lerdthanangkul and Krochta, 1996; McHugh and Senesi, 2000; Pérez-Gago et al., 2002). Pérez-Gago et al. (2002) studied the effect of whey protein isolate-beeswax coatings on fresh cut apple pieces during storage at 5 and 20°C. Results showed the coating application did not reduce weight loss in fresh-cut apples, due to the high RH of the products. Lerdthanangkul and Krochta (1996) studied the effect of whey protein isolate, sodium caseinate, sodium caseinate-beeswax emulsion coatings, commercial cellulose and also mineral-oil based coatings on green bell peppers during storage at 10°C, 80 to 85% RH for 20 days. Only mineral-oil based coating significantly reduced moisture loss.

Changes in fruit firmness

Firmnesses of both wrapped and non-wrapped Japanese persimmon were measured at five different zones (Figure 1) and a total firmness was calculated for each fruit (Table 2). Fruit firmness was measured for 17 days at 25°C and for 28 days at 10°C.

Firmness of non-wrapped fruits stored at 10 and 25°C decreased significantly after 7 and 3 days of storage, respectively ($p<0.05$) (Table 2). Wrapping of the fruits had no significant effect on firmness and the wrapped fruits showed a similar trend as that of non-wrapped ones ($p>0.05$) when stored at 10°C. The wrapped fruits showed higher firmness than non-wrapped fruits ($p<0.05$) stored at 25°C. The higher moisture loss of wrapped fruits stored at 25°C caused the product to become hard and tough (Debeaufort et al., 1998). After 17 days of storage at 25°C, the fruits rapidly lost their physical integrity. There are many studies in literature showing that the effects of beginning of the climacteric phase on fruit quality (Vargas et al., 2008; Ramin, 2008). Thus, the starting of the climacteric phase of Japanese persimmon may cause a sharp decrease in firmness.

Appearance of the fruits

Photographs of the fruit were taken before analysis to compare the effect of wrapping on the appearance of Japanese persimmons. There was a significant difference between appearance of wrapped and non-wrapped fruits at the end of the storage period. Figure 3 reflects this difference for the fruits stored 17 days at 10°C. The most important difference between wrapped and non-wrapped fruits was the decreased microbial growth in wrapped fruit (Figure 3). This may have been caused by an increase in metabolites having known antifungal activities, like acetaldehydes. Acetaldehyde, a natural aroma component, is present in almost every fruit. It accumulates during ripening even under aerobic conditions, but to a much greater extent under partially or totally anaerobic conditions. Partially anaerobic conditions often occur during fruit ripening and under storage conditions, for example, through coating with waxes or other films, or in modified and controlled atmospheres (Pesis, 2005). The MC-WP film might have caused an accumulation of this kind of metabolite, which is produced during storage, between the film and persimmons.

Figure 3. Wrapped and non-wrapped Japanese persimmons stored 17 days at 10°C.

Conclusions

In this research, Japanese persimmons were wrapped with MC-WP based edible films to extend their shelf life. The scientific literature contains numerous reports on the production and evaluation of properties of edible films but food applications of these are scarce.

This study showed that MC-WP films may not be best suited to control the weight and firmness of the Japanese persimmon. However, the wrapping helps to keep appearance of the fruits. Furthermore, the applications of these films for packaging of other food products such as cheese or in the separation of layers of heterogeneous food systems like in pizzas may be more promising.

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