

COMPARISON OF SWEET ORANGE (*CITRUS SINENSIS*) AND LEMON (*CITRUS LIMONUM*) ESSENTIAL OILS ON QUALITIES OF FRESH-CUT APPLES DURING STORAGE

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Abstract: Fresh-cut apple is one of the most popular minimally processed products with limited shelf-life. The objective of this research was to study the effect of sweet orange (*Citrus sinensis*) and lemon (*Citrus limonum*) essential oils in pectin-based edible coatings on quality of fresh-cut Jazz apples. Apple pieces were coated with pectin (2 % w/v), whey protein (1 % w/v), and sweet orange essential oil or lemon essential oil (0.10 - 0.15 % w/v). Then, weight loss, color, firmness, microbial growth, and difference in sensory qualities were determined during storage at 4±2 °C. Coating with both sweet orange and lemon essential oils tended to reduce changes of fresh-cut apples in weight loss, color, texture and microbial counts when compared to uncoating or coating with pectin alone. In sensory evaluation, the panelists noticed slight differences between coated and uncoated samples on color and texture while the differences on apple flavor were not detected. Pectin-based edible coating with 0.10 % lemon essential oil provided potential results on preserving fresh-cut apples.

Keywords: *edible coating, essential oils, minimally processed apples, physical properties, sensory evaluation*

INTRODUCTION

Fresh fruits and vegetables with minimal processing such as fresh-cut fruit and premixed salad are preferred as snack foods for consumers who are concerned about their health [1]. Minimally processed fruits and vegetables account for 48 % of fruit and vegetable market in USA [2]. Due to the increasing demand of ready-to-eat fruits and vegetables in restaurants, dining commons, fast food restaurants, and retail markets, the estimated sales of these products are more than \$10 billion per year [3, 4]. One of the challenges during production and distribution of fruits and vegetables with minimal preparation is their limited shelf-life, especially after peeling, trimming and cutting [3]. Apple either consumed as whole or fresh-cut is one of the most popular products [1]. Minimally processed apples (cv. *Jonagored*) have a very short shelf-life of 3 days during storage at 4 °C mainly because of the browning surface, which is undesirable for consumption [5]. During production, natural protective skin of the fruit is removed by peeling and cutting. These processes release intracellular products causing texture and color change, increasing respiration rate, ethylene production, microbial spoilage, and eventually limited shelf-life of this fresh-cut product [6].

Edible coating, which is a thin layer of edible material, is one of potential methods to extend the shelf-life of fresh-cut products by creating a barrier on a surface to minimize water loss and respiration rate during storage [7]. Moreover, the application of edible coating on fresh-cut fruits can decrease color change and ripening while some research has proved that edible coating can be used as a carrier of antioxidant agents, antimicrobial agents, texture enhancers, nutraceuticals, flavor and volatile precursors [8]. There are currently more than 1,000 companies producing edible films with estimated sales of more than \$100 million a year [4]. The development of suitable edible coatings for each variety of fresh-cut fruit is important due to different characteristics of the product [9]. Hydrocolloid is one of the main components of edible coatings for minimally processed fruits and vegetables, especially protein, polysaccharide, and lipid [8]. Pectin is a colloidal polysaccharide with high molecular weight, which is generally used as a thickening agent, stabilizer, encapsulating agent, and inhibitor of crystallization. Moreover, pectin and its derivatives, such as pectate and amidated pectin, have been applied in edible coatings on fresh or minimally processed fruits and vegetables to provide a barrier to O₂ and oil, as well as a prevention of aroma and texture losses [10]. Pectin alone or incorporated with other compounds to create edible coatings has been studied to preserve the quality and extend the shelf-life of various fresh-cut fruits, such as apples, carrots, potatoes, and melon [11 – 14].

Essential oils are liquid extracted from plant parts, such as seeds, buds, flowers, leaves, fruits, roots, bark, wood, or herbs. They have been studied for antifungal, antibacterial, antiviral, and insecticidal activities in several products, for examples, cosmetics, perfumes, sanitary products, natural remedies, and food products [15]. Orange peel essential oil contains various compounds, such as limonene, β -myrcene, β -pinene, α -pinene, citral Z, and citral E, since there are a lot of glands or oil sacs in epidermis of exocarp on orange peel. The previous research showed that essential oil extracted from orange (*Citrus sinensis* var. *Valencia*) peel is effective in inhibition of *Aspergillus flavus* at minimum inhibitory concentration of 16.000 mg·L⁻¹ [16]. Citrus (*Citrus* sp.) peel essential oil also contains Limonene, linalool and citral as main volatile compounds. This essential oil has been reported its antimicrobial effect against *A. niger*, *A. flavus*,

Penicillium verrucosum, and *P. chrysogenum* [15]. A recent study has investigated the antimicrobial activity of edible film produced from pectic extract (0.7 - 1 %) and Mexican lime essential oil (0 - 1 g·L⁻¹) against *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* [17]. Recently, there have been a few studies on application of edible coating from orange and lemon essential oils in fresh-cut apples [18 – 20]; however, most research has focused on the antimicrobial activities of these essential oils with a few studies of their effects on quality and sensory characteristics of the product. Therefore, the main objective of this research is to study the effect of pectin, incorporated with sweet orange (*Citrus sinensis*) and lemon (*Citrus limonum*) essential oils, on quality attributes of fresh-cut Jazz apples during storage at 4 °C.

MATERIALS AND METHODS

Materials

Jazz apples (*Malus domestica*) were purchased from Big C Supercenter Public Company Limited (Nonthaburi, Thailand) stored at 4±2 °C, and used within 24 h. Food-grade low methoxyl pectin (Union Chemical 1986 Co., Ltd., Bangkok, Thailand), sweet orange (*Citrus sinensis*) and lemon (*Citrus limonum*) essential oils (Saflora, Vilnius, Lithuania) were used for the coating formulations. Food-grade calcium chloride (Qingdao Huadong Calcium Producing Co, Ltd., China) was used in a final dip to induce the cross-linking reaction.

Sample preparation and treatment procedures

After purchasing, the whole apples were initially immersed in calcium hypochlorite solution (200 mg·L⁻¹) for 2 min, rinsed with running distilled water for 2 min, and air-dried for 5 min. Following good manufacturing practices using a sterilized hand-operated apple corer and knife, the apples were peeled, cored, and cut into pieces (3 x 3 x 2 cm³). In order to block out the variation among samples, fresh-cut apples were randomly selected and immediately immersed in 1 of the 6 different solutions (water as control; 2 % w/v pectin; 2 % w/v pectin + 0.10 % w/v sweet orange; 2 % w/v pectin + 0.15 % w/v sweet orange; 2 % w/v pectin + 0.10 % w/v lemon; 2 % w/v pectin + 0.15 % w/v lemon) for 2 min and air-dried for 2 min. During preparation of coating formulations, 1 % w/v whey protein (Chemipan Corporation Co., Ltd., Bangkok, Thailand) had been added as an emulsifier in every solution, except control and 2 % pectin. After that, every sample except control was immersed in aqueous solution of 2 % w/v calcium chloride for 2 min and then air-dried for 5 min. Samples from each treatment were packaged in a releasable storage bag (18 x 20 cm²) (Big C Supercenter Public Company Limited, Nonthaburi, Thailand) and stored at 4±2 °C for further analysis. The preparation process was independently repeated on 3 separate days as replication.

Weight loss

Weight loss was measured with assumption to correspond with water loss at 7 days of storage to determine the effectiveness of pectin coating as a moisture barrier. The weight of three apple pieces in each treatment was monitored and the weight loss percent relative to initial weight was calculated and reported.

Color

Color of the apple surface at 0, 3, 6, and 9 days of storage were directly determined with a colorimeter (Color Quest 45/0, Hunter Associates Laboratory, Inc., Reston, VA). Before measurement, the instrument was calibrated using a standard white and black reflector plates. Three apple pieces per treatment were measured and four readings were made in each replicate by changing the position of the sample. The color determination was conducted at room temperature. Browning index was calculated from (Equation 1) [21]:

$$\text{Browning index} = \frac{100}{0.17} \left(\frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} - 0.31 \right) \quad (1)$$

Firmness

Firmness of apple pieces was measured by compressing samples with Texture analyzer TA.XT2i (Stable Micro Systems Ltd., United Kingdom) at 0 and 7 days of storage [22].

Microbiological analysis

Total viable counts (TVC) were determined by the pour-plate method [23] at 0, 3, 6, and 9 days of storage. 10 g of each sample was aseptically weighted and homogenized with 90 mL of sterile peptone water (Merck, KGaA, Germany) for 1 min using a stomacher (Funke-Gerber, Germany) at a speed of 230 rpm. The homogenized samples were serially diluted (1 : 10) in sterile peptone water. 1 mL of sample from serial dilutions was added to each duplicate sterile Petri dish. Plate Count Agar (PCA) was added to each Petri dish and samples were then incubated at 37 ± 2 °C for 48 h. After incubation, colonies were counted and microbiological data was expressed as logarithms of number of colony-forming units ($\log \text{CFU} \cdot \text{g}^{-1}$).

Sensory evaluation

The best treatments (from instrumental and microbiological analysis) were selected. A difference-from-control test was then performed to detect difference between samples at 0 and 7 days of storage when compared to a standard which was a fresh-cut apple piece without coating. Thirty partially trained panelists were screened from juniors and seniors in the Department of Agro-Industrial, Food, and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand. Every panelist was initially asked to evaluate and remember the

standard, labeled with “S”. Then, they were asked to rate the degree of difference in color, apple flavor, and texture between the sample and the standard. The line scale for rating included 3 = extremely more than the standard, 0 = no difference from the standard, -3 = extremely less than the standard. All samples were labeled with random 3-digit codes and presented in random order.

Statistical analysis

All analyses were run in triplicate, except microbiological analyses, which were run in duplicate. Data were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics 24 (IBM Corporation, Armonk, NY). Duncan’s multiple range test (DMRT) was used to determine significant differences among means (significance was defined at $p < 0.05$).

RESULTS AND DISCUSSIONS

Weight loss

Weight loss can be used to indicate water loss and freshness of fresh-cut fruit [24] since water from fruit migrates to the environment with lower relative humidity during storage [25]. Edible coatings can produce higher relative humidity at the surface of fresh-cut fruits, leading to reduction of water loss [26]. In this research, weight loss of control and apples coated with pectin alone or incorporated with 0.10 % sweet orange at 7 days of storage was not significantly different ($p \geq 0.05$) while the samples coated with pectin incorporated with 0.15 % sweet orange, 0.10 or 0.15% lemon had significantly lower weight loss than the control ($p < 0.05$) (Table 1). This may be because polysaccharide alone is not efficient enough to reduce the weight loss. The previous study also showed that pectin coating did not provide a good barrier for water loss of fresh-cut apple during storage since fresh-cut fruit has higher rate of water loss when compared to whole fruit and polysaccharide-based coating can be permeable to water [18]. However, emulsifiers can be used in coating solutions to improve stability, wettability, and adhesion to the fruit surface and cross-linking agents can also react with polysaccharides and form a solid polymeric matrix on food surface [10]. The recent study also observed the lowest weight loss in fresh-cut apples coated with edible film prepared from *trans*-glutaminase, whey protein and pectin when compared to uncoated samples and samples coated with whey protein or soy protein isolate during 10 days of storage at 4 - 6 °C [13].

Color

Appearance and color are important factors indicating freshness and flavor of fresh and processed fruits and vegetables during purchasing [27]. The intensity of brown color of fresh-cut fruits can be indicated by browning index [22]. The browning index of control and apples coated with pectin incorporated with various concentrations of essential oils during storage are presented in Table 2. All samples with edible coatings had significantly lower browning index when compared to the control ($p < 0.05$). The edible

coatings incorporated with both sweet orange and lemon essential oils tended to have fewer changes in browning index than the control and sample coated with pectin alone at the initial day of storage and during storage. This may be because the lipid droplets of essential oils increase the intensity of light scattering, leading to more diffuse reflectance [28].

Browning in fresh-cut apples happens after peeling and cutting, leading to broken tissue cells and releasing of enzymes, such as polyphenol oxidases (PPOs) [29]. In the presence of oxygen, this enzymatic browning reaction is related to the oxidation of monophenols to diphenols, which oxidize later into *o*-quinones. These *o*-quinones can polymerize into brown pigments with the presence of amino acids. Moreover, the quinones from browning reaction may oxidize other polyphenols in oxidation reactions [30]. Basically, edible coatings can slow down the browning reaction because of their role of oxygen barrier. The orange peel essential oil microemulsion and nanoemulsion (at 0.5 – 1.0 %) in pectin-based coating also minimize the decrease of lightness on minimally-processed sliced orange during storage at 4 °C [31]. Additionally, another study showed the positive effect of edible coating formulated with citral (up to 250 mg·L⁻¹) on inhibition of lightness reduction on fresh-cut apple surface during 4 days of refrigerated storage [19].

Table 1. Weight loss of control and apples coated with pectin alone or incorporated with essential oils after 7 days of storage at 4 ± 2 °C

Treatment	Weight loss [%]
Control	6.50 ^a ± 0.48
2 % pectin	5.13 ^{ab} ± 1.43
2 % pectin + 0.10 % sweet orange	6.33 ^a ± 1.31
2 % pectin + 0.15 % sweet orange	2.96 ^b ± 1.76
2 % pectin + 0.10 % lemon	2.63 ^b ± 1.71
2 % pectin + 0.15 % lemon	3.01 ^b ± 1.17

Different lowercase letters (a, b) indicate significant difference between treatments at $p < 0.05$

Table 2. Browning index of control and apples coated with pectin alone or incorporated with essential oils during storage at 4 ± 2 °C

Treatment	Days of storage			
	0	3	6	9
Control	^B 58.73 ^a ± 3.27	^B 62.22 ^a ± 0.59	^B 58.40 ^a ± 0.94	^A 78.40 ^a ± 3.53
2 % pectin	^D 47.52 ^b ± 0.24	^B 53.35 ^b ± 0.09	^C 51.31 ^b ± 0.06	^A 57.65 ^b ± 1.35
2 % pectin + 0.10 % orange	^D 35.45 ^c ± 0.99	^B 45.95 ^{bc} ± 0.50	^C 38.79 ^c ± 1.41	^A 50.96 ^{bc} ± 1.58
2 % pectin + 0.15 % orange ^{NS}	35.25 ^c ± 0.45	44.50 ^c ± 8.97	39.95 ^c ± 4.77	50.70 ^{bc} ± 8.05
2 % pectin + 0.10 % lemon	^C 32.55 ^c ± 0.58	^B 39.74 ^c ± 0.91	^{BC} 36.23 ^c ± 1.25	^A 45.09 ^c ± 3.65
2 % pectin + 0.15 % lemon	^B 33.69 ^c ± 6.61	^A 53.83 ^b ± 6.93	^B 38.49 ^c ± 4.40	^A 58.03 ^b ± 6.21

Different lowercase letters (a, b, c) indicate significant difference between treatments at $p < 0.05$

Different uppercase letters (A, B, C, D) indicate significant difference between days of storage at $p < 0.05$

Firmness

One of important factors affecting consumer acceptability is texture. Hardness or firmness is widely used as a texture parameter for whole and fresh-cut fruits [32]. Moreover, softening of fruit texture can indicate the deterioration during storage due to starch hydrolysis and pectin degradation during fruit ripening [33]. Firmness of control and apples coated with pectin incorporated with various concentrations of essential oils during storage are presented in Table 3. The firmness of control significantly decreased with storage time and it was significantly lower than coated samples at the last day of storage ($p < 0.05$). All coated samples maintained the same firmness during storage, except samples coated with 0.15 % sweet orange or 0.15 % lemon, which had higher firmness at 7th day of storage. During storage, the firmness of the control decreased due to degradation of cell wall by pectin solubilization and depolymerization, as well as the decrease of turgor pressure while the edible coatings can play an important role as a barrier to water vapor, resulting in prevention of turgor loss in fresh-cut apples [8]. In addition, the crosslinking between calcium chloride and coating polymers may be another reason why edible coatings can retain firmness of samples when compared to the control [33]. These similar results were found in the study of alginate-based edible coatings enriched with eugenol (0.1 - 0.2 %) and citral (0.15 - 0.3 %) essential oils on minimally processed strawberry [33], as well as the study of alginate-based edible coatings incorporated with lemongrass essential oil (0.1 - 0.5 %) on fresh-cut pineapple [34].

Table 3. Firmness of control and apples coated with pectin alone or incorporated with essential oils during storage at 4 ± 2 °C

Treatment	Days of storage	
	0	7
Control	394.61 ^b ± 11.97	250.06 ^c ± 25.45
2 % pectin	412.67 ^b ± 26.98	353.36 ^b ± 55.45
2 % pectin + 0.10 % orange	483.36 ^a ± 44.76	428.65 ^a ± 28.04
2 % pectin + 0.15 % orange	366.48 ^b ± 51.80	447.13 ^a ± 27.32
2 % pectin + 0.10 % lemon	477.26 ^a ± 52.83	387.49 ^{ab} ± 55.88
2 % pectin + 0.15 % lemon	377.56 ^b ± 37.17	463.07 ^a ± 72.04

Different lowercase letters (a, b, c) indicate significant difference between treatments at $p < 0.05$

Microbiological analysis

Total viable counts (TVC) of control and apples coated with pectin incorporated with various concentrations of essential oils during storage are presented in Table 4. The initial number of bacteria in every sample was under 2 logCFU·g⁻¹, indicating good quality of fresh apples and suitable sanitation used during sample preparation. Lower than 2 logCFU·g⁻¹ of total aerobic bacteria was also observed on fresh-cut Golden Delicious apples purchased from a local produce provider with processing under sterile conditions [35]. During storage, all samples coated with edible coatings containing essential oils significantly slowed down the growth of bacteria during storage, when compared to the control and the sample coated with pectin alone. The phenolic compounds in citrus essential oil can be an important factor for its antimicrobial activity

due to the interaction between hydrophilic part and polar part of cell membrane while the lipophilic structure could alter the structure of cell membranes and mitochondria of microorganisms, resulting in the leakage of ions and the loss of cell components. The membrane permeability, reduction of membrane potential, and loss of cell contents can lead to loss of macromolecules, decrease of intracellular ATP, and eventually cell death [2, 36]. The previous research revealed the positive effect of commercial orange (*Citrus sinensis*), lemon (*Citrus lemon*), and Mandarin (*Citrus reticulata*) from Spain against Gram-positive and Gram-negative bacteria [37]. Another study also showed the reduction of microbial spoilage in strawberry coated with alginate-based edible coatings enriched with eugenol (0.1 - 0.2 %) and citral (0.15 - 0.3 %) essential oils [33].

Table 4. Total viable counts ($\log \text{CFU} \cdot \text{g}^{-1}$) of control and apples coated with pectin alone or incorporated with essential oils during storage at $4 \pm 2^\circ \text{C}$

Treatment	Days of storage			
	0 ^{ns}	3	6	9
Control	^D 1.35 ± 0.06	^C $2.49^a \pm 0.23$	^B $3.98^a \pm 0.12$	^A $4.46^a \pm 0.18$
2 % pectin	^D 1.26 ± 0.06	^C $1.73^b \pm 0.15$	^B $2.80^c \pm 0.16$	^A $3.51^b \pm 0.25$
2 % pectin + 0.10 % orange	^D 1.31 ± 0.06	^C $1.65^b \pm 0.08$	^B $2.15^d \pm 0.16$	^A $2.84^c \pm 0.14$
2 % pectin + 0.15 % orange	^C 1.24 ± 0.12	^B $1.58^b \pm 0.10$	^A $2.54^c \pm 0.26$	^A $2.61^{cd} \pm 0.14$
2 % pectin + 0.10 % lemon	^D 1.31 ± 0.05	^C $1.62^b \pm 0.11$	^B $3.13^b \pm 0.06$	^A $2.64^{cd} \pm 0.15$
2 % pectin + 0.15 % lemon	^C 1.24 ± 0.13	^B $1.63^b \pm 0.21$	^A $2.08^d \pm 0.11$	^A $2.36^d \pm 0.21$

Different lowercase letters (a, b, c, d) indicate significant difference between treatments at $p < 0.05$

Different uppercase letters (A, B, C, D) indicate significant difference between days of storage at $p < 0.05$

Sensory evaluation

Since samples coated with pectin incorporated with 0.10 % and 0.15 % of each essential oil showed similar results in color and microbial counts, as well as the initial sensory evaluation from the authors, the apples coated with pectin incorporated with 0.10 % sweet orange and lemon were selected for difference-from-control test compared to the control sample. The difference-from-control test was done to evaluate whether the partially trained panelists ($n = 30$) could discriminate the coated sample from the fresh-cut uncoated apple, used as a standard. Table 5 presented sensory scores from difference-from-control test of control and coated samples. Both uncoated and coated samples were not significantly different in apple flavor ($p \geq 0.05$); therefore, edible coatings did not have noticeable effect on flavor of the product. Similarly, some previous studies also found no effects of edible coatings incorporated with essential oils on flavor and/or aroma of fresh-cut fruits during sensory evaluation [18, 33, 38]. In color attribute, the panelists noticed darker color on the surface of the sample coated with the edible coating containing lemon essential oil before storage while the sample coated with the edible coating containing orange essential oil had lighter surface after 7 days of storage. This positive effect on color attribute was also found in application of orange peel essential oil microemulsion and nanoemulsion in pectin-based coating on fresh-cut orange [31]. However, the panelists noticed firmer texture of apples coated with the edible coating with lemon essential oil when compared to those with orange essential oil while they were not significantly different from the control at 7th day of storage ($p \geq 0.05$). The similar results were found in minimally processed strawberry coated with alginate-base edible coating containing 0.15 and 0.3 % citral essential oil

during storage at 0.5 °C [33] and fresh-cut Fuji apples coated with carnauba-shellac wax containing lemongrass oil during storage at 1 ± 1 °C [38].

Table 5. *Sensory Scores* from difference-from-control test of control and apples coated with pectin incorporated with essential oils during storage at 4 ± 2 °C*

Days of storage	Treatment	Attributes		
		Color	Apple flavor ^{ns}	Texture
0	Control	$0.00^{bc} \pm 0.63$	0.38 ± 0.50	$-0.25^b \pm 1.18$
	2% pectin + 0.10 % orange	$0.50^{ab} \pm 0.97$	0.50 ± 1.37	$-0.13^b \pm 1.20$
	2% pectin + 0.10 % lemon	$1.00^a \pm 1.51$	0.50 ± 1.46	$1.06^a \pm 1.44$
7	Control	$-0.31^c \pm 1.30$	0.19 ± 0.98	$1.56^a \pm 1.36$
	2% pectin + 0.10 % orange	$-1.13^d \pm 1.09$	-0.13 ± 1.50	$-0.81^b \pm 1.11$
	2% pectin + 0.10 % lemon	$0.06^{bc} \pm 0.93$	-0.13 ± 2.19	$0.94^a \pm 0.93$

* 3: extremely more than the standard (fresh uncoated sample); 0: no difference from the standard; -3: extremely less than the standard

Different lowercase letters (a, b, c, d) indicate significant difference between samples for the same attribute ($p < 0.05$)

ns indicates no significant difference between treatments for the same attribute ($p \geq 0.05$)

CONCLUSIONS

Coating fresh-cut apples with sweet orange and lemon essential oils could reduce quality changes in weight loss, color, texture and microbial counts when compared to uncoating or coating with pectin alone. In sensory evaluation, the panelists noticed slight differences between coated and uncoated samples on color and texture without noticing the differences on apple flavor. The potential essential oil to incorporate with pectin-based edible coating were 0.10 % lemon essential oil since this mixture of coating efficiently reduced the changes in weight loss, browning index, firmness, and microbial growth of samples while the panelists did not notice the differences in color apple flavor, and texture when compared to the uncoated sample during storage at 4 ± 2 °C.

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