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RESEARCH ARTICLE

Whey Protein Concentrate Coating Incorporated with Modified Atmosphere Packaging for Extending Tangerines Shelf-Life: Physicochemical, Microbiological and Sensory Evaluation Through Refrigerated Storage

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Abstract

In this study, tangerines (Dancy) were packaged in modified atmosphere packaging to prevent microbial spoilage and quality degradation. Different concentrations of WPC (0, 5, 10, and 15%) were coated on tangerines and packed in polyethylene bags under MAP (5% oxygen, 15% carbon dioxide, and 80% nitrogen). During the 60-day storage period, the tangerines were kept in the refrigerator. During storage, tangerine samples showed an increase in bacterial, mold and yeast counts, soluble solids, weight loss, pH, color change, and O_2 consumption and CO_2 production. However, they decreased in acidity, ascorbic acid, firmness, and sensory properties. As a result of the WPC coating (10%) and packing in MAP, tangerines exhibited the lowest pH, soluble solids, weight loss, O_2 consumption, and CO_2 production ($P<0.05$). The color change in tangerines coated with WPC was the lowest. Sensory scores of samples were increased by WPC coating. Microbial and fungal counts were also lowest in these samples. WPC coating and MAP conditions by delaying aging caused the maintenance of ascorbic acid, acidity, and firmness of tangerine samples. After 60 days of storage, the Lightness and Chroma index of tangerines coated with 5 and 10% WPC incorporated with MAP were within acceptable levels. Extending the shelf-life of tangerines was evidently achieved through the use of WPC coating and a controlled atmosphere (MAP) environment. To obtain a deeper understanding of the tangerines' shelf-life, it is advisable to conduct research over an extended duration or under more accelerated conditions.

Keywords Tangerine · Whey protein · Modified atmosphere packaging · Quality properties · Microbial quality

Introduction

The citrus fruit has a great taste and a high nutritional content, making it one of the most widely consumed fruits in the world [1]. Fruits such as citrus are consumed worldwide for various health-benefiting properties, including vitamins, folic acid, carotenoids, antioxidants, and flavonoids. Flavonoids and limonoids in citrus exhibit numerous disease-preventing properties, including anticancer, lipid-lowering, and cardiovascular protective properties [2]. The bioavailability of these compounds is reduced by unfavorable postharvest strategies and inadequate storage facilities [3]. As a result of senescence, particularly granulation, citrus nutrients and flavors often deteriorate during postharvest storage [4]. Citrus fruits generally have a short shelf-life and are prone to postharvest losses [5]. Citrus fruit is also 'non-climacteric'; in other words, ripening, texture, and compositional

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changes occur slowly. A continuous decline in respiration and extremely low levels of ethylene production are also observed during fruit development. As a result, it must be matured on the tree before harvest [2]. Generally, long intervals between harvest and citrus consumption can cause extensive waste through fungal, bacterial, and physiological spoilages. For this reason, adequate postharvest procedures (time and type of harvest, handling, processing, storage, and transport) are crucial to supplying high-quality fruit to local and distant markets, extending their commercial life, and reducing postharvest losses [6].

1. *Postharvest processing methods*: The passage mentions several methods for postharvest processing, including wax coating, hot water or air treatment, biocontrol agents, modified atmosphere packaging (MAP), and hot water or air treatment. These methods are used to extend the shelf-life and maintain the quality of fruits and vegetables after they have been harvested [7–10].
2. *Edible coatings*: Edible coatings have gained attention as a promising postharvest processing method. These coatings are considered appealing, safe, and effective in preserving produce quality. They provide a protective layer on the surface of fruits and vegetables [11–13].
3. *Biopolymers*: Biocompatible and biodegradable biopolymers are highlighted as excellent choices for coating materials. These materials are environmentally friendly and have properties that make them suitable for use as coatings [14].
4. *Drawbacks of commercial waxes*: Traditional commercial waxes have been used to coat fruits, especially citrus fruits, to reduce water loss and shrinkage. However, these synthetic materials have drawbacks, such as limiting of gases exchange on the fruit's surface. This limitation can lead to anaerobic respiration, ethanol production, and off-flavor sensations, which can negatively affect the quality of the produce [15].
5. *Growing demand for edible coatings*: Due to concerns about environmental sustainability and human health, there is a growing demand for edible coatings and films as alternatives to synthetic waxes. These coatings aim to maintain the quality of fruits and vegetables while addressing the limitations associated with traditional wax coating [15]. The passage emphasizes the importance of finding sustainable and effective postharvest processing methods, such as edible coatings made from biodegradable biopolymers, to preserve the quality of fruits and vegetables while addressing environmental and health concerns associated with synthetic materials like commercial waxes. Whey protein concentrate coating can act as semi-permeable barrier against aroma, fat, water vapor, and oxygen. Thermal denaturation at high temperatures of 65 °C can cause the unfolding of whey protein via breaking disulfide bonds and exposing hydrophobic sulfhydryl and amino groups of lysine and glutamine amino acids, which leads to an increase in steric accessibility that improves mechanical properties [16, 17]. MAP is the practice of manipulating the atmosphere to provide an optimal atmosphere for preserving food quality and increasing shelf-life. Using permeable polymeric films, MAP extends the shelf-life of fresh or minimally processed foods by creating a modified atmosphere around the product. Gases such as oxygen, carbon dioxide, and nitrogen are used in MAP, which play a crucial role in the respiration of fresh fruits and vegetables [18]. The use of MAP also improves the shelf-life of fruits and vegetables by controlling ethylene production, respiration, and ripening, as well as slowing down the browning reaction (due to the decrease in oxygen levels and increase in carbon dioxide levels in food packaging) [19]. This type of packaging is also helpful for reducing respiration, inhibiting or delaying enzymatic, physiological reactions, and preventing product quality loss [20]. Therefore, MAP with excellent conditions has been extensively investigated for food products along storage. The rate of respiration and consumption of organic acids and sugars is retarded during these conditions [21]. Citrus is one of the most economically important crops all around the world as well as Iran, and in terms of citrus cultivation area, Iran has ninth rank in the world (around 2.8 million tons are produced per year). Of the total amount of citrus produced in Iran, 18% is related to tangerines, which is equivalent to 500,000 tons [22]. Therefore, this study was focused on the assessment of the extension of the shelf-life of tangerine using whey protein concentrate coating under modified atmosphere packaging at refrigerator temperature (4 °C) for 60 days.

Materials and Methods

Materials

The mature and fresh '*Dancy*' tangerines with uniform morphological properties were collected from Citrus Research Institute—Ramsar (Mazandaran, Iran). Analytical reagent grades of chemicals are used, purchased from Merck Company (Darmstadt, Germany). This whey protein concentrate was obtained from Sigma-Aldrich (Sigma, Switzerland). The polyethylene zip bags were purchased from Armani Company (Tehran, Iran).

Preparation of Edible Coating Based on WPC

The WPC solutions with the concentration of 5, 10, and 15% (w/v) were prepared by dissolving the whey protein powder of 5 g, 10 g, and 15 g (80%, Merck Company, Germany) in 100 ml of distilled water in a hot water bath of 90° for 30 min. To homogenize the solution, glycerol as a plasticizer substance was added to the WPC solution in the ratio of 1:30 [17].

Coating of Tangerine Fruits

The tangerines were washed with distilled deionized water. The fresh fruits were coated by dipping into the aqueous solutions for 2 min and then at room temperature the surface was dried for 4 h. The control tangerines (uncoated) were placed in distilled water. In order to drain the extra coating solution, the tangerines were placed on a stainless steel sieve and air dried at 20 ºC for 90 min.

Treatments and Storage Procedure

The effects of modified atmosphere packaging on tangerines during storage time were evaluated by packing coated and uncoated tangerines were packed in polyethylene-based laminated bags (PE/PA/PE) with a thickness of 80 microns (6 numbers each) each under two different conditions inclusive MAP (5% O_2, 15% CO_2, and 80% N_2), atmospheric conditions in 3 replications and sealed with a heat-sealing machine. A total of 8 groups of tangerine samples were analyzed, including Control (without any coatings—air conditions), T1 (without any coatings—MAP conditions), T2 (coated with WPC(5%)—air conditions), T3 (coated with WPC (5%)—MAP conditions), T4 (coated with WPC (10%)—air conditions), T5 (coated with WPC (10%)—MAP conditions), T6 (coated with WPC (15%)—air conditions), and T7 (coated with WPC (15%)—MAP conditions). During the 2-month storage period, the samples were stored at 4 °C with 85 ± 5% RH. At the beginning of the experiment and after 20 days of storage, coated tangerines were tested for microbial, physicochemical, textural, color, and sensory characteristics (4 times analysis).

Evaluation of Weight Loss

To determine the weight loss, the weight of the control and coated fruits was measured at the storage days (0, 20, 40, and 60). The weights changes were calculated using Eq. 1 [22]:

$$\%\text{Weight loss} = \frac{W_t}{W_0} \times 100 \tag{1}$$

where W_0 is the initial weight; W_t is the fruit weight after an indicated period of storage.

Evaluation of Titratable Acidity

Titratable acidity (TA) of the samples (grams of citric acid per 100 ml of juice) was determined using titration potentiometric with 0.1 N sodium hydroxide (NaOH) at an endpoint pH of 8.2 [23].

Evaluation of Total Content of Soluble Solids

The fruit juice was tested for soluble solids content (TSS) with a digital refractometer (PR-101, Atago, Tokyo, Japan). The concentration of soluble solids (°Brix) was reported based on degree of brix unit (gram per 100 g of sample) [22].

pH

The pH of the samples was determined using a pH meter (Metrohm 691, Switzerland) at room temperature of 20 °C [23].

Evaluation of Ascorbic Acid

The concentration of ascorbic acid in tangerine juice was measured by a spectrophotometer based on milligrams of vitamin C per 100 mL of fruit juice [24].

Evaluation of Firmness

The firmness of the tangerine fruits was evaluated using a texture analyzer (TA-XT Plus, Stable Micro Systems, England) with a 5 mm diameter cylindrical probe, 1 mm/s rate, 1 mm/s pretest rate, 5 mm/s posttest rate, and 20 mm penetration depth of the probe. The force injected into the tissue (*N*) was measured at three points of the surface (*A*). The first point was in the center of the bottom of each fruit; the second and third points were 90° of difference from the first point. The force required for penetration in terms of Newton was considered the firmness of the tissue [11].

Internal Atmosphere Analysis

An oxygen–carbon dioxide analyzer was used to determine the headspace gas composition (%O_2 and %CO_2) of packages (Gases Analyzers, Model Oxybaby, WITT Company Germany) before opening the packages on the sampling days. An instrument needle was inserted through a rubber septum attached to the lidding material to analyze gas composition [23].

Microbial Analysis

To determine the microbial count, homogenized transgenes (10 g sample) per replicate were diluted in 90 ml of sterile peptone water. Triplicate dilutions were performed. Incubated at 30 °C for 2 days, total aerobic mesophilic bacteria were counted using the pour plate method (PCA, Merck, Germany). Pour plate culturing on PCA and incubation at 7 °C for 10 days determined the total number of psychotropic bacteria. Yeasts and molds were counted using the surface plate method on potato dextrose agar (PDA, Merck, Germany). Yeast and mold counts were determined after 5 days of incubation at 25 °C. The results of each test were expressed as Log colony-forming units (CFU) per g [23].

Color

The peel color of tangerine samples was measured by a colorimeter (Model CR-410, Minolta, Osaka, Japan). The results were expressed in $L*$, Chroma, and Hue scales, in which $L*$ represents luminosity or brightness on a black (0) to white (100) axis. Equations 2 and 3 were used to calculate Chroma and Hue angles [27]:

$$\text{Chroma} = \sqrt{a^{*2}+b^{*2}} \quad (2)$$

$$\text{Hue}=\tan^{-1}\frac{b^*}{a^*} \quad (3)$$

Sensory Evaluation

Sensory properties, including texture, color, and flavor were determined by 40 trained panels (20 female and 20 male) using the five-point hedonic scale of 1–5. Scale ranging from “extreme dislike (1)” to “extreme like (5)”. In each session, three samples were presented to the panelists [23].

Statistical Analysis

For each of the eight treatments, three replications were performed based on a completely randomized design. One-way analysis of variance (ANOVA) was used to analyze the results, and Duncan’s test was used to compare means at a 95% confidence level ($P<0.05$). SPSS Software version 21.0 was used for all statistical analyses (ver. 21, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Effect of Coating on Weight Loss

Effect of coating on weight loss. The loss of weight is a significant concern since it affects shelf-life. As a result of water loss and dry matter consumption, the weight loss percentage increased during storage [27]. Stomatal transpiration and direct evaporation by epidermal cells accounted for most water loss in fruits [26]. Weight loss was significantly differed between the 0th, 20th, 40th, and 60th days. The lowest amount of weight loss was shown by the T5 groups, which is due to the decrease in water evaporation compared to the control sample. The weight loss percentage of the T2, T3, T4, and T7 groups was lower than T6, T1, and control groups. The highest amount of weight loss was related to uncoated fruits, because there was no obstacle on the surface of the fruit to prevent weight loss in this group ($P<0.05$) (Fig. 1).

Ali et al. found that different moisture levels between fruit surfaces and environments and respiration of fruit skin cause pressure differences and weight loss in fresh fruits and vegetables [27]. In cold storage, fresh fruits loose water since their moisture content is over 80% [29]. Khorram et al. reveal that evaporation time is highly dependent on temperature, storage time, and the thickness of the fruit’s skin. Furthermore, fruit’s weight loss is associated with the process of respiration, moisture transfer, and some oxidation processes during the ripening that lead to shrinkage and reduce the marketability of the product. Therefore, fruit coating can provide an opportunity to reduce enzymatic browning, nutrient deficiency, wrinkling, delayed fruit ripening, and maintain the quality of the fruit. In addition to reducing respiration rate, water loss, and oxidation reactions, coatings can act as a semi-permeable barrier against oxygen,

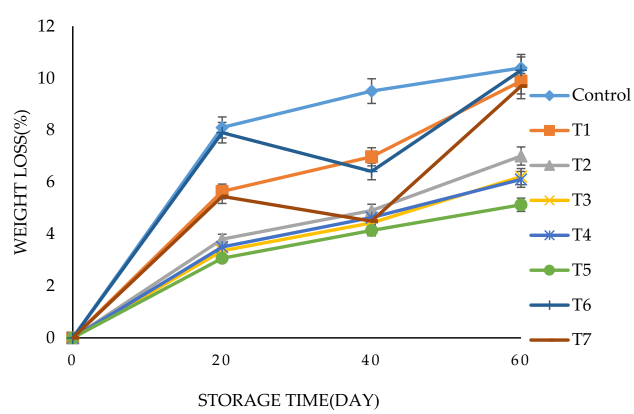

Fig. 1 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the weight loss of tangerine fruit in storage duration

carbon dioxide, moisture, and solute movements [22]. Furthermore, the protection of water content in fruits is one of the main advantages of MAP-based packaging that preserves the high-level water vapor pressure around the fruits and reduces water loss [28, 29]. Other studies on tangerines and oranges reported increased weight loss due to fruit juice loss during postharvest storage by increasing storage time. The loss in fruit juice depends on the storage period and storage temperature [22, 23, 30–34].

Titratable Acidity (TA), Total Content of Soluble Solids (TSS) and pH

In order to evaluate the nutrition, taste, and flavor of citrus fruits, the TSS and TA are the key nutritional determinants [34]. During storage, the acidity of the samples decreased (Table 1). The titratable acidity of the tangerine samples on the 20th and 40th days was not significantly different, but the acidity of the samples on the 60th day was lower. T4 and T5 showed the highest titratable acidity levels. The respiration rate decreased compared to the control sample due to the acidity of this sample being similar to fresh fruit acidity. T2, T3, T4, and T5 groups did not show any significant differences. Furthermore, the T6, T7, and control groups did not show any significant differences. The control sample had the lowest acidity level ($P < 0.05$). Hazrati et al. report that organic acids are the principal components of TA. These acids are used as substrates for enzymatic respiration. Therefore, TA concentration is considered an essential predictor of fruit respiration [35]. Tangerines have a lot of organic acids, including oxalic, tartaric, malic, lactic, citric, and ascorbic acids. Among them, citric acid occupies is first place, followed by the acid malic [22], although, respiration reduces organic acids in fruits during storage by converting them to sugars edible coatings preserving organic acids, by modifying the internal atmosphere and reducing the rate of respiration of the fruit [23, 36, 37]. Decreased titratable acidity of oranges and tangerines during storage was also recorded in other studies either [22, 23, 31, 32, 38].

Table 1 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) packaging on the TA, TSS, pH of tangerine fruit in storage duration

Treatments	Storage time (day)			
	0	20	40	60
TA				
Control	0.69^{a}	0.49^{f}	0.46^{k}	0.41^{p}
T1	0.69^{a}	0.45^{d}	0.48^{j}	0.42^{o}
T2	0.69^{a}	0.61^{c}	0.57^{i}	0.49^{n}
T3	0.69^{a}	0.63^{e}	0.61^{h}	0.51^{m}
T4	0.69^{a}	0.61^{d}	0.60^{j}	0.54^{m}
T5	0.69^{a}	0.68^{b}	0.66^{g}	0.58^{l}
T6	0.69^{a}	0.53^{e}	0.52^{k}	0.46^{n}
T7	0.69^{a}	0.54^{e}	0.55^{h}	0.47^{n}
TSS				
Control	10.96^{l}	12.02^{h}	13.05^{d}	14.12^{a}
T1	10.96^{l}	11.45^{j}	12.41^{g}	13.45^{c}
T2	10.96^{l}	11.70^{i}	12.66^{e}	13.91^{b}
T3	10.96^{l}	11.15^{k}	11.72^{j}	12.25^{h}
T4	10.96^{l}	11.65^{i}	12.50^{f}	13.80^{b}
T5	10.96^{l}	11.10^{k}	11.60^{j}	12.15^{h}
T6	10.96^{l}	11.86^{h}	12.96^{d}	13.96^{b}
T7	10.96^{l}	11.30^{j}	12.35^{g}	13.30^{c}
pH				
Control	3.67^{n}	3.85^{f}	3.89^{d}	4.02^{a}
T1	3.67^{n}	3.83^{g}	3.87^{e}	3.91^{c}
T2	3.67^{n}	3.75^{j}	3.77^{i}	3.83^{gh}
T3	3.67^{n}	3.73^{k}	3.73^{k}	3.82^{h}
T4	3.67^{n}	3.71^{l}	3.72^{kl}	3.81^{h}
T5	3.67^{n}	3.69^{m}	3.69^{m}	3.74^{b}
T6	3.67^{n}	3.84^{fg}	3.89^{d}	3.92^{b}
T7	3.67^{n}	3.83^{g}	3.78^{i}	3.91^{c}

Values represent means ± standard deviations. Means within a column with the same lowercase letters are not significantly different ($P < 0.05$)

MAP modified atmosphere packaging, *TA* titratable acidity, *TSS* total soluble solids, *WPC* whey protein concentrate

As shown in Table 1, the amount of soluble solids increased during storage for all samples. On the 0, 20th, 40th, and 60th days, there was a significant difference in dissolved solid content between the samples. The lowest amount of dissolved solids is related to groups T4, T3, T5, and the highest amount is related to the control sample. The increase of soluble solids in the T1 and T6 groups was higher than in the T2 and T7 groups ($P < 0.05$). Sugars, acids, vitamins, minerals, and some soluble pectin are the main components of TSS in fruit juice [39]. During the storage of fruits, soluble solids increase due to the decomposition of cell wall structures (pectin, cellulose, and hemicellulose) by galactosidase, and a decrease in the fruit water content [23]. Togrul and Arslan recorded the reduction of soluble solids using sugars and acids during respiration along storage, which disagrees with the result of the present study. The coating can ensure the stabilization of soluble solids by providing a reducing in respiration, water losses, gas exchanges, and ethylene production [40], increasing the soluble solids is directly related to increasing the enzyme activity that causes starch hydrolysis to soluble sugars. Changes in soluble solids depend on several factors, such as the amount of fruit sugars, acidity, and soluble pectin in the fruit [36]. Under modified atmospheric conditions, with reduced respiratory activity, carbohydrates are less commonly used, and the soluble solids exhibit a stabilization amount due to the lower activity of certain enzymes. The higher content of soluble solids in control samples compared to coated samples is probably due to the loss of surface water in the control samples under modified atmospheric conditions [28, 29]. Bal et al. [30] and Nie et al. [34] found a decrease in TSS values for strawberry samples according to harvest value. Research findings by Chen et al. [32] and Khorram et al. [22] also showed an increase in soluble solids of tangerine and orange samples during storage.

The pH analysis results showed that the pH of samples increased during 60 days of storage. The pH level of tangerines showed a significant difference on 0 and 20th days. The pH level of the samples on days 20 and 40 was not significantly different. T4 and T5 groups had the lowest pH levels, while the control sample had the highest ($P < 0.05$). In groups T2, T3, T4 and, T5, there was no significant difference. In comparison to the T6, T1, and T7 groups, there was no significant difference between them (Table 1). Acid breakdown during respiration contributes to the pH of fruits [23, 40]. Rapisarda et al. reported that the acidity of orange samples was decreased during storage, but the pH was not significantly changed. Therefore, they did not find a clear correlation between pH and buffering capacity of juice [41]. Furthermore, coatings with different substances and methods have different effects on pH due to the changes in metabolic activities, especially respiratory rate [42]. Under modified atmospheric conditions, organic acids are commonly used less than that of the general condition. Therefore, the respiratory activity of the fruits is decreased due to acidity changes via pH variations [29]. Khorram et al. [22] and Tagrul and Arsalan (2004) also reported an increase in the pH of oranges and tangerines during storage.

Ascorbic Acid Content

As presented in Fig. 2, the amount of ascorbic acid decreased during the storage period. Ascorbic acid concentrations were highest in day 0 samples. The samples of the 20th and 40th days, respectively, had significantly less

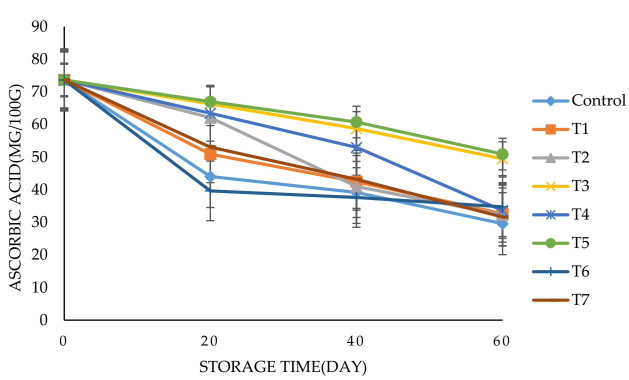

Fig. 2 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the ascorbic acid of tangerine fruit in storage duration

ascorbic acid. Ascorbic acid levels decreased in both samples, but in the T4, T3, and T5 groups, significantly less than in the control group. It was found that ascorbic acid reduction in the T1, T6, and T7 groups was more significant than that in the T2. Preservation of ascorbic acid is due to the reduction of oxygen permeability of coatings. Low oxygen reduces the oxidation rate of ascorbic acid ($P < 0.05$). After harvest, fruits are subjected to many oxidation events that result in the loss of ascorbic acid, accelerating cell aging and producing free radicals. As a result, ascorbic acid content is a valuable measurement of fruit quality during storage. Nevertheless, postharvest procedures can reduces ascorbic acid levels in fruit because ascorbic acid is water-soluble and thermosensible [43]. Reduction of oxygen permeability by coatings procedure prevents the activity of the oxidation-related enzymes and reduces vitamin C oxidation, which is very useful to maintain the nutritional value of fruits [44]. Furthermore, the oxidation of vitamin C depends on the type of packaging, temperature and storage conditions, the amount of oxygen dissolved, and the degree of penetration of the packaging material against oxygen. The higher the amount of O_2 in the package's headspace, the more significant decreases in ascorbic acid [45]. Results of Baswal et al. [31] and Chen et al. [32] showed a decrease in the amount of ascorbic acid during storage. Rapisarda et al. [41] reported the amount of vitamin C in Tarocco oranges between 60 and 90 mg per 100 ml of fruit juice. The concentration of ascorbic acid decreased during storage, so that at the end of storage, it was between 40 and 50 mg [41]. Khorram et al. also reported a decrease in the ascorbic acid content of orange samples during storage time [23].

Firmness

The hardness of the fruit is one of the critical factors in its acceptance as a fresh fruit or vegetable [46–48]. The tissue firmness is significantly related to water loss and biochemical changes in the cell wall, the intermediate layer, and cell membrane (cellulose, hemicellulose, and pectin) [29, 36, 37]. The results showed that the tissue firmness index decreased during storage. The tissue hardness of samples on days 0, 20th, 40th, and 60th showed a significant difference. It was found that the T5 group had more tissue hardness than the others, which is related to the coating decreasing the activity of cell wall enzymes. Groups T2, T3, and T4 had significantly stiffer tissue than groups T1, T6, T7, and control. Control samples had the softest texture after 60 days of storage ($P < 0.05$) (Fig. 3). The skin texture of the fruits became soft and crushed during the storage of the fruits. Tissue firmness may be affected by enzymatic activity and cell wall destruction, parenchyma degradation, and pectin dissolution in the intracellular fluid [44]. The softening of the fruit depends on increasing the number of enzymes,

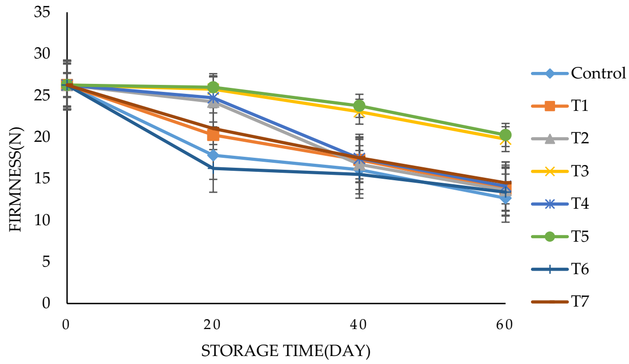

Fig. 3 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the firmness of tangerine fruit in storage duration

Table 2 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) packaging on the gas composition (O_2, CO_2) and color values (L*, Chroma, Hue angle) of tangerine fruit in storage duration

Treatments	Storage time (day)			
	0	20	40	60
$\%O_2$ (KPA)				
T1	5^{a}	3.55^{e}	2.45^{j}	1.32^{m}
T3	5^{a}	3.81^{c}	2.85^{g}	2.25^{k}
T5	5^{a}	4.15^{b}	3.25^{f}	2.55^{i}
T7	5^{a}	3.65^{d}	2.61^{h}	1.41^{l}
$\%CO_2$ (KPA)				
T1	15^{i}	19.62^{e}	22.20^{c}	32.81^{a}
T3	15^{i}	17.31^{gh}	19.85^{e}	21.90^{c}
T5	15^{i}	16.15^{h}	17.41^{g}	18.91^{f}
T7	15^{i}	18.85^{f}	21.25^{d}	28.72^{b}
L* value				
Control	69.41^{a}	67.55^{f}	63.71^{l}	62.41^{o}
T1	69.41^{a}	68.65^{d}	66.26^{i}	64.20^{k}
T2	69.41^{a}	67.75^{e}	65.61^{j}	63.61^{n}
T3	69.41^{a}	68.85^{c}	67.20^{g}	66.74^{hi}
T4	69.41^{a}	68.02^{e}	65.80^{j}	63.81^{n}
T5	69.41^{a}	69.13^{b}	67.41^{g}	66.94^{h}
T6	69.41^{a}	67.65^{e}	64.25^{k}	63.55^{n}
T7	69.41^{a}	68.75^{c}	66.83^{h}	64.53^{l}
Chroma				
Control	66.88^{a}	64.90f	59.49^{k}	58.23^{p}
T1	66.88^{a}	66.17^{d}	64.34^{i}	59.61^{n}
T2	66.88^{a}	65.68^{e}	63.53^{j}	58.66^{o}
T3	66.88^{a}	66.42^{c}	64.91^{h}	63.04^{l}
T4	66.88^{a}	65.97^{d}	63.86^{j}	58.70^{o}
T5	66.88^{a}	66.58^{b}	65.25^{g}	63.08^{l}
T6	66.88^{a}	65.12^{f}	59.55^{k}	58.51^{p}
T7	66.88^{a}	66.31^{c}	64.61^{h}	59.91^{m}
Hue angle				
Control	70.02^{a}	69.07^{f}	66.73^{k}	64.83^{p}
T1	70.02^{a}	67.83^{d}	66.11^{j}	63.34^{o}
T2	70.02^{a}	69.28^{e}	67.91^{i}	65.35^{n}
T3	70.02^{a}	67.94^{c}	66.64^{h}	65.20^{m}
T4	70.02^{a}	69.31^{d}	68.08^{j}	65.56^{m}
T5	70.02^{a}	68.14^{b}	66.83^{g}	65.41^{l}
T6	70.02^{a}	69.36^{e}	66.22^{k}	65.09^{p}
T7	70.02^{a}	68.08^{b}	66.40^{h}	63.61^{n}

Values represent means ± standard deviations. Means within a column with the same lowercase letters are not significantly different ($P < 0.05$)

MAP modified atmosphere packaging, *WPC* whey protein concentrate

including polygalacturonase, beta-galactosidase, and pectin methyl esterase activity [49]. The coating method can reduce pectin methyl esterase activity, pectin lyase, and polygalacturonase [23]. Furthermore, the coating prevents the increase in the ethylene concentration due to reducing the amount of respiration of the fruit and preserves the firmness of the fruit tissue [50]. The typical composition of MAP includes oxygen, carbon dioxide, and nitrogen. O_2 and CO_2 ratios play a critical role in protecting food commodities. In addition to slowing the ripening of fruits and vegetables, lowering the oxygen level and raising the carbon dioxide level can also slow respiration and ethylene production, as well as slowing many compositional changes associated with ripening [18]. Polygalacturonase enzyme activity is limited by low levels of O_2 and high levels of CO_2 during storage [51]. Pearson correlation analysis showed a significant ($P < 0.05$) correlation between tissue firmness and soluble solids ($R = -0.939$), as well as between tissue firmness and weight loss ($R = -0.899$). Based on this study and Baswal et al. [31] and Khorram et al. [22], similar results were found.

Internal Atmosphere Composition

Study results showed that carbon dioxide increased and oxygen decreased during storage. Oxygen levels were highest in the T5 group, while carbon dioxide levels were lowest. CO_2 percentages were highest, and oxygen percentages were lowest in the control group. According to the critical amount of oxygen gas and carbon dioxide (< 2, 20), the control group and T1, T6, and T7 were out of the critical limits from the 40th day onwards, groups T2, T3, T4, and T5 until the 60th day did not go beyond the critical limits ($P < 0.05$) (Table 2).The result showed a decreased consumption of nutrients in fruits after harvest because postharvest physiological processes were restricted. A high CO_2 content in fruits would lead to anaerobic respiration, which would possibly adversely affect the quality and taste of the fruits [52]. According to previous studies, the metabolic activity continues in harvested fruits that absorb O_2 and release CO_2, acetylene (C_2H_2), and aromatic compounds in the ripening process [40]. Inappropriate control of gaseous compounds could lead to anaerobic respiration, increased physiological decay, and decreased shelf-life [37]. As the result, the respiration rate of the packaged fruits reduced, followed by increasing Carbon dioxide levels. Thus, semi-permeable coatings around fruit reduce respiratory exchange and gas exchange and modify the atmosphere [11]. Khorram et al. [22] and Karacay et al. [23] have found similar results.

Microbiological Evaluations

The microbiological assessment showed that counts of mesophilic (Fig. 4), psychotropic (Fig. 5), bacteria strains, and fungal strains (yeast and mold) (Fig. 6) increased during storage. The microbial count was significantly differed on days 0, 20, 40, and 60. Mesophilic, psychotropic bacteria, mold, and yeast were found in the lowest number in the T5 groups. As compared to T6, T1, and control groups, T2, T3, T4, and T7 groups showed a lower number of mesophilic bacteria, psychotropic, mold, and yeast. The highest number of mesophilic, psychotropic bacteria, mold, and yeast was found in uncoated tangerines ($P < 0.05$). The molds and yeasts in fruits are part of the natural flora and can grow at lower pH values. However, hygienic fruit washing can delay spoilage, especially by yeasts. In addition, washing with 200 ppm chlorine before peeling, maintaining a pH of 3.5, and using MAP at low storage temperatures help to maintain microbial quality. In studies, it has been demonstrated that high levels of carbon dioxide inhibit aerobic microorganisms and yeasts [20].

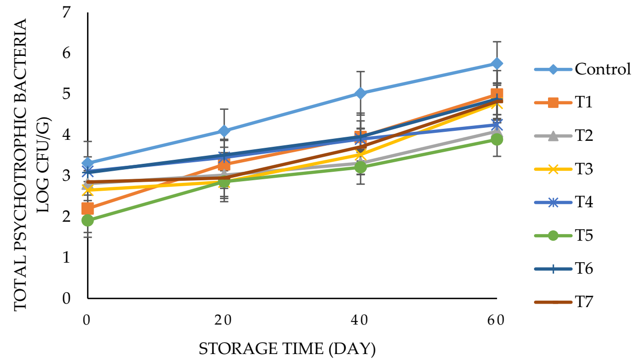

Fig. 5 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the total psychotrophic aerobic bacteria of tangerine fruit in storage duration

Color

Maintaining the inherent color of fresh fruit, often used as a quality indicator, has a significant impact on consumer acceptance [37]. Study results indicated that the color quality indices (L^*, Chroma, and Hue angle) decreased during storage. During the 20th day, the T6 group and the control group maintained their brightness and Chroma index based on the standard values of three brightness indices, Chroma, and color angle (65–70, < 60, 80 >). T2 and T4 groups and T1 and T7 groups maintained these indicators until the 40th day. At the end of the storage time, only the brightness and Chroma index (C*) of the T5 and T3 groups were within the standard range. Although the value of color angle (H˚) decreased during the storage period, it did not deviate from the standard in any of the treatments ($P < 0.05$), (Table 2). After harvesting in the ripening process, yellow, red, or orange colors are produced due to the reduction of chlorophyll and the synthesis of carotenoids in fruits [27]. Fruits exposed to warm temperatures (25–5 °C) during prevailing ambient conditions, coupled with low relative humidity (65–70%), will show a reduction in hue due to peel browning. According to the reports, decrease in respiration and modified atmosphere cause the delay in the change of fruit color and ethylene content. Furthermore, the synthesis of carotenoids and lycopene probably lead to a change in hue

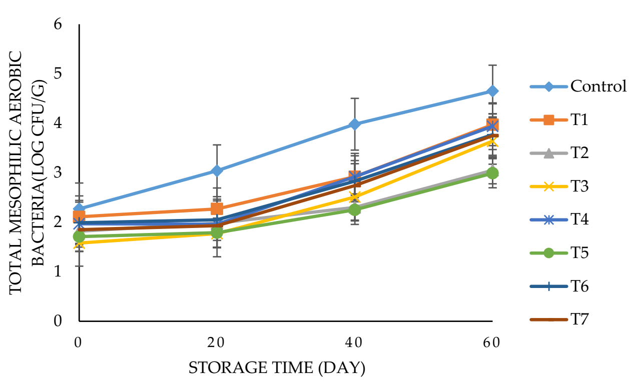

Fig. 4 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the total mesophilic aerobic bacteria of tangerine fruit in storage duration

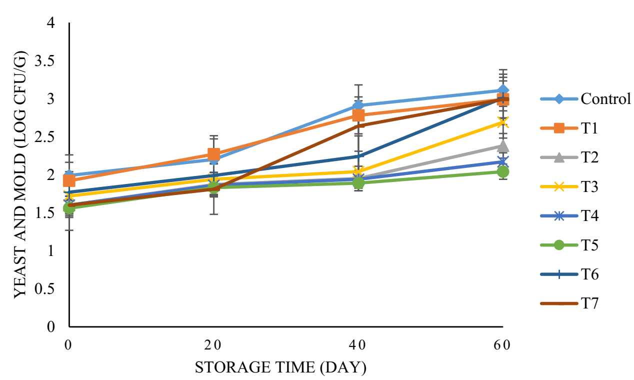

Fig. 6 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the total yeast and mold aerobic bacteria of tangerine fruit in storage duration

and Chroma angles [25]. One of the factors that reduces the brightness during storage is the oxidation of pigments [23, 53]. The coating can reduce the brightness loss of the samples, delay in ripening, and prevent color changes by reducing water loss. CO_2 Concentrations above 1% can delay the ripening of fruits by preventing the synthesis of ethylene [25]. Although, the increase in a^* of the packaged samples in normal conditions indicates carotenoid synthesis during the ripening process, the ripening processes of the packed samples under a low oxygen atmosphere are almost stopped [54]. Ramezanian et al. [53] and Karacay et al. [23] achieved similar results. One of the factors is the decrease in brightness during the oxidation of pigments. Saberi et al. [33] reported that the orange color was not affected by storage time.

Sensory Evaluation

As shown in Table 3, although the overall acceptance of all samples decreased during the storage period. According to the acceptance limit of the sensory characteristics of the control and T6 groups, they were accepted until the 20th day. T5 groups received the highest sensory scores. The control sample had the lowest sensory scores. Groups T2, T4, T7, and control group were acceptable until the 40th day. T3 and T5 groups maintained the quality of their sensory characteristics until the 60th day ($P < 0.05$). Tietel et al. reported that Flavor compounds are reduced with the increase in the storage time of mandarin [38]. In tangerine, the sensation of off-flavors develops faster than that of orange and grapefruit due to its high respiration rate, i.e., 3–3.5 [55]. In mandarins, the flavor of the fruit decreases due to the reduction of sesquiterpenes and monoterpenes, and the oxidation of short chains of fatty acids [39]. The concentration of ethanol in citrus is covered with composite coatings is higher than that of simple coatings because of low gas permeability [33]. Commercial waxes reduce weight loss and shrinkage, but the use of these coatings can cause off-flavor (through biochemical pathways of ethanol fermentation (acetaldehyde, ethanol, ethyl acetate, and acetic acid), fatty acid catabolism through the LOX pathway ((*E*)-2-octanal, (*E*)-2-hexanol, ethyl dodecanoate, ethyl hexanoic acid and ethyl propanoate), and the amino acid catabolism of mainly leucine and isoleucine decomposition (ethyl 2-methyl propanoate, ethyl 2-methyl butanoate, 3-methyl butanol and ethyl 2-butenoate) [39]. Furthermore, the most important advantages of the modified atmosphere are the reduction of respiration rate, ethylene production, softening of the fruit, and change in composition of the cell wall. Fresh fruits and vegetables have an active metabolism; they use oxygen for their activities and produce carbon dioxide during respiration. The metabolic activities cause the senescence and deterioration of the fruit. The crispness of fruits is related to the turgor pressure. Packaging under modified atmosphere conditions prevents the loss of water and crispness of fruits. Therefore, edible coatings and modified atmosphere packaging can act as barriers against moisture and resist moisture diffusion during cold storage, providing good adhesion to

Table 3 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) packaging on the sensory properties (flavor, color, texture, and overall acceptability) of tangerine fruit in storage duration

Treatments	Storage time (day)			
	0	20	40	60
Texture				
Control	4.60^{a}	3.65^{g}	2.85^{i}	1.75^{n}
T1	4.60^{a}	4.10^{e}	3.35^{h}	2.15^{l}
T2	4.60^{a}	4.30^{d}	3.30^{h}	2.35^{k}
T3	4.60^{a}	4.50^{c}	4.00^{ef}	3.55^{g}
T4	4.60^{a}	4.40^{cd}	3.35^{h}	2.35^{j}
T5	4.60^{a}	4.56^{b}	4.00^{ef}	3.60^{g}
T6	4.60^{a}	3.60^{g}	2.95^{i}	1.80^{m}
T7	4.60^{a}	3.95^{f}	3.35^{h}	2.20^{kl}
Color				
Control	4.70^{a}	3.60^{hi}	2.70^{l}	2.00^{n}
T1	4.70^{a}	4.10^{f}	3.30^{j}	2.20^{mn}
T2	4.70^{a}	4.20^{e}	3.70^{h}	2.30^{m}
T3	4.70^{a}	4.40^{c}	3.80^{gh}	3.50^{i}
T4	4.70^{a}	4.30^{de}	3.85^{g}	2.50^{l}
T5	4.70^{a}	4.60^{b}	3.90^{g}	3.70^{h}
T6	4.70^{a}	3.60^{hi}	2.80^{k}	2.10^{n}
T7	4.70^{a}	4.00^{f}	3.35^{j}	2.35^{m}
Flavor				
Control	4.85^{a}	3.55^{g}	2.65^{j}	1.52^{n}
T1	4.85^{a}	3.90^{e}	3.25^{i}	1.70^{mn}
T2	4.85^{a}	4.15^{cd}	3.25^{i}	1.85^{l}
T3	4.85^{a}	4.30^{bc}	3.45^{h}	3.25^{i}
T4	4.85^{a}	4.30^{bc}	3.50^{gh}	2.00^{kl}
T5	4.85^{a}	4.45^{b}	3.75^{f}	3.38^{h}
T6	4.85^{a}	3.70^{f}	2.72^{j}	1.70^{mn}
T7	4.85^{a}	4.05^{d}	3.25^{i}	1.85^{l}
Overall acceptability				
Control	4.60^{a}	3.35^{ef}	2.70^{g}	1.60^{k}
T1	4.60^{a}	3.85^{d}	3.15^{f}	1.95^{i}
T2	4.60^{a}	4.00^{c}	3.40^{ef}	2.25^{h}
T3	4.60^{a}	4.35^{bc}	3.85^{d}	3.40^{ef}
T4	4.60^{a}	4.25^{c}	3.60^{e}	2.20^{h}
T5	4.60^{a}	4.55^{b}	3.90^{d}	3.50^{ef}
T6	4.60^{a}	3.45^{ef}	2.75^{g}	1.70^{ij}
T7	4.60^{a}	3.90^{c}	3.20^{f}	2.05^{i}

Values represent means ± standard deviations. Means within a column with the same lowercase letters are not significantly different ($P < 0.05$). Modified atmosphere packaging;

WPC whey protein concentrate

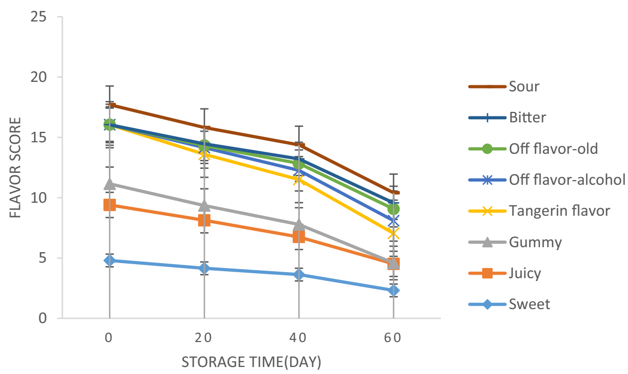

Fig. 7 The effect of whey protein concentrate coating and packaging (MAP and atmosphere condition) on the flavor of tangerine fruit in storage duration

fruit surfaces [28]. During storage, fruit flavor degraded due to a gradual decrease in tangerine flavor and an increase in non-flavor accumulation (Fig. 7(. Saberi et al. [33] stated that although no significant reduction in orange's sensory properties was observed in the first three weeks of storage, but scored significantly lower at the end in fourth week.

Overall, tangerine fruit coated with whey protein concentrate and packaged under modified atmosphere conditions showed a significant delay in the changes of weight, titratable acidity, total soluble solids (TSS), ascorbic acid, internal atmosphere composition, firmness, microbial quality, color, pH and sensory evaluation.

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Declarations

Conflict of Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare no conflict of interest.

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