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Alginate coating with orange essential oil on extend shelf life of fresh cut Pomelo (*Citrus grandis* L. Osbeck cv. Khao Yai)

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Abstract The result indicated that the use of coatings can reduce the weight loss and respiration rate. The appearance of a non-treated coating in fresh cut pomelo had distinctly separated pulp as compared to a treated coating in fresh cut pomelo, according to color change of pulp was measured by L*, a*, b*, and Hue value. Regarding, the study of chemical change as total soluble solid (TSS), total titratable acidity (TA) and ascorbic acid raised that the chemical change was depended on storage time, which the coating had not affected on chemical change. It was found that the fresh cut pomelo in a treated coating with 1% alginate combined with 0.5% orange essential oil inhibited as the increasing in total plate count, yeast and mold during storage. The total plate count, yeast and mold were reduced because the orange essential oil expressed to control the microbial growth. Hence, the use of alginate coating with 0.5% orange essential oil can preserve the quality and extend the shelf life.

Keywords: Alginate coating, Orange essential oil, Microbial growth and Postharvest quality

Introduction

Pomelo is *Citrus maxima* (Burm.) Merrill or *C. grandis* L. Osbeck., from the family Rutaceae and has many common names, including Pomelo, Pummel, Shaddock, Pumpelmoes, Pomplemose, etc. Pomelos are considered non-climacteric fruits, which are those that have sluggish physical and chemical changes as well as low respiration rates and low levels of ethylene synthesis (Kale and Adsule, 1995). Although the pomelo is regarded as a significant commercial fruit in Thailand, its enormous size and thick peel make it difficult to consume, which has an impact on consumer decision-making. Therefore, fresh cut pomelos are crucial to raising the value of agricultural products. The major problem of fresh cut pomelo is softness, wetness, and separated petals of the fruit during storage, resulting in a short shelf life. Hence, one of the key elements in

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raising the value of the product is the investigation of various strategies for extending the shelf life.

Alginate is a naturally occurring anionic polymer, typically obtained from brown seaweed, and has been extensively investigated and used for many biomedical applications, due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca^{2+} (Gombotz and Wee, 1998 and Parreidt *et al.,* 2018). As a result, alginate is utilized as a coating on fruit surfaces, where its main purpose is to reduce water loss. It controls respiratory function and has the ability to prolong shelf life. There was a study of the effect of alginate-based coatings and cinnamon essential oil in mango fruit. It found that the use of alginate-based coatings can delay the physical and chemical changes, and improve quality (Yin *et al.,* 2019). Additionally, alginate is also used with various forms of calcium (Alginate-based edible coatings) to increase the strength of the coating and is used together with essential oil or probiotic in coatings on vegetables, fruits, and processed foods to reduce microbial growth during storage (Hadidi *et al.,* 2021, Gaowa *et al.,* 2023 and Prietsch *et al.,* 2023).

Essential oils, which are also known as volatile or ethereal oils, are aromatic, viscous liquids derived from various plant parts, including buds, seeds, leaves, twigs, barks, fruits, and roots. The chemical composition of essential oils varies depending on the type of plant, growing season, location, and harvesting time. According to reports, the oil glands of Citrus species contain a significant amount of essential oil with chemical compositions including β-myrcene, g-terpinene, i-limonene, o-cymene, β-linalool, and cyclohexane (Yang and Kang, 2013). There was a study of the usage of orange peel oil in combination with fruit coatings in fresh cut mangoes (*Mangifera indica* L.). It was discovered that it can impede chemical and physical changes such firmness, color changes, and weight loss (Rodríguez *et al.,* 2015). In addition, red raspberries fruits were coated with alginate and lemon essential oil. It was found that the fruits coated with alginate and 0.2% lemon essential oil were able to preserve their color and inhibit the growth of mold and yeast (Gomes *et al.,* 2017).

Therefore, the purpose of this study was to investigate the possibility of extending the shelf life and quality of fresh cut pomelo by coating it with alginate and adding orange essential oil.

Materials and methods

Alginate coating

The 1% (w/v) sodium alginate solution was homogenized for 30 minutes at 18,000 rpm at 100 °C and sterilized for 20 minutes in an autoclave at 121 °C.

To produce the cross-linking reaction required for gel formation, an alginate solution containing 2% (w/v) calcium chloride, 1% (w/v) ascorbic acid, and 1% (v/v) glycerin was added.

Plant material and treatment

Pomelo (*Citrus grandis* L. Osbeck cv. Khao Yai) was obtained from cultivation in Nakhon Pathom, Thailand, and transported to the laboratory of the Faculty of Animal Sciences and Agricultural Technology, Silpakorn University. The processing was managed in selecting, washing in tap water, peeling, and selecting pieces that were similar in size and color. The fresh-cut pomelo was coated with an alginate solution and orange essential oil (commercial food grade). It was divided in 5 treatments, and 3 replications, as follows: 0 (control), alginate, alginate with 0.1% orange essential oil, alginate with 0.3% orange essential oil, and alginate with 0.5% orange essential oil. The fresh-cut pomelo was packed in a polyethylene terephthalate (PET) plastic box, 12x15 cm (6 pieces per box, approximately 250-270 g), and stored at 10 °C for 21 days. To investigate the quality change during storage, samples were randomly selected from each treatment at 0, 7, 14, and 21 days. The sample was kept fresh while the remainder was frozen and stored at -20°C prior to extraction and analysis.

Weight loss

Weight loss was measured as a reduction in the weight of the produce during storage and was expressed as the percentage of weight change compared with the initial weight, namely, the weight loss rate.

Color

Nine pieces of fresh cut pomelo were randomly sampled for analysis of color changes. The color of fresh cut pomelo was measured by a color meter (Model Mini Scan EZ, Hunter Lab), the data were assessed in L*, a*, b* and Chroma values.

Visual appearance

The visual appearance was evaluated based on the appearance of petals by visual observation using a five-point scale as follows: 5: Fleshy petals are not separated; The cyst is firm, and there is no wetness; 4: Fleshy petals are separated by no more than 10% of the total petal area; The cyst is firm and there is no wetness; 3: The petals are separated by 10-30% of the total petal area; The cyst has started to shrink, but is still not wet; 2: The petals are separated by more than 30% of the total petal area; The cysts in the petals began to willt clearly; and there is a slight wetness; 1: The appearance is unacceptable; The petals are separated by over 50% of the total area of the petals; The cyst has clearly visible wetness.

Total soluble solid (TSS): Total titratable acidity (TA) ratio

The method of A.O.A.C. (1990) was used to determine the total soluble solids (TSS) and titratable total acidity (TA) for each sample in 3 replications. The TSS determination from the juice was performed by direct reading in a digital refractometer (Atago Pocket Refractometer PAL-1) and expressed as %Brix. The TA determination, measured as % citric acid, was determined by titration with a 0.1 N sodium hydroxide solution and phenolphthalein indicator.

Ascorbic acid content

A 100 grams of pomelo pulp and place in a blender and filter and take a 5 ml of pomelo juice in 125 ml erlenmeyer flask, containing 5 ml of Metaphosphoric acid (HPO_3) solution. The sample was titrated with 2, 6-Dichlorophenol indophenol until the endpoint was pink solution. The titration value was calculated and compared with the ascorbic acid standard solution.

Respiration rate

Respiration rate and ethylene production were measured at storage temperature by sealing each glass bottle (1,000 ml in volume), containing with weighed 4 pieces of pomelo for 2 hours, and then sampling 5 ml of headspace gas using gas-tight plastic syringe and injected into the Gas Chromatography (model GC 6890, Agilent Technologies) for respiration (CO_2 production) with a thermal conductivity detector (TCD). The three columns in the GC column are as follows: HS-Q 80/100 0.5mx0.20mmID 1/8in OD, HayeSep Q 80/100 2.0mx0.53mmID 0.74mm OD and MS-13x 45/60 0.1ftx0.2mmID 1/8 in OD. The respiration rate was expressed as mg CO2 $kg^{-1}h^{-1}$.

Total plate count, Yeast and Mold

Total plate count was measured using plate count agar, while yeast and mold growth were measured using potato dextrose agar. A 25 g of sample was put in an erlenmeyer flask, containing 225 ml of 0.1% sterile peptone water. Pipette 1 ml each into 5 tubes containing 9 ml of sterile peptone water to obtain a dilution of 10^{-1} to 10^{-6}. The plates were incubated at 37 °C for 2 days, while yeast and mold were incubated at 42°C for 5 days. The number of colonies in each plate with the highest number of colonies less than 250 colonies, stating the results in Colony Forming Units (CFU/g).

Experimental design and statistical analysis

Completely randomized design (CRD) was used throughout the whole experiment. All the data was analyzed with a one-way analysis of variance (ANOVA). Mean separations were performed by Tukey HSD (Tukey's honestly significant difference). Differences at $p \leq 0.01$ were considered statistically significant.

Results

Weight loss

The weight loss in fresh cut pomelos was significantly different. The weight loss in fresh cut pomelos without alginate coating was a rapid increase at 14 days of storage (4.144%) and continued to increase until the last day of storage (14.503%), while fresh cut pomelo coated with alginate together with orange essential oil in other treatments had an average weight loss rate throughout storage of 0.071-0.226% (Figure 1). In addition, it was found that coating and the essential oil concentration was no effect on the rate of weight reduction.

Color

After 14 days of storage, there was a considerable difference in the L* a* b* and Chroma values. The b* value of fresh cut pomelo that was coated with alginate and 0.3% orange essential oil was higher than that of fresh cut pomelo that was not coated (control). However, there was no discernible difference in L* a* and Chroma values between fresh cut pomelo coating with alginate and essential oil. When coated and uncoated fresh cut pomelos were compared, the coated fruits had greater L*, a*, b*, and Chroma values than the uncoated fruits (Figure 2).

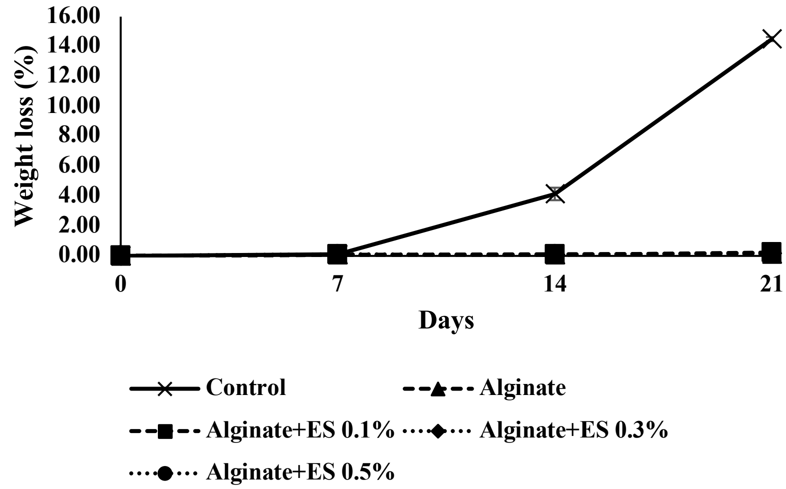

Figure 1. Percentage of weight loss in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

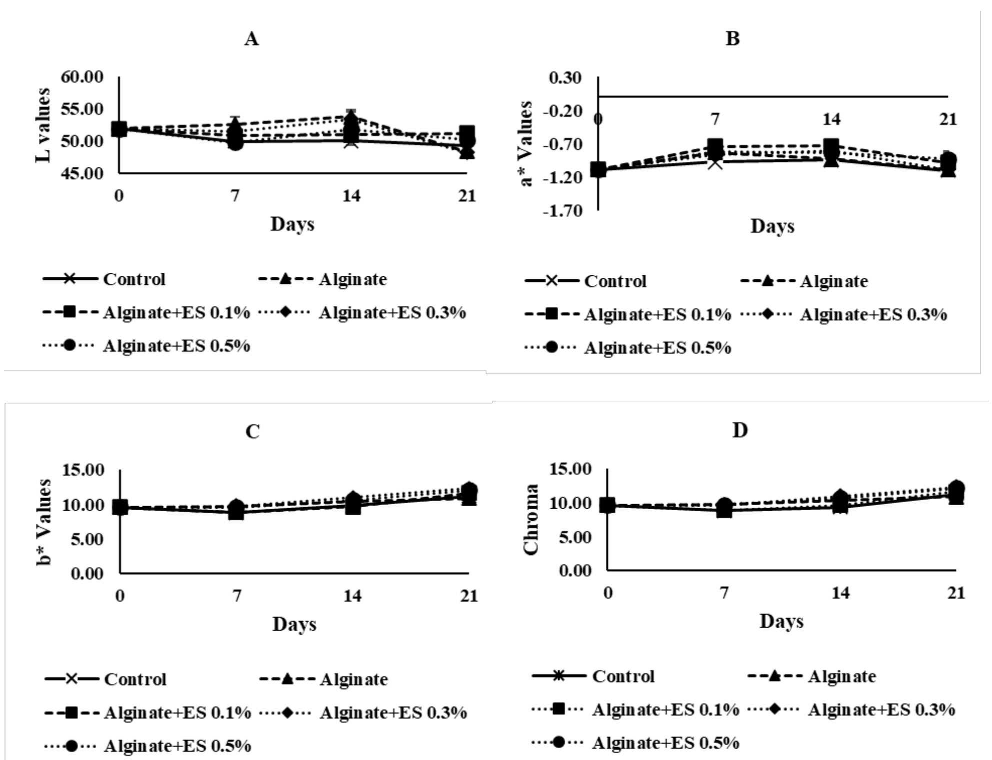

Figure 2. The color in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Visual appearance

The visual appearance of fruits during storage serves as an indicator of their quality. A high score indicated good quality of fresh cut pomelo; The appearance score ranged from 1 to 5 points (Figure 3). During the initial week of storage, fresh cut pomelo coated with alginate and varying concentrations of orange essential oil displayed indications of firmness in the fleshy petals, no separation, no wetness, and no disease. The visual appearance score was 5.00 points on average. On the other hand, the average score for fresh cut pomelos without an alginate coating was 4.33 points, indicating only a little crack in the cyst.

Fresh cut pomelos that were uncoated and coated with alginate and orange essential oil at concentrations of 0, 0.1, 0.3, and 0.5% were examined, after the fruit had been stored for 14 days. The results showed that coating can prolong the shelf life and quality of fresh cut pomelos for consumption, with average scores of 2.57, 3.56, 3.40, 4.00, and 4.00, respectively. Compared to coated fresh cut pomelos, uncoated fresh cut pomelos had more symptoms of petal cracking. The high rate of water loss in the uncoated fresh cut pomelos led to a decrease in the adherence of each cyst in the pulp. Therefore, this finding indicated a relationship between the coating and the pulp's quality of adhesion in the pomelo fruit.

On the final day of storage, the uncoated fresh cut pomelo developed mold and yeast, which resulted in a zero-point visual score. The first factor influencing a consumer's purchase decision is appearance.

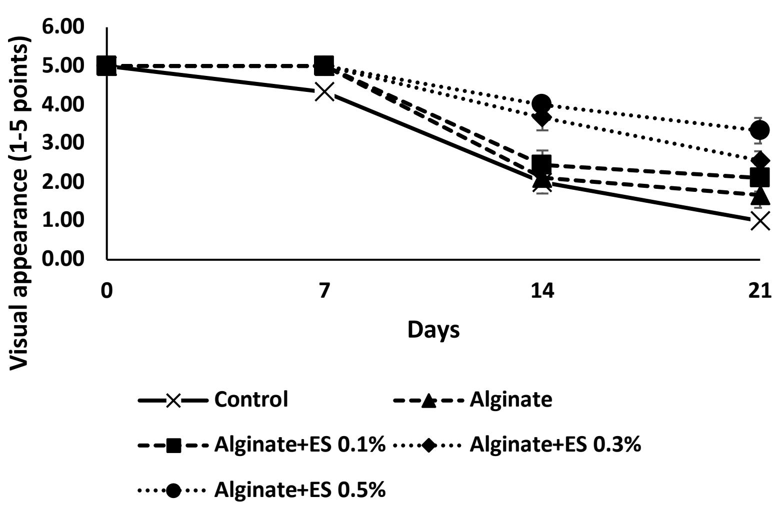

Figure 3. The visual appearance in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Total soluble solid (TSS) and Total titratable acidity (TA) ratio

The soluble solids are an indicator of the sweetness of the fruit. Fresh cut pomelos had a total soluble solid (TSS) in the juice equal to 10.75 Brix at the first time, after 14 days of storge, TSS decreased. The coated fresh cut pomelos had a lower TSS value than uncoated fresh cut pomelos (Figure 4A).

The results showed that the TA in fresh cut pomelos rapidly decrease until the 14 days of storage. Comparing the TA in each treatment, there were no significant differences. At the beginning of the experiment, the amount of titrated acid was 1.23% and decreased was 0.55-0.75% at the final day of storage (Figure 4B).

The TSS:TA ratio in all treatments was rapidly increasing until 14 days of storage, and then was constant. The TSS:TA ratio of fresh cut pomelo coated with alginate and orange essential oil at concentrations of 0.1, 0.3, and 0.5% was lower in the final days of storage than that of fresh cut pomelo without coating (Figure 4C).

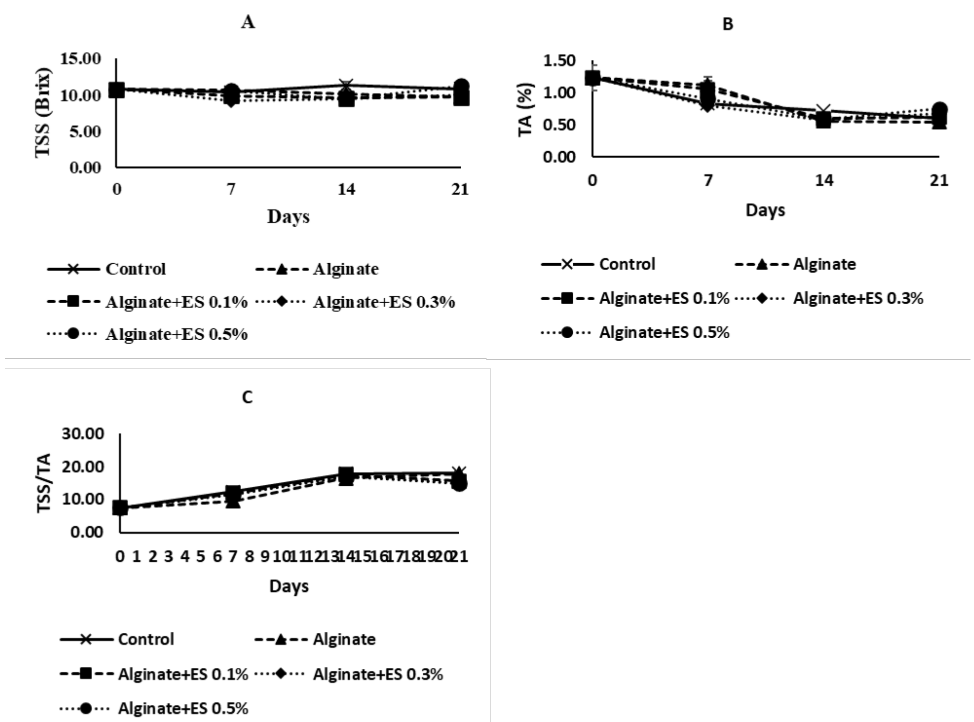

Figure 4. The total soluble solid (TSS), total titratable acidity (TA) and the total soluble solid: total titratable acidity ratio in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Ascorbic acid content

On the first day, fresh cut pomelo had 45,61 mg.100 g^{-1} of ascorbic acid. It was discovered that throughout storage, the ascorbic acid concentration was constant during the first week and began to rise in all treatments from day 14 (Figure 5). After being stored for 14 days, fresh cut pomelo without coating had the maximum ascorbic acid level (105.6 mg.100g^{-1}), while coated pomelo had an ascorbic acid content ranging from 66.61 to 75.83 mg.100g^{-1}.

At the 21 days of storage, the ascorbic acid concentration of fresh cut pomelo that was not coated rapidly declined (68.42 mg.100 g^{-1}), but fresh cut pomelo that was coated had a slight decrease (60.05–64.01 mg.100 g^{-1}). There was no statistical difference between coating and uncoating.

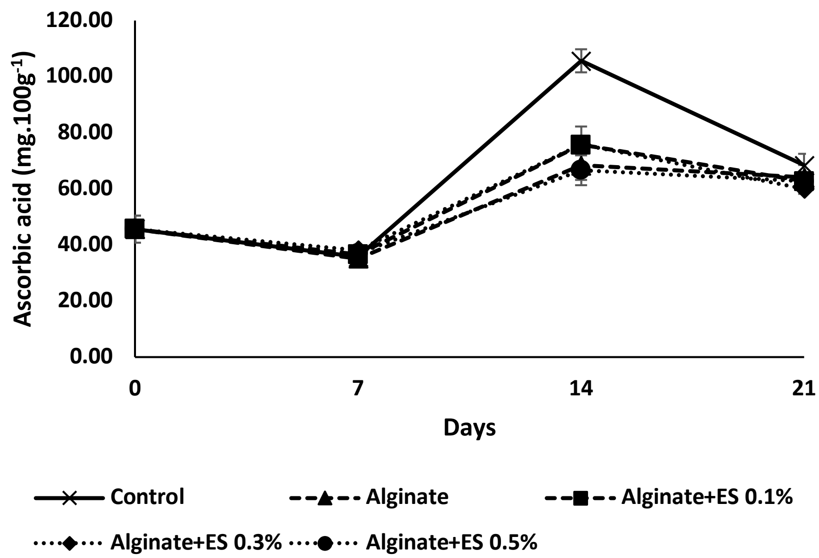

Figure 5. The ascorbic acid content in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Respiration rate

The change of respiration rate was shown in Figure 6. Fresh cut pomelo recorded an average respiration rate of 169.39 mg CO_2/kg.hr on the first day of storage. Following that, the respiration rate decreased and stayed that way for the duration of the storage. The uncoated fruits had the highest respiration rate when compared to the fruits that had been coated with alginate and orange essential oil at different concentrations. There was no statistically significant difference observed while using various concentrations of orange essential oil. These findings demonstrated that the coating has the ability to reduce the respiration rate. The concentration of orange essential oil, however, had no effect in decreasing the rate of respiration.

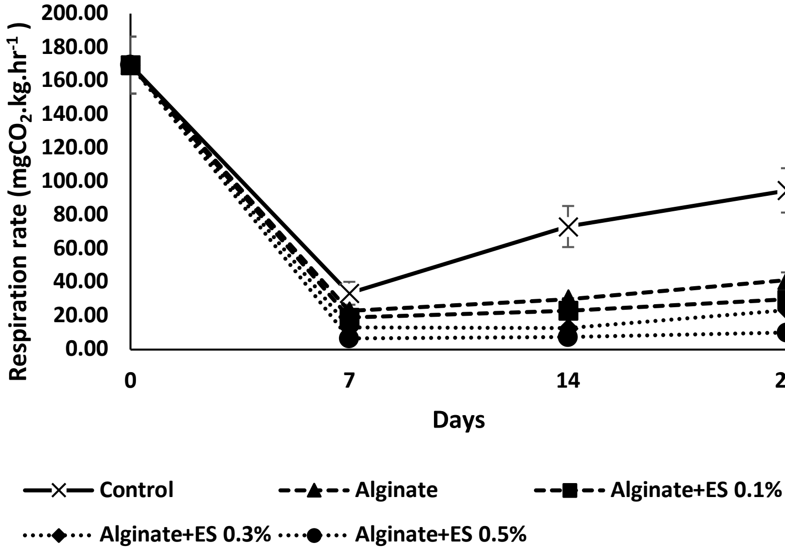

Figure 6. The respiration rate in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Total plate counts, yeast and mold

The development of yeast, mold, and total plate counts during fresh cut pomelo storage is a measure of all the microorganisms that impact shelf life and quality (Figure 7). The total plate counts are an indicator of the total amount of bacteria. At the beginning of the experiment, there were 2.69 log CFU/g. The microbial count of the uncoated fresh cut pomelo increased to 3.87 log CFU/g, after it was stored for a week. Subsequently, it experienced an exponential growth, culminating in 6.84 log CFU/g on the 14 days and 7.17 log CFU/g on the final day. A comparison of total plate counts between fresh cut pomelos coated with alginate and orange essential oil revealed that the coated fruits had a lower total plate counts than the untreated fruits. The fresh cut pomelo, which had been coated with an alginate solution and 0.5% (v/v) orange essential oil, had the lowest TPC on the final storage day (5.55 log CFU/g).

On 14 days of storage, the highest amounts of mold and yeast were found in fresh cut pomelos coated with alginate and orange essential oil at concentrations of 0.0, 0.1, and 0.3%, respectively. In contrast, no yeast or mold growth was discovered on the fresh cut pomelos that were coated with alginate and 0.5% orange essential oil, however there was an increase on the last day.

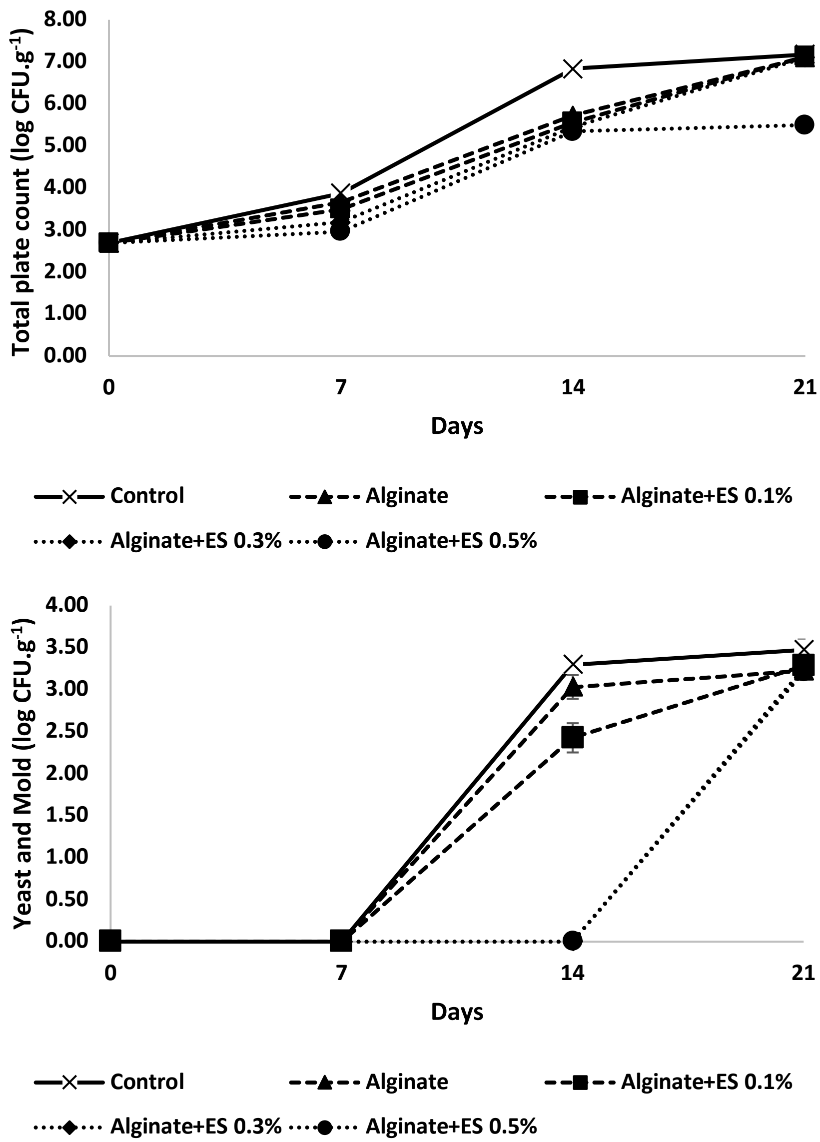

Figure 7. The total plate count, yeast and mold in fresh cut pomelo stored at 10 °C for 21 days. Each data point is the mean ± SE of three replicate samples.

Discussion

Pomelo fruit is categorized as a non-climacteric fruit and has a low respiration rate of 5–10 mg CO_2/kg.hr. There will cause numerous metabolic changes and increase respiration and dehydration rates when processed in fresh cut fruit. Fresh cut fruits and vegetables typically have a higher respiration rate because the wound of the process stimulated the transpiration occurrence, which slows down weight loss. Dehydration of fruits affects their shape and appearance, such as deformation and wilting. The results showed that coatings help prevent weight loss in fresh cut pomelo fruit because they slow down the pace at which carbon dioxide and oxygen are exchanged, which lowers respiration and reduces dehydration. Duong *et al*. (2023) claimed that coating rose apples with alginate before preserving them at a low temperature may decrease weight loss.

The increase in TSS of uncoated fresh cut pomelos, maybe due to water loss, or alterations in the pectin content of the cell wall, may be the cause of a rise in TSS. High molecular weight pectin exists in an insoluble state called protopectin, which breaks down in a lower molecular weight material that dissolves in water, producing a more concentrated cell-filled solution (Conrad, 1926; Kameshwar and Qin, 2018).

At the beginning of the experiment, the amount of titrated acid was 1.23% and decreased was 0.55-0.75% at the final day of storage. The decrease in TA in fresh cut pomelos during storage is consistent with experiments in mandarin oranges (Ennab *et al.,* 2020). In addition, it is possible that important organic acids in the anaerobic respiration involves the decomposition of malate and pyruvate to ethanol and acetaldehyde, resulting in a reduction in TA (Pesis, 2005; Vanoli *et al.,* 2015).

Fresh cut pomelos coated with alginate and orange essential oil showed reduced respiration rates compared to uncoated pomelos because the coating can regulate the exchange of carbon dioxide and oxygen. Glycolysis is impacted by changes in carbon dioxide and oxygen levels. A study revealed that the presence of carbon dioxide gas lowers the pH within the cell, decreasing phosphofructokinase activity (PFK), the most significant regulatory enzyme in the glycolysis path, and ultimately preventing the glycolysis pathway from proceeding (Mathooko, 1996).

The outcome showed that the most effective way to prevent bacterial and fungal growth is to combine alginate with 0.5% concentration of orange peel essential oil. The concentration of orange peel essential oil directly affects how well it inhibits the growth of germs and fungi. The antibacterial qualities of citrus essential oil contribute to the decrease of bacteria during storage. There was reported that the Citrus species has the chemical composition that can reduce the microbial growth such as β-myrcene, γ-terpinene, ι-limonene, o-cymene, β-linalool, cyclohexane (Edogbanya *et al.,* 2019). Yang and Kang (2013) reported that research has demonstrated the antibacterial activity of orange peel oil against the following bacteria: *Bacillus cereus, Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella enteritidis* and *Pseudomonas aeruginosa*.

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