Enhanced Knowledge in Sciences and Technology Vol. 2 No. 1 (2022) 069-078


**EKST**

Homepage: http://publisher.uthm.edu.my/periodicals/index.php/ekst
e-ISSN : 2773-6385

# Effect of Pectin Coating Enriched with Oregano Essential Oil on the Fresh-Cut Papaya Quality

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DOI: https://doi.org/10.30880/ekst.2022.02.01.008
Received 20 June 2021; Accepted 29 November 2021; Available online 1 August 2022

**Abstract**: Fresh-cut papaya has been found to deteriorate more readily than intact fruit resulting in increased surface browning, water loss, textural breakdown and off-flavour growth. Thus, the purpose of this study was to evaluate the effect of adding oregano essential oil to pectin coating on the physicochemical properties of fresh-cut papaya during 12 days of storage at 4°C. Yeast and molds growth as well as sensory evaluation were also done in this study. The incorporation of 0.75% (v/v) oregano (*Origanum vulgare*) essential oil (OEO) to the 2.25% (w/v) pectin coating of the fresh-cut papaya resulted in low physicochemical quality changes of up to 34% only when compared to the control samples, which achieved up to 75% physicochemical quality changes. The fresh-cut papaya coated with 2.25% (w/v) of pectin and 0.75% (v/v) OEO still under the critical limit of yeast and molds growth, which is 5 log CFU/ml, even has been stored for 12 days at 4°C, while the uncoated fresh-cut papaya has passed the critical limit at day 8 of storage. Although the coated fresh-cut papaya was accepted by the panellists, future research on the removal of unacceptable odour from the coatings may be needed to improve the sensorial quality of the coated papaya.

**Keywords**: Edible Coating, Essential Oil, Oregano, Papaya, Pectin Coating

## 1. Introduction

The Malaysian papaya industry is facing critical postharvest losses due to the fruit's short shelf-life. Papaya is unable to withstand long distant markets due to its fast-ripening attribute and poor keeping quality [1]. Around 30–50% of the harvested papaya never reach the consumers mainly because of the postharvest spoilage [2]. The papaya has short shelf life which soften rapidly at room temperature after being harvested. Besides that, it has been reported that cold storage can cause symptoms of chilling injury, including wooliness, flesh translucency, flesh bleeding and internal breakdown [3].

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Edible coatings have great interest of producers and distributors of fresh fruits due to their capabilities to increase the shelf life of fruits by reducing respiration. The implementation of edible coatings to fresh fruits can minimize changes in quality and loses in value, by changing and regulating the internal environment of the individual fruit [4]. One of the edible coating materials is pectin, which is the derivatives from polysaccharides. Pectin is an excellent candidate for film forming applications because it is naturally present in plant cell walls and available as a byproduct of the food and agricultural industries [5]. Pectin is made up of a polymer of α-1→4-linked D-galacturonic acid units, some of which are partially esterified with methanol at the C-6 carboxyl group and may be esterified with acetyl groups at C-2 or C-3 [6]. Based on the degree of methyl-esterification (DM), pectin is graded as high-methoxyl (HMP) or low-methoxyl (LMP) pectin. Other types of coatings, such as lipid-based coatings have been reported to result in the formation of an extra brittle and thicker coating, which may damage the appearance and gloss of the coated fruits. Meanwhile, protein-based coatings have low mechanical strength [7, 8].

The enhancement of shelf life of fruits is very important as even a few day extension of shelf life could represent a significant economic advantage for fruit industry. Essential oils (EOs) are natural compounds extracted from plants and generally recognized as safe (GRAS) food additives. The incorporation of essential oil into the edible coating will act as an antioxidant or antimicrobial agent for the fruits. As EOs can be used as active ingredients in biodegradable active films, they have received much attention especially due to their safety and simultaneous antioxidant and antimicrobial activities which will protect the quality of the papaya [9]. Oregano (*Origanum vulgare*) essential oil has showed it effectiveness in maintaining the quality of the fruit due to the presence of Carvacrol, an active phenolic compound which reported to have antifungal properties [10]. The integration of edible coatings with EOs are also important as carriers for a wide variety of food additives, including anti-browning agents, antioxidants, antimicrobials, colorants, colours, nutrients and spices [11].

Therefore, this study aimed to evaluate the effect of application of pectin coating incorporated with oregano essential oil on the physicochemical properties, microbial growth and sensory attributes of fresh-cut papaya.

## 2. Materials and Methods

### 2.1 Materials

Papayas (*Sekaki papayas*) were bought from local supplier at Bukit Gambir, Muar, Johor, Malaysia. Papaya with uniform shape, size was selected and only papaya with 45%-50% yellow skin was selected [12]. Papaya with less than 10% of total surface area affected by shape and skin defects was also accepted. High methoxyl pectin, esterified potassium salt from citrus fruit with 66-69% esterified (Dangshan Haisheng, Anhui, China), glycerol (EvaChem, Selangor, Malaysia), calcium chloride (EvaChem, Selangor, Malaysia), tween 20 (EvaChem, Selangor, Malaysia), oregano (Origanum vulgare) essential oil (Soap Cart, Malaysia) and filter paper (Whatman No 1, Sigma-Aldrich), peptone (Himedia, M618-500g, India), 0.85% sodium chloride (Himedia, MB023-1KG, India), Potato Dextrose Agar (Himedia, MH906, India), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution (Alfa Aesar, 1898-66-4, United States) were purchased and used in this study.

### 2.2 Preparation of fresh-cut papaya

Papayas were cleaned by immersion in sodium hypochlorite (NaOCl) solution (200 ppm) for 15 min, rinsed with distilled water and air-dried. Then, the papayas were peeled with a sharp stainless steel knife and cut into half prior to removing their seeds. The papaya flesh was cut into a cylinder (5 cm diameter and 1.5 cm height) using a moulder.

2.3 Designing pectin-oregano essential oil emulsion formulation

The percentages of pectin and oregano essential oil (OEO) in the formulations were the independent variables in this study. The percentage of these variables were designed using simplex-lattice mixture design from the statistical software (Design Expert® Version 6.0.4). The range of pectin was 1 – 3% (w/v) and for OEO was 0 – 3% (v/v) [10]. Table 1 shows six coating formulations labelled as F1-F5 and C1 acts as a control which contains distilled water only. C2 is an uncoated sample and act as a control too.

**Table 1: Coating formulations**

| Samples | Percentage of pectin (w/v) | Percentage of OEO (v/v) |
|---|---|---|
| F1 | 3.00 | 0.00 |
| F2 | 2.25 | 0.75 |
| F3 | 1.50 | 1.50 |
| F4 | 0.75 | 2.25 |
| F5 | 0.00 | 3.00 |
| C1 (distilled water only) | 0 | 0 |
| C2 (uncoated) | 0 | 0 |

2.4 Preparation of pectin-oregano essential oil formulation

1.5% (w/v) of pectin powder was added into 400 ml of distilled water and heated at 70 °C with continuous stirring for 1 hour to achieve complete dispersion of pectin. Next, glycerol 2% (v/v) (56 ml) was added as plasticizer to the pectin solution. Tween 20 2% (v/v) (8 ml) was added as emulsifier prior to OEO incorporation. The solution was cooled to 25°C. 1.5% (v/v) of OEO was added to incorporate into coating solutions. The amount of pectin powder and OEO were depending on the coating formulations presented in Table 1. The complete mixtures were subjected to magnetic stirring for a few minutes and pectin-oregano essential oil (PEC-OEO) emulsion was formed. 8 ml of 2% (w/v) calcium chloride (CaCl) was prepared with distilled water and used to crosslink the polymers as a separate solution.

2.5 Coating of fresh-cut papaya

The cut papaya flesh was first immersed in PEC-OEO formulated at different concentrations stated in Table 1 for 2 min. Excess of coating material was allowed to drip off for 30 sec before the cut papaya was immersed in 2% (v/v) of calcium chloride solution for one minute. The samples were left dripping dry for 30 sec again. The samples were placed in polypropylene trays and wrapped with the transparent polyethylene wrapping films and stored in refrigerator at 4 ± 4°C [13].

2.6 Physicochemical analysis of the fresh-cut papaya

Physicochemical analysis that included weight loss, firmness, total soluble solids (TSS) content, pH, colour and antioxidant were determined at day 0, 4, 8 and 12 of storage period and the analysis were done in triplicates.

Weight of the three samples from each treatment were measured on each day of analysis using a laboratory electronic balance (TXB622L, Shimadzu, Japan) with accuracy of 0.001 g [10]. All the samples were weighted before the storage as the initial weight of samples. Weight loss were determined and expressed as percentage weight loss using Eq. 1.

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100 \qquad Eq.1$$

The firmness of each sample was evaluated using a texture analyzer (TA.XT2, Texture Technologies Corp., NY). The samples were subjected to a puncture test at a constant speed of 50 mm/min using a 5-mm-diameter, round-tipped puncture probe at their geometric centre where it was defined as the force penetrating 5 mm into the samples at room temperature [10].

The TSS content of each sample on each day of analysis were observed by using a hand refractometer (0 –32% Brix, ERMA Inc. Tokyo, Japan) by crushing a small part of sample and the juice was extracted. The obtained reading was recorded in degree brix (˚Brix) [14].

The pH was measured using a pH metre, which has been calibrated with buffer solutions with pH 4.0, 7.0 and 10.00 for each use. 10 g of papaya sample was homogenized using a blender with a 50 ml of distilled water. The tip of the pH metre probe was immersed inside the solution and the readings were read when 'READY' appeared on the screen.

A colorimeter (Mini Scan XE Plus, HunterLab, USA) was used to determine the colour of samples on each day of analysis throughout the storage period. The device was calibrated with black and white tiles before analyzing the samples for colour. Next, the samples were covered with transparent plastic and the surface of the device was pressed on the samples. The results displayed on the screen were in terms of L* (lightness), a* (green-red) and b* (yellow-blue) values.

The antioxidant capacity of fresh-cut papaya was studied through the determination of free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The fresh-cut papaya samples were centrifuged at 4,500 rpm for 15 min at 4°C (Centrifuge AVANTI J-25, Beckman Instruments Inc., Fullerton, CA, USA) and filtered through a Whatman No 1 paper. An aliquot of 0.01 ml of the supernatant was mixed with 3.9 ml of methanolic DPPH of 0.025 g/l and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in a dark area for 30 min. The absorption of the samples was measured with UV-Vis spectrometer at 517 nm against a blank of methanol without DPPH. The results were expressed as stated in Eq. 2.

$$\text{DPPH}\ (\%) = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of sample}} \times 100 \quad Eq.2$$

2.7 Selection of the best formulation

The selection of the best formulation was done by the percentage of changes of physicochemical analysis. Formulation with the lowest changes in percentage of weight loss, firmness, TSS content, pH, colour and DPPH value was chosen at the end of 12 days of storage at 4°C in the refrigerator. The results were expressed using Eq. 3. The selected formulation was continued with microbiological and sensory analysis.

$$\text{Percentage changes} = \frac{\text{Final value} - \text{Initial value}}{\text{Initial value}} \times 100 \quad Eq.3$$

2.8 Microbiological analysis

10 g of fresh-cut papaya from each treatment was transferred into a laboratory glass bottle with screw cap. The samples were diluted with 90 mL of saline peptone water, which consists of 1.5% peptone water and homogenized by shaking the sample solution. 1 ml of the solution was added to the next dilution. 0.1 ml was poured onto Potato Dextrose Agar (PDA) to quantify the yeasts and molds. The PDA was incubated at 37 ± 2°C for 5 days. The results number of colony forming units (CFU) was obtained after incubation time using Eq. 4. Two replicate analyses for each sample were carried out periodically throughout storage.

$$\text{Log CFU/g} = \log \frac{\text{Number of colonies counted} \times \text{Dilution factor}}{\text{Volume of aliquots in ml}} \times 100 \quad Eq.4$$

2.9 Sensory analysis

10 trained panellists were recruited among the Universiti Tun Hussein Onn Malaysia (UTHM) students to do the tests. The selected formulation sample was labelled as 239 and the uncoated sample was labelled as 741. Both samples were served in a tray presented to each panellist at room temperature (25±1 °C). The sensory evaluation was carried out using a 15-cm line scale, where the sample attributes tested including odour, colour and firmness. Plain water was used for palate cleansing between samples [10].

2.10 Statistical analysis

All data were expressed in mean and standard deviation. The significance difference between each quality parameters were subjected to one-way ANOVA from Microsoft Excel 2010 (15.5, Microsoft Corporation, USA) and Tukey's test using statistical software (Minitab®, version 19, State Collage). P-value analysis to determine the differences among means at a 5% significance level was done and the data were analyzed as the effect of independent variables which is concentration of pectin and oil on different quality parameters of coated fresh-cut papaya [10].

## 3. Results and Discussion

3.1 Physicochemical properties of the fresh-cut papaya

Figure 1 presents the percentage of weight loss for all samples. The incorporation of OEO to the samples has shown lower weight loss due to the lipidic composition of essential oils, which helps to decrease the permeability of water vapor [14]. The weight loss is considered as a measure of freshness to both fruits and vegetables [15]. The low percentage of weight loss in the coated papayas indicates the presence of pectin in the emulsions, as its hydrophobic nature helps the matrix to be reinforced against moisture loss and cell wall destruction [16]. Coating gives good mechanical efficiency and barrier capability to resist leakage of moisture.

Figure 2 shows that the firmness was best maintained on samples with the use of coatings ($p < 0.05$). At the end of storage, F2 recorded the highest firmness of 832.63 ± 19.28 g, while F4 and F5 had the lowest change in firmness with 497.54 ± 57.93 g and 497.56 ± 57.94 g, respectively. The fresh cut fruit is extremely perishable since their tissues are exposed. Due to this lack of protective cover against cell wall destruction caused by respiration and chilling injury, tissue becomes prone to degradation. In avoiding the substance against softening, calcium chloride can be used for crosslinking the polymers since the calcium chloride has been commonly used as a firming agent for fruit tissues. It interacts in the cell wall to form calcium pectate with pectic acid, which facilitates molecular bonding between the cell wall constituents [16].

Figure 3 illustrates the pH values for all samples on the day 0, 4, 8 and 12 of storage. It has been observed that the incorporation of the OEO resulted in lowering the pH and also slowed the pH changes with the final value of day 12 ranging from 5.11 ± 0.08 to 5.45 ± 0.04. On the other hand, pH of the control samples (C1 and C2) had reduced to 5.27 ± 0.07 and 5.38 ± 0.03, respectively probably because of depicted high microbial and fungal growth [10]. At day 0, both control samples, C1 and C2 showed higher values of 5.98 ±0.06 and 6.01 ± 0.03, respectively compared to the coated samples. This is due to the presence of OEO in the coated samples, where the acidic properties of OEO has contributed to the lower pH values.

Figure 4 presents the TSS content of all samples during storage. The TSS content is relatively related to the concentration of organic acid and soluble sugar balance as high TSS values indicated high amount of these components. There is no significant difference ($p>0.05$) between the TSS values of all samples. This is possibly attributable to being treated with pectin which is a polysaccharide itself which may have led to the soluble solid material [10]. The impressive semi-permeable coating around the fruit

is altering the inner environment by restricting the amount of oxygen for macromolecular respiration and degradation. Therefore, the reduced respiration rates decelerate metabolite synthesis and usage, resulting in a slower rate of increase in TSS [15].

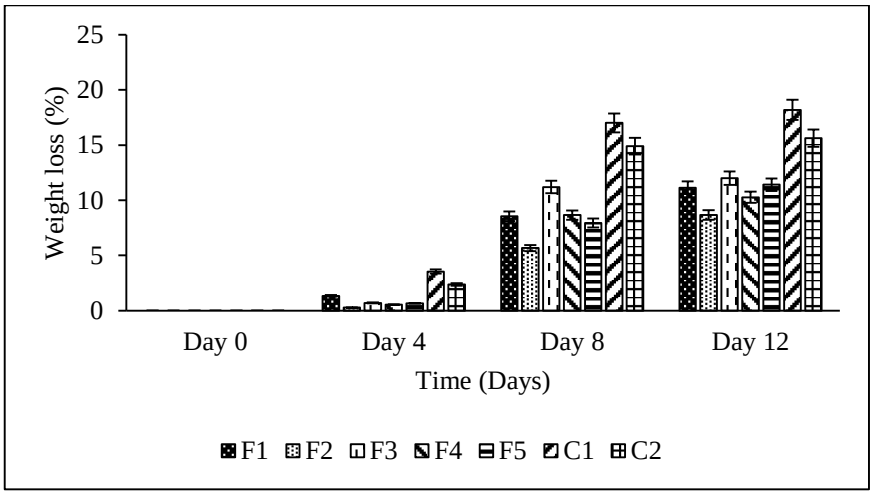


**Figure 1: The percentage of weight loss**

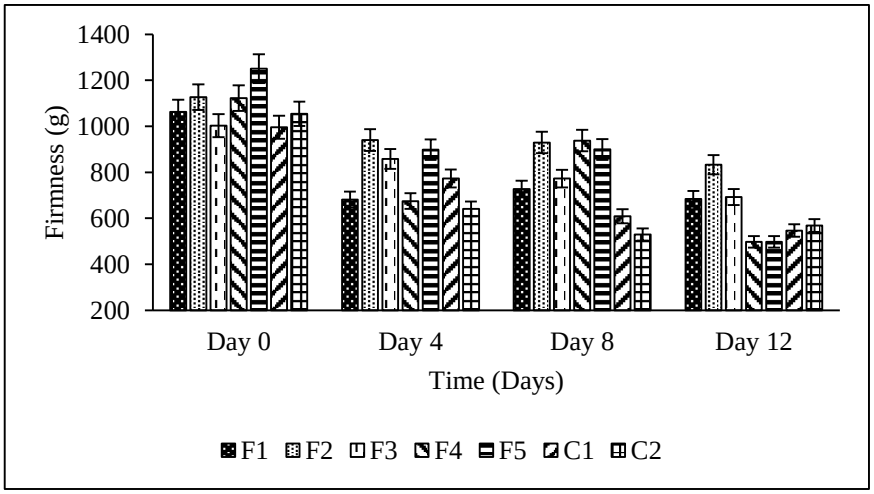


**Figure 2: The values of firmness**

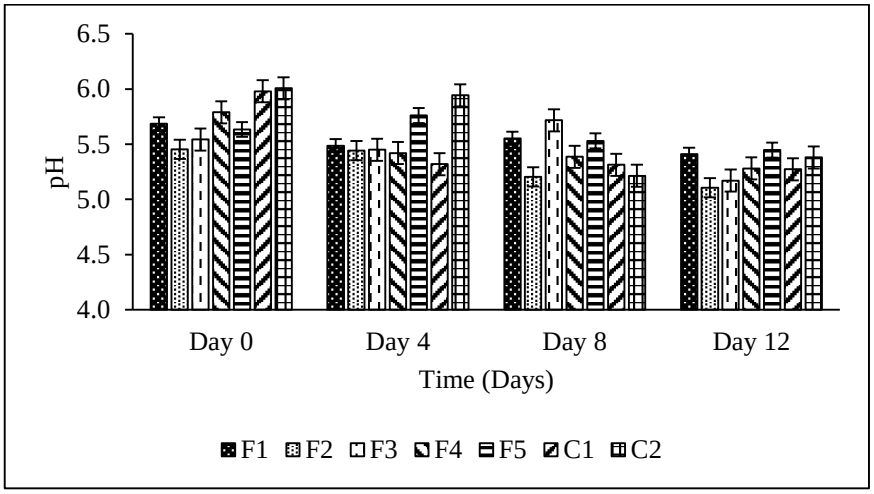


**Figure 3: The pH values**

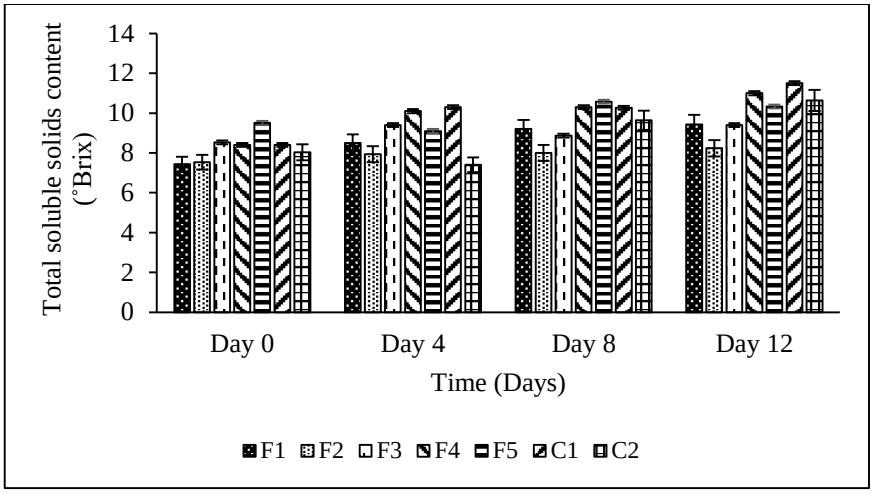


**Figure 4: The TSS content**

Figure 5 shows the lightness (L*), redness or greenness (a*) and yellowness or blueness (b*) values for all samples during storage. Based on Figure 5(i), F3 recorded the highest L* of 56.12 ± 2.90 ($p < 0.05$) on the day 12 of analysis, which explained the formulation has the brightest colour. The darkening of the samples, where the decreasing trend of L* indicated ripeness of papaya, was consistent with the findings of Narsaiah et al. [18]. Based on Figure 5(ii), the values of a* decreased continuously over the storage time. Based on Figure 5(iii), F1 to F5 samples possess high ability to preserve the yellowness compared to the control samples because the coatings inhibit oxidative or enzymatic browning [17].

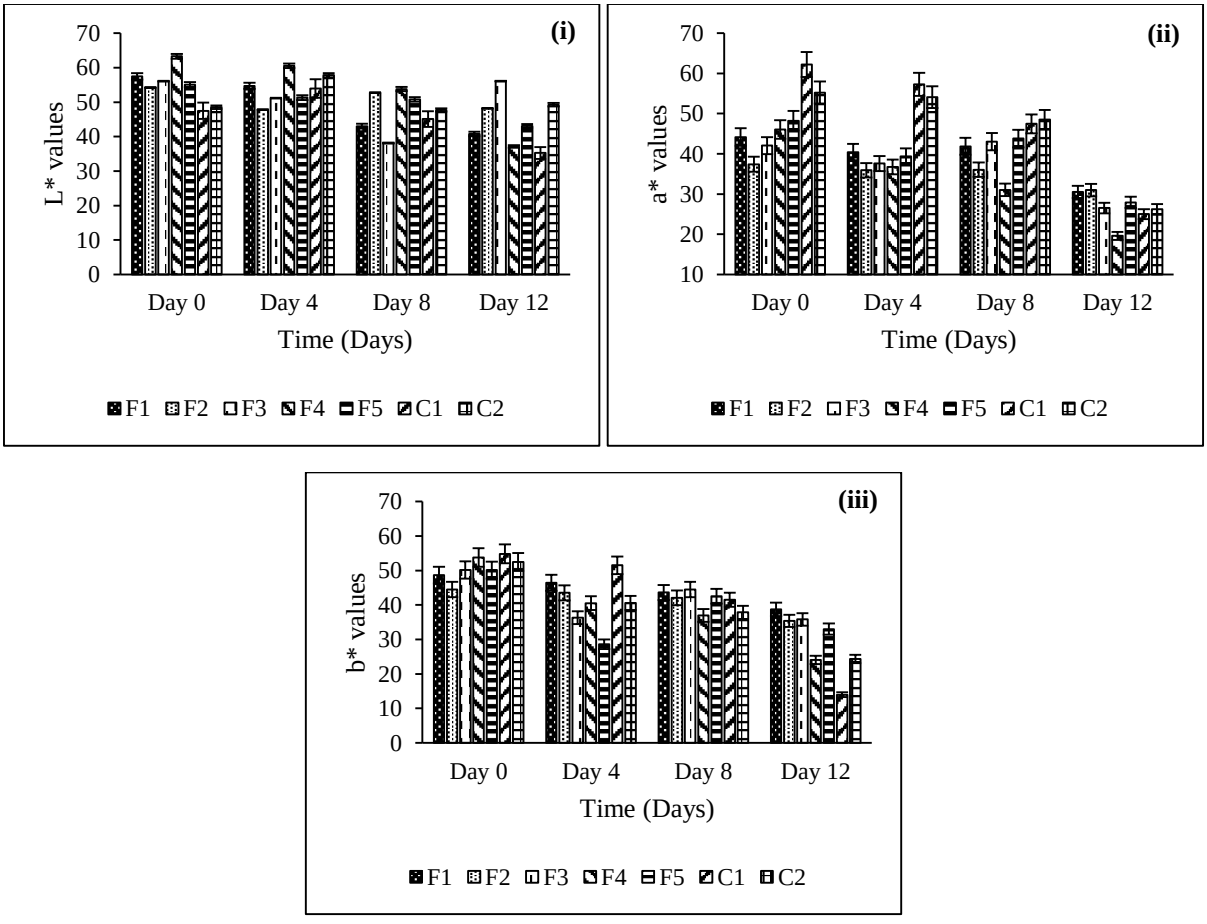


**Figure 5: The (i) L*, (ii) a* and (iii) b* values**

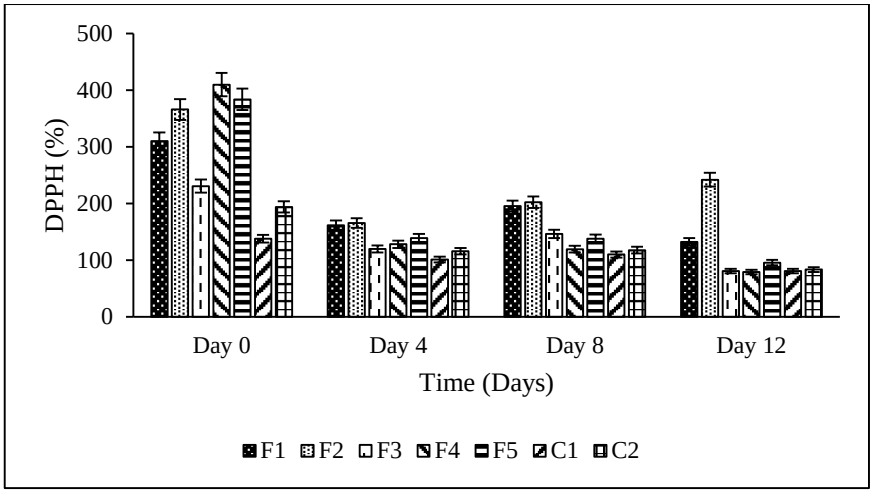


**Figure 6: The percentage of DPPH**

DPPH is a preventive antioxidant activity and the scavenging activity can be used as the indication of prevention from increased oxidative stress and hydroxyl radical scavenging activity. Figure 6 shows that the antioxidant activity was reduced along the storage of the fresh-cut papaya. During storage, cell defences against the harm induced by free radicals caused the antioxidant activity to decline [17]. F2 showed the highest percentage of antioxidant 241.86 ± 4.93 at the end of 12 days of storage at 4°C. At day 0, all treated samples showed higher percentage of antioxidant compared to C1 and C2, while F4 owns the highest DPPH value as it contains 3% of OEO.

3.2 Selection of the best formulation

Table 2 presents the changes in physicochemical properties for all samples after 12 days of refrigeration at 4°C. The best formulation was analysed by taking into account the most suitable edible coatings that had the lowest percentage of changes on day 12 values when compared to day 0 in all physicochemical analysis. F2 recorded the lowest value of changes by 8.68% hence, it shows the best result in retaining the weight of the samples. Moreover, F2 also recorded the lowest percentage change in firmness which was -26.10%. The percentage differences for colour analysis were -11.14% for L* values, -17.08% for a* and b* values -20.44%. Percentage different for DPPH was -33.87%. Although the TSS content and pH values for F2 are not the lowest, the values are still acceptable and close to the lowest values. In conclusion, F2 is the selected formulation in this study. The F2 contains 2.25% (w/v) pectin provided moisture barrier and incorporated with 0.75% (v/v) OEO which believed to decrease the respiration [9].

**Table 2: The percentage difference of the physicochemical properties**

| Samples | Weight loss (%) | Firmness (%) | TSS (%) | pH (%) | L* (%) | a* (%) | b* (%) | DPPH (%) |
|---|---|---|---|---|---|---|---|---|
| F1 | 11.14 | -35.65 | 26.91 | -4.87 | -28.98 | -30.90 | -20.36 | -57.42 |
| F2 | 8.68 | -26.10 | 9.29 | -6.36 | -11.14 | -17.08 | -20.44 | -33.87 |
| F3 | 12.00 | -30.92 | 8.73 | -3.31 | 0.09 | -36.92 | -28.61 | -65.01 |
| F4 | 10.27 | -55.68 | 30.95 | -8.81 | -41.36 | -57.46 | -55.29 | -80.72 |
| F5 | 11.41 | -60.22 | 10.16 | -6.73 | -21.79 | -42.13 | -34.16 | -75.13 |
| C1 | 18.19 | -45.12 | 71.64 | -11.82 | -25.80 | -59.80 | -74.56 | -41.47 |
| C2 | 15.64 | -46.08 | 60.30 | -10.43 | 1.46 | -52.55 | -53.59 | -57.00 |

### 3.3 Microbial growth

Table 3 shows the yeast and molds colony count for both coated and uncoated samples. The critical limit for yeast and molds for papaya fruit was 5 log CFU/ml [11]. Both F2 and control samples showed result less than 5 log CFU/ml at day 0 which was 2.18 ± 0.16 and 2.37 ± 0.10 respectively. It was observed that the result for day 4 portrayed the same trend (< 5 log CFU/ml) for both of the samples. However, F2 samples were managed to stay below the limit at day 8 with 3.04 ± 0.38 log CFU/ml and day 12 with 4.30 ± 0.11 log CFU/ml. Therefore, the shelf-life of the control sample was less than eight days and F2 extended the shelf life of fresh-cut papaya until day 12.

**Table 3: The yeast and molds colony count**

| Samples | Yeast and molds colony count (log CFU/ml) | | | |
|---|---|---|---|---|
| | Day 0 | Day 4 | Day 8 | Day 12 |
| F2 | 2.18 ± 0.16 | 2.33 ± 0.07 | 3.04 ± 0.38 | 4.30 ± 0.11 |
| Uncoated | 2.37 ± 0.10 | 2.56 ± 0.15 | 5.58 ± 1.06 | 6.48 ± 0.47 |

### 3.4 Sensory evaluation

Table 4 shows the results for sensory evaluation. Based on the observation, there was a slight pungent odour detected in the coated sample. The odour presented might be contributed by the OEO, where strong aroma has adversely prevailed over the original odour of papaya. However, the odour of F2 still the most preferred by the panellists compared to the uncoated sample due to its volatile nature. This finding is consistent with the study done on the fresh-cut pineapple [1] and fresh-cut papaya [10]. The firmness and colour of both the coated and uncoated samples did not differ significantly ($p > 0.05$).

**Table 4: The sensory evaluation**

| Sample | Odour | Firmness | Colour |
|---|---|---|---|
| F2 | 11.20 ± 1.02 | 8.90 ± 1.08 | 10.70 ± 2.76 |
| Uncoated | 7.30 ± 2.35 | 8.60 ± 1.06 | 13.50 ± 3.12 |

## 4. Conclusion

The incorporation of OEO to pectin coating diluted the intense and penetrating effect of the oil hence helped to maintain the physicochemical and microbiological quality of the fresh-cut papaya more than the uncoated fresh-cut papaya throughout 12 days of storage. The treatment with distilled water only contributed to faster deterioration of the papaya's quality. In summary, the 2.25% (w/v) of pectin and 0.75% (v/v) OEO is the most effective combination to retard the degradation rate of weight loss, firmness, pH, TSS content, colour and DPPH of fresh-cut papaya stored for 12 days at 4°C.

## Acknowledgement

The authors wish to thank Universiti Tun Hussein Onn Malaysia for the facilities provided and financial support.

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