Tomato peel-cutin based film mitigates the deterioration of calamansi (*Citrofortunella microcarpa*)

I N Madzuki¹, J M Tan¹, N A A Mohamad Shalan² and N S Mohd Isa³

¹ Faculty of Chemical Engineering Technology, University Malaysia Perlis (UniMAP), Perlis, Malaysia.
² Department of Coaching Science, Faculty of Sport Science and Coaching, Universiti Pendidikan Sultan Idris (UPSI), Perak, Malaysia
³ Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Terengganu, Malaysia.

E-mail: iffahmadzuki@unimap.edu.my

Abstract. The plant cuticle, cutin is the main component in tomato peel. The cutin was obtained through 3% (w/v) NaOH extraction by autoclave. The extract was then mixed with pectin, forming a film suspension before being used as a coating for calamansi. The calamansi samples were grouped (n=20) into: pectin; 1:1 pectin/cutin; 2:3 pectin/cutin; 3:2 pectin/cutin; and uncoated control. The calamansi were stored at 25°C for 10 days and were analysed for physio-chemical changes every two days. Calamansi samples coated with pectin/cutin showed a significant reduction in firmness and Brix as early as day 2 and the percentage of citric acid at day 6. After 10 days of storage, the 2:3 pectin/cutin treatment samples showed minimum changes in weight loss (23.52±3.20%), firmness (27.11±3.10 N), total soluble solids content (7.55±0.21°Brix), titratable acidity (25.60±0.32% citric acid) and colour. Therefore, it can be concluded that the 2:3 pectin/cutin film coating was the best treatment for harvested calamansi in attenuating the deterioration process.

1. Introduction
Petroleum-derived polymers food wrap is widely used in grocery and household items to maintain the quality of vegetables and fruit mainly from oxidisation and to prevent any contact with contaminants. However, the use of plastic made from petroleum-derived polymers had raised environmental concerns as this material is non-biodegradable and non-renewable. Only less than 5% of petroleum-derived polymers are recycled [1]. The average time taken to breakdown plastic food wraps is up to hundreds to thousands years [2]. Chemicals in plastic food wraps such as thalates and Bisphenol A (BPA) had caused a bad impact on human health and the environment. Eyes irritation, breathing difficulties and vision failure are examples of health problems that arise due to thalates and Bisphenol A (BPA) [3]. Moreover, the accumulation of food wraps as trash has seriously burdened the environment.

The use of edible film instead of plastic wraps may offer a better alternative to plastic food wraps without affecting the environment. Edible film is composed of a thin layer of edible material applied between different layers of components or on the food surface to maintain the quality food products and prolong its shelf life by providing additional external barrier [4][5]. The edible film is categorized as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA), mainly used for fresh fruit and vegetables to helps in preventing the deterioration of the quality which affected by oxidation, moisture loss, microbial spoilage and flavour loss [5][6].
The study of edible coating from agro-industrial wastes such as lettuce, cucumber, carrot and watermelon have been conducted previously [7]. According to the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT), tomato is the second largest primary vegetable crop after potato with the amount of 182.3 million tons in 2018 only [8]. Tomato pomaces or residues such as fibre, seed and peel are produced during tomato puree, paste, juice or sauce production. Tomato peel is considered as a waste product, although it contains abundant of nutrients and high in antioxidant [9]. The main component of tomato peel is cutin, a high molecular weight lipophilic substance that plays a role in controlling water loss, transporting substances across tissues and act as protection against pathogen and insect [10] [11].

Calamansi, a citrus fruit that is widely found in tropical countries particularly in Southeast Asia. The average diameter is up to 4.5 cm with a green or orange coloured thin peel with strong citrus flavour. Calamansi is high in vitamin C and may contain coumarin, tannins, caffeic and sinapic acid as shown in the most citrus family [12][13][14]. It has wide application and received high demand from various industries including food, drink, and even cosmetic, showing its high potential for commercialization. However, the constraint of technology and packaging method had caused limited capacity to produce the volume needed by the market [15]. It was reported that weight loss and vitamin C of citrus fruit significantly degraded as early as day 3 during storage [16][17]. One of the roles of food packaging is preserving the food products from chemical influence [18], which may be solved by environmental friendly packaging cutin edible film. Therefore, this study investigates the ability of tomato cutin biofilm in improving shelf life and slowing down the deterioration process of calamansi.

2. Experimental

2.1. Preparation of Tomato Peels Powder
Tomatoes were purchased from a hypermarket in Kangar, Perlis. The tomatoes were blanched for 60 seconds in boiling water and the peels were collected. The cleaned peels were dried at 40°C and were then milled into powder form and kept in polyethylene bags at room temperature (25°C) before using.

2.2. Cutin Extraction
Cutin was extracted according to [19] with slight modifications. The cutin was extracted by 3% (w/v) NaOH at 121°C for 20 min by using an autoclave. The extraction process was quintuplicate by removing and adding fresh NaOH. The solution was then filtered and the pH filtrate was adjusted to pH 5-6 with 6 M HCL. Lastly, it was centrifuged at 8650 rpm for 30 min and the collected pellet was dried at 25°C.

2.3. Film Forming and Coating Preparation

2.3.1. Film Forming Suspension
The film suspension was prepared from pectin (35 mg/ml in ultrapure water) and mixtures of pectin (35 mg/ml in ultrapure water) and cutin (25 mg/ml in ultrapure water at ratio 1:1, 3:2 and 2:3). The prepared suspension was then refluxed in an ultrasonic bath with 50% frequency for 90 min at 80°C and degassed at 30°C by using ultrasonic bath before use.

2.3.2. Coating on Fruits
The calamansi (Citrofortunella macrocarpa) with uniform size and colour was purchased (Kangar, Perlis), cleaned and air-dried. The fruits were divided into five groups: untreated control; coated with pectin, coated with 1:1 pectin/cutin (1P1C), coated with 2:3 pectin/cutin (2P3C), coated with 3:2 pectin/cutin (3P2C) and a positive control stored in a refrigerator. All calamansi samples except for positive control were stored at room temperature (25°C). The samples were analysed for weight loss, total soluble solids content, titratable acidity, colour and firmness every 2 days during 10 days of storage.
2.4 Physico-chemical analysis

2.4.1. Weight Loss
The weight loss was recorded for every 2 days and calculated as Equation (1):

\[
\% \text{ Weight Loss} \left( W_L \right) = \frac{W_i - W_f}{W_i} \times 100
\]  

(1)

where \( W_L \) (%) is weight loss, \( W_i \) (g) is the initial weight of fruits (g), and \( W_f \) (g) is the current weight of fruits.

2.4.2. Colour
The colour of the sample was determined by a chromameter (HunterLab, USA) with CIE L*a*b* colour space. The colour of each fruit was measured at three different sites. Colour was expressed as \( L^* \) (black to white), \( a^* \) (red to green), and \( b^* \) (blue to yellow).

2.4.3. Firmness
The firmness of the fruits was analysed by using a Texture Analyzer TA.XT Plus (Stable Microsystem, Surrey, UK) according to [29] with modifications. The penetration test was carried out by using a cylindrical probe (P/2). The measurement will be performed in triplicate. The firmness of fruit was measured in newtons (N).

2.4.4. Total Soluble Solids Content
The total soluble solids content was determined by using a digital handheld refractometer (ATAGO, Japan). A drop of calamansi juice was put on the refractometer prism for measurements. The reading (°Brix) was recorded.

2.4.5. Titratable Acidity
10 mL of 50% calamansi juice was titrated against 0.1 M NaOH with phenolphthalein as an indicator. The titratable acidity was expressed as percent citric acid and was calculated by using Equation (2).

\[
\% \text{ citric acid} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{Volume made up} \times \text{Acid miliequivalent factor}}{\text{The volume taken for estimation}} \times 100
\]  

(2)

where miliequivalent factor of citric acid is 0.064.

2.5. Statistical Analysis
All data were analysed by JMP Pro 13 statistical software and were presented as mean±standard deviation (SD). Statistical significance was determined using analysis of variance (ANOVA) followed by Tukey’s test (HSD). The significance level for the difference in the result was determined at \( p<0.05 \). 

abcd means significantly different (\( p<0.05 \)) within the storage period in the same treatment. ABCD means significantly different (\( p<0.05 \)) within the treatments in the same day.

3. Results and Discussion

3.1. Cutin edible film.
Cutin is a lipid-based substance which can be found abundantly in tomato peels up to 65% w/w [10]. It has high molecular weight and is made of hydroxylated C16 and C18 amino acids cross-linked by ester bonds [20]. The cutin yield extracted in this study is 28.22%, in line with the previous study [21]. The high lipophilic properties of cutin play a role in controlling water loss [22], but unable to form a stable film. Thus, the addition of pectin to the cutin as a binding agent improves the film stability [23]. Pectin
is the most complex class of polysaccharides, normally used in food products as a gelling, emulsifying and thickening agent.

3.2 Physical changes
The physical changes of fresh produce during storage include dryness, hardening of the skin and colour changes. Weight loss is not only from the process of vapour diffusion driven by the skin resistance and water vapour pressure gradient but also occur through respiration [24]. The weight loss is caused by water loss through transpiration which results in shrivel and wilt appearances that can lead to poor marketability. The changes in weight loss of calamansi with the different treatment over storage period in the room and refrigerated conditions were shown in Figure 1. The significant (p<0.05) weight loss begins on Day 2. The calamansi coated with 2P3C film alleviates almost 50% of weight loss compared to the untreated control, shown from Day 6 to Day 10. The moisture loss resulted in turgidity and texture loss causing an increase in firmness [25]. All of the calamansi samples showed a dramatical increase in firmness over the storage period in both room and refrigerated conditions as shown in Figure 2. There were significant (p<0.05) increased in the firmness for all treatment on Day 2 compared to Day 0 at room condition. On Day 4, the 2P3C group (17.17±3.68 N) showed a significant (p<0.05) reduction in firmness compared to untreated control (23.96±3.58 N) and pectin film (22.65±3.40 N). The result shows that the 2P3C provides the best coating properties that prevent moisture loss and the formation of leathery texture. The edible film controls the weight loss by preventing moisture migration and reducing respiration rate during storage period [26][27].

Calamansi, a non-climacteric fruit whereby the ripening process is almost stopped after harvest. The brown colour of the non-climacteric fruit is one of the signs of senescence. The deterioration of chlorophyll pigments during processing and storage can lead to colour changes [28]. Green coloured calamansi is preferable compared to yellow as it has a slower deterioration process and a lower risk of mechanical damage. Therefore, the green calamansi was chosen in the current study. The effect of different treatments on the colour of calamansi showed in Table 1. The increase in L* indicates the development of lighter brownish colour during the storage period, but the value is not significant among the groups. The least changes in a* value from day 0 to day 10 is showed by the refrigerated group followed by 2P3C, 3P2C, pectin, uncoated control and 1P1C. Meanwhile for the b* value, the sample with the least increment is 2P3C, followed by 1P1C, 3P2C, pectin, refrigerated and lastly uncoated control. The biofilm preserves the colour changes and discolouration of fruit and vegetables [29]. The edible coating helps in reducing oxygen and carbon dioxide transmission, resulted in the extension of the food shelf life [30][29].

3.3 Chemical changes
Fruit undergo chemical changes during ripening and senescence. This includes changes in acidity and soluble solids. The total soluble solids (TSS), measured as degree Brix is mainly used to measure the degree of sweetness of food product and is one of the important measurements for fruit maturity. TSS is indirectly related to hydrolytic activities of polysaccharide (starch) concentration of cell wall [31]. The TSS of calamansi during storage was shown in Figure 3. All treated group shows a significant (p<0.05) inhibition of the TSS increment as compared to untreated as early as Day 2. Calamansi coated with 2P3C shows the least increment in TSS, better than the positive control throughout the storage period. The calamansi coated with 2P3C also shows the least reduction of citric acid content from day 2 to 10 and the citric acid content is significantly (p<0.05) higher than uncoated calamansi, as shown at day 6 onwards (Figure 4). A significant (p<0.05) reduction of citric acid content was only shown by the untreated group on Day 2 as compared to Day 0. The untreated group also shows the highest citric acid loss throughout the storage period, although the amount is not significant (p>0.05) as compared to the treated groups on Day 2 and 4. Citric acid is an organic acid accounted for the sour taste of calamansi and is also widely found in fruit especially citrus fruit. The citric acid is commonly used in food and
Figure 1. Changes in weight loss of calamansi with different treatments

Figure 2. Changes in firmness of calamansi with different treatments
| Type of Treatment/storage period (day) | Control (uncoated) | Pectin | 1P1C | 2P3C | 3P2C | Refrigerated |
|---------------------------------------|--------------------|--------|------|------|------|--------------|
|                                       | L* (0: black, 100: white) | a* (-a*: green, +a*: red) | b* (-b*: blue, +b*: yellow) | |
| (i)                                   |                   |        |      |      |      |              |
| 0                                     | 58.25± 3.42 AbAa  | 54.97± 3.16 AbAa | 53.49± 1.71 AaAa | 56.67± 0.76 AbAa | 55.72± 1.06 AbAa | 60.74± 1.54 Ba |
| 2                                     | 61.48± 4.69 AaAa  | 57.58± 1.84 AbAb | 54.12± 2.38 AaAa | 58.82± 3.66 AaAa | 57.44± 4.70 AaAa | 62.22± 5.61 AaAa |
| 4                                     | 65.05± 3.27 AaAa  | 57.94± 3.28 AbAb | 57.71± 10.13 AaAa | 60.85± 3.17 AaAa | 58.59± 2.43 AaAa | 63.99± 5.59 AaAa |
| 6                                     | 68.84± 6.17 AaAa  | 59.46± 1.42 AbAb | 59.73± 4.25 AaAa | 60.74± 1.42 AaAa | 59.99± 0.28 AaAa | 64.08± 9.01 AaAa |
| 8                                     | 64.04± 8.04 AaAa  | 59.24± 1.32 AbAb | 59.87± 3.97 AaAa | 60.77± 2.29 AaAa | 61.75± 3.27 AaAa | 61.55± 8.78 AaAa |
| 10                                    | 62.12± 8.42 AaAa  | 64.22± 6.13 AbAb | 60.81± 2.03 AaAa | 61.54± 0.30 AaAa | 62.94± 0.29 AaAa | 59.86± 3.26 AaAa |
| (ii)                                  |                   |        |      |      |      |              |
| 0                                     | -8.12± 3.02 AaAa  | -8.87± 3.18 AaAa | -8.56± 3.42 AaAa | -6.63± 1.63 AaAa | -5.56± 1.39 AaAa | -6.42± 3.10 AaAa |
| 2                                     | -6.75± 2.43 AbAa  | -6.81± 2.41 AaAa | -6.65± 1.57 AbAb | -5.62± 2.25 AaAa | -4.55± 2.10 AbAb | -5.62± 4.50 AaAa |
| 4                                     | -4.28± 1.94 AbAb  | -6.19± 4.79 AaAa | -3.88± 1.09 AbAb | -5.17± 2.70 AaAa | -2.80± 2.81 AbAb | -5.64± 1.14 AaAa |
| 6                                     | -3.09± 2.55 AbAa  | -4.34± 4.16 AaAa | -2.46± 0.80 AbAb | -3.17± 1.90 AaAa | -1.95± 1.14 AbAb | -3.71± 1.24 AaAa |
| 8                                     | -2.20± 2.41 AbAa  | -2.78± 3.23 AaAa | -1.07± 1.01 AcAc | -2.00± 2.85 AbAb | -1.09± 0.95 AcAc | -3.25± 1.16 AaAa |
| 10                                    | -0.73± 1.97 AbAb  | -1.65± 3.04 AaAa | -0.33± 0.60 AaAa | -0.08± 1.57 AbAb | 1.18± 1.27 AbAb  | -2.56± 1.71 AaAa |
| (iii)                                 |                   |        |      |      |      |              |
| 0                                     | 8.63± 4.00 AaAa   | 6.34± 9.43 AaAa | 2.92± 2.91 AaAa | 6.02± 3.85 AaAa | 3.07± 2.86 AaAa | 3.99± 1.90 AaAa |
| 2                                     | 9.90± 4.66 AaAa   | 7.03± 8.91 AaAa | 4.13± 2.24 AaAa | 6.33± 4.32 AaAa | 4.43± 2.26 AaAa | 3.35± 1.66 AaAa |
| 4                                     | 11.20± 3.95 AaAa  | 9.76± 9.73 AaAa | 5.01± 2.90 AaAa | 7.40± 2.87 AaAa | 4.42± 3.34 AaAa | 5.29± 2.22 AaAa |
| 6                                     | 15.99± 7.75 AaAa  | 11.98± 10.7 AaAa | 6.47± 0.90 AaAa | 9.69± 3.34 AaAa | 6.93± 5.54 AaAa | 7.67± 4.35 AaAa |
| 8                                     | 16.04± 10.55 AaAa | 12.93± 9.55 AaAa | 8.47± 0.95 AaAa | 10.83± 2.60 AaAa | 8.73± 2.93 AaAa | 9.39± 3.42 AaAa |
| 10                                    | 19.26± 14.91 AaAa | 13.54± 11.39 AaAa | 9.36± 1.70 AaAa | 11.62± 3.42 AaAa | 9.87± 4.65 AaAa | 11.20± 4.20 AaAa |

This result are presented as mean±SD (n=3). abcd means significantly different (p<0.05) within the storage period in the same treatment. ABCD means significantly different (p<0.05) within the treatments in the same day.
beverages to impart taste and flavour. Therefore, the high citric acid content in calamansi is favourable in most cases. This study shows the ability of pectin/cutin as a coating material that prevents the increase of TSS and citric acid loss. The results obtained from this study indicate the potential use of edible film composed of cutin/pectin in slowing down the ripening process and prolong the shelf life of food. Cutin is a hydrophobic insoluble polyester layer of plant cell walls that plays an important role in permeating gasses solutes and moisture [32]. These properties help in minimizing the respiration rate by reducing the water diffusion rates which resulted in arresting the TSS increment and preserving the citric acid content [11].

Figure 3. Changes in total soluble solids (TSS) of calamansi with different treatments

Figure 4. Changes in acidity of calamansi with different treatments
4. Conclusion
This study was conducted to evaluate the bioactive properties of cutin, extracted from tomato peel as a coating biofilm in preserving calamansi. The cutin is extracted from tomato peels by using autoclave heat treatment with a yield of 28.22%. The biofilm with the highest cutin content group, 2P3C was found as the best combination in extending the calamansi shelf life by minimizing the weight and citric acid loss, preventing an increase in firmness and TSS content, while maintaining the colour. Based on the weight loss and firmness result, it can be said the combination of coating material 2:3 pectin/cutin mitigates the deterioration process for at least 50%. This study reveals the potential use of pectin/cutin edible film as a coating for perishable food produce.

References
[1] Sutherland et al. 2010 A horizon scan of global conservation issues for 2010 Trends in Ecology and Evolution 25 1–7
[2] Tudor VC, Marin A, Vasca DZ, Micu MM and Smadescu DI 2018 The influence of the plastic bags on the environment Mater. Plast 55 595–9
[3] Proshad R, Kormoker T, Islam MS, Haque MA, Rahman MM and Mithu MMR 2017 Toxic effects of plastic on human health and environment: A consequences of health risk assessment in Bangladesh Int. J. Heal. 6 1
[4] Galus S and Kadzińska J 2015 Food applications of emulsion-based edible films and coatings Trends in Food Science and Technology, 45(2) 273-83.
[5] Bourtoom T 2008 Edible films and coatings: characteristics and properties Int. Food Res. J. 15(3) 237–48
[6] Food Drug Administration FDA 2019 Food Additive Status List
[7] Andrade RMS, Ferreira MSL and Gonçalves ÉCBA 2016 Development and characterization of edible films based on fruit and vegetable residues J. Food Sci. 81(2) E412-8
[8] Food and Agriculture Organization Corporate Statistical Database FAOSTAT 2020
[9] Elbadrawy E and Sello A 2016 Evaluation of nutritional value and antioxidant activity of tomato peel extracts Arabian Journal of Chemistry 9 S1010–8
[10] Benítez et al. 2018 Valorization of tomato processing by-products: Fatty acid extraction and production of bio-based materials Materials (Basel) 11 11
[11] Li Y, Beisson F, Koo AKJ, Molina I, Pollard M and Ohlrogge J 2007 Identification of acyltransferases required for cutin biosynthesis and production of cutin with suberin-like monomers Proc. Natl. Acad. Sci. U. S. A. 104(46) 18339–44
[12] Kiy N and Suryanto 2019 Efek lama perendaman ekstrak kalamansi (citrus microcarpa) terhadap aktivitas antioksidan tepung pisang goroho (Musa Spp.) Chem. Prog. 4 27–33
[13] Xu G, Ye X, Liu D, Ma Y and Chen J 2008 Composition and distribution of phenolic acids in Ponkan (Citrus poonensis Hort. ex Tanaka) and Huyou (Citrus paradisi Macf. Changshanhuyou) during maturity J. Food Compos. Anal. 21(5) 382–9
[14] Kelebek H 2010 Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (Citrus paradisi) cultivars grown in Turkey Ind. Crops Prod. 32(3) 269-74
[15] Garcia CRA 2017 Calamansi is hot in Seoul, but can local suppliers deliver? | BusinessWorld.
[16] Nasrin TAA, Rahman MA, Arfin MS, Islam MN and Ullah MA 2020 Effect of novel coconut oil and beeswax edible coating on postharvest quality of lemon at ambient storage J. Agric. Food Res. 2 100019
[17] Ajibola VO, Babatunde O and Suleiman S 2009 The Effect of Storage Method on the Vitamin C Content in Some Tropical Fruit Juices Trends Appl. Sci. Res. 4(2) 79–84
[18] Marsh K and Bugusu B 2007 Food packaging - Roles, materials, and environmental issues: Scientific status summary J. of Food Science 72(3) R39–55
[19] Cicognini T 2015 Extraction method of a polyester polymer or cutin form the wasted tomato peels and polyester polymer so extracted WO 2015/028299 AI
[20] Domínguez E, Heredia-Guerrero JA and Heredia A 2011 The biophysical design of plant cuticles: An overview New Phytologist 189 938–49.
[21] Cifarelli A, Cigognini I, Bolzoni L and Montanari A n.d. Cutin isolated from tomato processing by-products: extraction methods and characterization
[22] Heredia A 2003 Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer Biochi. et Biophys. Acta 1620 1-7
[23] Manrich A, Moreira FKV, Otoni CG, Lorevice MV, Martins MA and Mattoso LHC 2017 Hydrophobic edible films made up of tomato cutin and pectin Carbohydr. Polym. 164 83–91
[24] Montanaro G, Dichio B and Xiloyannis C 2012 Fruit Transpiration: Mechanisms and Significance for Fruit Nutrition and Growth Advances in Selected Plant Physiology Aspects 10 233-50
[25] Paniagua AC, East AR, Hindmarsh JP and Heyes JA 2013 Moisture loss is the major cause of firmness change during postharvest storage of blueberry Postharvest Biol. Technol. 79 13–19
[26] Salehi F 2020 Edible Coating of Fruits and Vegetables Using Natural Gums: A Review Int. J. of Fruit Sci. 1-20
[27] Tomás SA, Bosquez-Molina E, Stolik S and Sánchez F 2005 Effects of mesquite gum-candelilla wax based edible coatings on the quality of guava fruit (Psidium guajava L.) J. De Physique. IV : JP 125 889–92
[28] Thompson AK 2010 Postharvest chemical and physical deterioration of fruit and vegetables Chemical Deterioration and Physical Instability of Food and Beverages 483–518.
[29] Dhall RK 2013 Advances in edible coatings for fresh fruits and vegetables: a review Critical Reviews in Food Science and Nutrition 53(5) 435–50
[30] Ghavidel RA, Davoodi M, Adib A, Asl, Tanoori T and Sheykholeslami Z 2013 Effect of selected edible coatings to extend shelf-life of fresh-cut apples Int. J. Agri. Crop Sci 6(16) 1171–8
[31] Hossain MA, Rana MM, Kimura Y and Roslan HA 2014 Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures Biomed Res. Int. 2014
[32] Pollard M, Beisson F, Li Y and Ohlrogge JB 2008 Building lipid barriers: biosynthesis of cutin and suberin Trends in Plant Science 13(5) 236–46

Acknowledgments
The authors wish to thank Universiti Malaysia Perlis for the financial and facility support in performing this study.