Alginate and Aloe vera gel-based edible coating for the storage stability enhancement of fresh-cut MD2 pineapple

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Abstract
Fresh-cut fruits are susceptible to deterioration due to tissue wounding during the preparation process which caused a shorter shelf-life of fresh-cut fruits. This study aimed to compare the storage stability of fresh-cut MD2 pineapples coated with alginate versus fresh-cut MD2 pineapples with Aloe vera gel-based coating. The physicochemical properties, microbiological changes, and quality changes of coated fresh-cut MD2 pineapples were analysed during low-temperature storage (5±1°C) under 85±10% of relative humidity for 16 days. Uncoated pineapples were used as the control and stored under the same storage conditions as the treated pineapples. The Aloe vera-coated pineapples showed a significantly (p < 0.05) lower amount of fluid loss, total plate count, as well as yeast and mould counts, as compared to the uncoated controls. The alginate-coated pineapples were brighter and more yellowish during storage compared to the control, therefore slightly extending the fruit shelf life. Overall, the Aloe vera gel-based edible coating was more effective at retaining the storage stability of fresh-cut MD2 pineapple, as it maintained its microbiological quality for up to 14 days and reduced fluid loss.

1. Introduction
Pineapple (Ananas comosus) regards as one of the major fruit crops in Malaysia alongside papaya, pomelo, banana, watermelon, jackfruit, and mango. MD2 pineapple is currently the most popular choice for fresh consumption out of the nine pineapple cultivars in Malaysia and has been identified as a target product for Malaysia’s Economic Transformation Program (MOA, 2020). Compared to other cultivars, the MD2 pineapple has a uniform bright gold colour, a sweeter taste, higher vitamin C content, lower fibre and lower acidity. It is also smaller in size but has thinner skin compared to other pineapple cultivars. Besides, MD2 pineapple has a longer shelf life that enables it to better retain its quality over long-distance shipping (Amar et al., 2015). Hence, consumers living in countries far away from Malaysia can enjoy pineapples fresh instead of canned ones.

The demand for convenient ready-to-eat food raises the market for fresh-cut fruits (Singh et al., 2018). However, the quality and safety of these fresh-cut fruits are still an issue of concern as consumers demand fresh-cut fruits that are high in nutritional value with no chemical preservatives and extended shelf life (Maringgal et al., 2019). The destruction of surface cells during fruit peeling and cutting provides a larger cut surface for microbial growth compared to the whole fruit, resulting in a shorter shelf life (Yousuf et al., 2018). The wounding of fruit tissues during processing also promotes the loss of nutritional content and enhances metabolic activities in the fresh-cut fruit, which, in turn, lead to the degradation in flavour, texture, and colour of the fruit, and later enzymatic browning (Singh et al., 2018), a condition that leads to quality deterioration where the fresh-cut pineapple starts to brown and soften (Montero-Calderón et al., 2008).

Edible coatings are thin layers of edible materials that are considered to have great potential to improve the safety and quality of food, as they provide the food with a selective barrier that protects it against external environmental conditions such as moisture, oxygen, and carbon dioxide (Fernandes et al., 2018; González-Saucedo et al., 2019). The edible coating is widely used...
in fruit storage to prevent the fruit from undergoing physical and mechanical damage, microbial spoilage, and loss of quality during the postharvest period, thus increasing its shelf life (Khan et al., 2019). Alginate is one of the most commonly used edible coatings, as it is easily prepared and is commonly available in the market (Abdallah et al., 2018). Previous studies have shown that alginate-based edible coatings are able to prevent loss of moisture and firmness, control the fruit’s respiratory rate, improve textural properties, and act as a carrier for bioactive components that improve the quality of fresh-cut fruit such as pineapple (Azaraksh et al., 2014), apple (Guerreiro et al., 2017), cantaloupe (Koh et al., 2017) and mango (Salinas-Roca et al., 2017).

*Aloe vera* is a tropical and subtropical plant that is widely known for its medicinal properties (Misir et al., 2014). Recently, *Aloe vera* gel gaining much interest as a potential functional ingredient in the edible coating due to its translucency, environmentally friendly and tasteless properties (Benítez et al., 2015). Besides, *Aloe vera* gel is rich in antimicrobial and antifungal compounds such as anthraquinones and emodine that inhibit the growth of microorganisms to prevent foodborne disease and extend the shelf life of postharvest fruits. (Rasouli et al., 2019; Mendy et al., 2019). Furthermore, it consists of essential oil that is used to enhance the visual appearance of fruits (Parven et al., 2020). *Aloe vera* gel-based edible coating can also control maturation development, delay oxidation, and reduce microorganism proliferation in kiwifruit slices (Benítez et al., 2015), plum (Martínez-Romero et al., 2017) and orange (Rasouli et al., 2019). However, most of these fruits are not fresh-cut and no specific data has been reported on the effect of alginate and *Aloe vera* gel-based edible coating on fresh-cut MD2 pineapple. Hence, this study was carried out to evaluate and compare the effect of alginate and *Aloe vera* gel-based edible coating on the storage quality (physicochemical and microbiological properties) of fresh-cut MD2 pineapple.

### 2. Materials and methods

#### 2.1 Fruit sample preparation

Fresh pineapples with uniform size, a regular shape, and a maturity index of 1 (mature green) were purchased from a local supplier (Johor, Malaysia). The maturity of the pineapple was determined based on its peel colour (Ding and Syazwani, 2016). The fruits and all the utensils in this study were washed and sanitized with a 0.1% (w/v) sodium hypochlorite solution. Then, the pineapples were cut into cubes of 2×2×2 cm with a sharp knife.

#### 2.2 Alginate-based edible coating formulation

The alginate-based edible coating was prepared based on Azaraksh et al. (2014). Sodium alginate powder [1.29% (w/v)] was dissolved in distilled water by heating the mixture using a stirring hot plate at 70 °C until the solution turned clear. Then, 1.16% (w/v) glycerol and 0.025% (w/v) sunflower oil were added to the formulation, making up a total volume of 500 mL consisting of alginate, glycerol, and sunflower oil with the remainder being distilled water. The solution was then homogenized with a Diax 900 homogenizer (Heidolph Instruments, Schwabach, Germany) for 5 mins at 24,000 rpm to form an emulsion, and then degassed with an 8891 ultrasonic cleaner (Cole-Parmer, Illinois, US).

The pineapple cubes were dipped into the alginate-based formulation for 2 mins after which excess coating materials were allowed to drip off. The pineapple cubes were then dipped into a 2% (w/v) calcium chloride solution containing 1% (w/v) ascorbic acid and 1% (w/v) citric acid for 2 mins. The samples were air-dried at ambient temperature (25±1 °C) for 1 hr.

#### 2.3 Aloe vera Gel-based edible coating formulation

The *Aloe vera* gel-based coating was prepared based on Misir et al. (2014) with slight modifications. Fresh *Aloe vera* leaves were washed thoroughly under running tap water and then patted dry using clean filter papers. The peels were discarded while the gel was finely ground using an MX-800S electric blender (Panasonic Corp., Osaka, Japan). The resulting mixture was filtered to remove fibre. The obtained *Aloe vera* gel was pasteurized at 65°C for 30 mins and then cooled down immediately at ambient temperature (25±1 °C) to enable the gel to stabilize. Then, the pineapple cubes were dipped into the gel for 5 min and then air-dried at ambient temperature (25±1 °C) for 1 hr.

#### 2.4 Storage conditions

The coated pineapples were packed individually in a transparent polypropylene bag, sealed tight, and stored at 5±1°C under 85±10% of relative humidity for 16 days. Uncoated samples were also packed and stored under the same conditions as the control. Headspace gas composition, fluid loss, firmness, colour, microbiological count, as well as coating homogeneity and adherence, were determined in 4-day intervals.

#### 2.5 Headspace gas composition

The headscape gas composition of the fresh-cut pineapple was determined based on Koh et al. (2017), using a 6600-headspace oxygen/carbon dioxide analyser.
The fluid loss of the fresh-cut pineapple was determined based on Fan et al. (2008). The weights of the sample and the juice accumulated in the bag were measured. The percentage (%) of fluid loss was calculated based on the following formula:

\[
\text{Percentage (\%) of fluid loss} = \frac{\text{weight of the fluid}}{\text{weight of sample}} \times 100
\]

2.7 Firmness

The firmness of the fresh-cut pineapple was measured via a puncture test using a TX-XT2i Texture Analyser (Stable Micro Systems Ltd, Surrey, UK). The samples were punctured with a 2-mm diameter cylindrical probe with a 1.0 mm/s pre-test speed, a 0.5 mm/s test speed, a 10 mm/s post-test speed, a 10 mm target distance, and a 5 kg load cell. The maximum peak measured during the test was recorded as the firmness (Rocculli et al., 2009) expressed in Newton (N).

2.8 Colour

The surface colour of the fresh-cut pineapple was measured using a Minolta Chroma Meter CR-300 (Konica Minolta Inc, Tokyo, Japan). The instrument was standardized against a white tile before measurements were taken. The value of L*, a*, and b* were recorded. The whiteness index (WI), the chromaticity (C*), and the hue angle (h°) of the samples were calculated using the following formulas:

\[
\text{WI} = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}
\]

\[
C^* = [(a^*)^2 + (b^*)^2]^{1/2}
\]

\[
h° = \tan^{-1}(b^*/a^*)
\]

2.9 Microbiological Analysis

Total plate count (TPC) and yeast and mould counts were calculated for the microbiological analysis according to the methods mentioned in Koh et al. (2017) with slight modifications. A piece of the sample (~10 g) was homogenized with 0.1% (w/v) sterile peptone water at a ratio of 1:10 (sample: peptone water) using a Bag Mixer 400 stomacher lab blender (Interscience, Saint-Germain-en-Laye, France). The pour plate method with Plate Count Agar as a medium was used for the TPC. The plates were incubated at 36±2°C for 2 days. The yeast and mould counts were determined using the spread plate method with Potato Dextrose Agar as the medium. The plates were incubated at 25±2°C for 5 days. The results were expressed as log_{10} colony forming units per grams (log_{10} CFU/g).

2.10 Coating homogeneity and adherence

The homogeneity and adherence of the coating to the surface of the fresh-cut pineapple were observed using a MEIJI EMZ-5TRD stereomicroscope (Meiji, Tokyo, Japan) with a magnification of 20×. The samples were cross sectioned using a razor blade and dyed with 0.05% (w/v) toluidine blue. The cross-sections were observed under the stereomicroscope, which was connected to a camera and a computer (Rojas-Graü et al., 2007).

2.11 Statistical analysis

All analyses were carried out in triplicates. The results were reported as mean±standard deviation (SD). A One-way Analysis of Variance (ANOVA) with Tukey’s test was performed to compare the means of the sample with different coatings and the mean of the control. The results were taken as significant when \(p < 0.05\).

3. Results and discussion

3.1 Headspace gas composition

The exchange of CO\(_2\) and O\(_2\) between the fruit sample and its environment indicates the fruit’s potential shelf life (Martínez-Ferrer et al., 2002). Table 1 shows the CO\(_2\) and O\(_2\) composition detected in the sample package headspace throughout 16 days of storage. The result shows that there was no significant (\(p > 0.05\)) difference in the CO\(_2\) and O\(_2\) composition of the coated samples and the uncoated samples during the same storage time. This finding is contrary to that of Koh et al. (2017), who reported that coated fresh-cut cantaloupes had lower CO\(_2\) production and lower O\(_2\) uptake than uncoated samples because of the barrier properties of the coatings. The result is probably related to the permeability of the polypropylene packaging to CO\(_2\) and O\(_2\), allowing these gases to pass through to the environment from the package headspace, as similar results were also observed by Rojas-Graü et al. (2007) for fresh-cut Fuji apples coated with alginate and gellan coatings.

The CO\(_2\) and O\(_2\) composition of the coated and uncoated fresh-cut pineapples increased by 1.41–1.61% and decreased significantly (\(p < 0.05\)) to 19.69–19.94%, respectively, on the 12\(^{th}\) day of storage. This production of CO\(_2\) and the consumption of O\(_2\) for the coated and uncoated samples during the storage period were due to the tissue respiration of the samples and the microbial growth on the samples (Ramos-Villarroel et al., 2014).
progressive increase in CO₂ and a decrease in O₂ have also been reported in pineapple (Rocculi et al., 2009), kiwifruit (Benítez et al., 2015), and cantaloupe (Koh et al., 2017). The CO₂ and O₂ compositions of all the samples were recorded to range from 0.70–1.61% and 19.0–20.8%, respectively, throughout the storage period (Table 1). The slow changes in the headspace CO₂ and O₂ compositions imply the low respiration rate of the samples, as CO₂ is a by-product of tissue respiration (Tadeo et al., 2018). The results in Table 1 also indicated that the O₂ composition of all the samples did not decrease to less than 2% (the fermentation threshold limit) during the entire storage period, which prevented anaerobic respiration and the possible formation of off-flavour, off odour, and/or accelerated ageing (Soliva-Fortuny et al., 2004).

3.2 Fluid loss

Figure 1 shows the fluid loss of the coated and uncoated fresh-cut pineapples throughout 16 days of storage. The highest weight loss throughout the storage period was observed in the uncoated samples (Figure 1). The weight loss of the uncoated samples reached 4.81% at the end of storage, which is approximately 1% higher than the alginate-coated samples (3.80%) and the Aloe vera coated samples (3.62%). The samples coated with Aloe vera gel showed a significantly (p < 0.05) lower fluid loss than the uncoated samples over the storage period. Oms-Oliu et al. (2010) reported that edible coatings could form a barrier on the fresh-cut fruit surface to reduce water vapour transmission, eventually resulting in decreased fluid loss. Besides, the coating could act as a sacrificial layer for water loss that had occurred before the fruit was coated (Azarakhsh et al., 2014). The hygroscopic properties of Aloe vera gel also allow a water barrier to form between the fruit and its surrounding environment, thereby reducing fluid loss (Morillón et al., 2002). Therefore, Aloe vera gel coating effectively serves as a protective barrier on the fresh-cut pineapple to restrict water vapour transmission and protect the fruit sample from mechanical injuries. Several kinds of research also reported how Aloe vera gel-based coating decreased the water loss in table grapes (Tripathi and Dubey, 2004) and papaya (Brishiti et al., 2013). The fluid loss of the coated and uncoated fresh-cut pineapple increased significantly (p < 0.05) during the 16 days of storage (Figure 1). This result is attributed to the transpiration and respiration of the fruit samples (Bierhals et al., 2011), and is also in agreement with those reported for strawberry and cantaloupe (Sogvar et al., 2016; Koh et al., 2017).

### Table 1. The effect of edible coating on the headspace gas composition of fresh-cut pineapple

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>Treatment</th>
<th>CO₂ composition (%)</th>
<th>O₂ composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(days)</td>
<td>Without Coating</td>
<td>Alginate Coating</td>
<td>Aloe vera Coating</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.79±0.22&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.73±0.27&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.81±0.28&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.71±0.12&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.63±0.09&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>0.79±0.12&lt;sup&gt;Ba&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>1.03±0.15&lt;sup&gt;Ba&lt;/sup&gt;</td>
<td>1.09±0.18&lt;sup&gt;ABa&lt;/sup&gt;</td>
<td>1.01±0.23&lt;sup&gt;ABa&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>1.61±0.36&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>1.60±0.49&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>1.41±0.31&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
<tr>
<td>16</td>
<td>1.48±0.12&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>1.53±0.49&lt;sup&gt;Aa&lt;/sup&gt;</td>
<td>1.37±0.12&lt;sup&gt;Aa&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD (n = 3). Values with uppercase superscript within the same column and values with lowercase superscript within the same row are significantly different (p < 0.05).
3.3 Firmness

Firmness is one of the key factors for determining fruit quality and the consumer acceptability of fresh-cut fruit (Misir et al., 2014). Results indicate that, at the end of storage, the fresh-cut pineapple coated with Aloe vera gel had the highest value of firmness (0.64 N), followed by the alginate-coated samples (0.61 N) and the uncoated samples (0.54 N). According to Oms-Oliu et al. (2010), this result could be due to the coating on the fruit surface reducing the transmission of water vapour from the fruit to the environment, in line with the fluid loss results. Similar observations were reported in Koh et al. (2017) on fresh-cut cantaloupe and in Martinez-Romero et al. (2017) on plum. The firmness of coated and uncoated fresh-cut pineapple decreased significantly (p < 0.05) during the 16 days of storage, ranging from (1.2–1.3) N in the beginning to (0.55–0.65) N at the end of storage. The firmness of the fresh-cut fruits is strongly dependent on the enzymatic hydrolysis of the cell wall substances of the fruit. Per the current results, Rocculli et al. (2009) also reported that increased polygalacturonase, β-galactosidase, and pectinesterase activities caused the tissue in pineapples to soften. This condition will stimulate senescence or cell degradation of the fruit samples. Senescence in fruit will lead to a loss of turgidity and changes in the cell wall structure and eventually cause the tissue of the fruit to soften (Gómez et al., 2012).

3.4 Colour

Colour is the most important visual attribute that directly influences the consumer’s perception of fruit quality (Misir et al., 2014). Table 2 shows the colour parameter (whiteness index, chromaticity, and hue angle) of the coated and uncoated fresh-cut pineapples during 16 days of storage. The alginate coating was more effective at maintaining the pineapple colour, yielding a significantly (p < 0.05) higher value of chromaticity and hue angle compared to the uncoated samples and the Aloe vera gel-coated samples throughout the storage period (Table 2). Chromaticity indicates colour saturation or intensity while the hue angle shows the yellowness of the fruit sample. Higher values of chromaticity and hue angles make the fruit sample appear brighter and more yellowish, respectively (Koh et al., 2017). The edible coating has been reported to act as a carrier for anti-browning agents such as ascorbic acid to prevent undesirable changes in the colour and appearance of fresh-cut fruits (Falguera et al., 2011). Thus, the colour of pineapple coated with alginates was more effectively maintained as it had undergone a slower enzymatic browning reaction during storage than the other treatments. The whiteness index, the chromaticity, and the hue angle of the coated and uncoated pineapples have previously been reported to decrease progressively during storage due to browning reactions (Antonioli et al., 2007). Similar observations were reported in Montero-Calderón et al. (2008) and Azarakhsh et al. (2014) on fresh-cut pineapple. The browning of fruit is

<table>
<thead>
<tr>
<th>Storage Time (days)</th>
<th>Without Coating</th>
<th>Alginete Coating</th>
<th>Aloe vera Coating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whiteness Index</td>
<td>Chromaticity</td>
<td>Hue angle</td>
</tr>
<tr>
<td>0</td>
<td>50.23±1.84^Aa</td>
<td>37.09±1.04^Aa</td>
<td>88.34±0.66^Ab</td>
</tr>
<tr>
<td>4</td>
<td>50.65±1.03^Aa</td>
<td>30.43±1.89^Bb</td>
<td>85.51±1.08^Bb</td>
</tr>
<tr>
<td>8</td>
<td>49.34±0.32^Aa</td>
<td>30.60±1.24^Bb</td>
<td>85.02±0.47^Bb</td>
</tr>
<tr>
<td>12</td>
<td>48.28±0.77^Aa</td>
<td>29.45±1.150^Bch</td>
<td>84.05±0.40^Bch</td>
</tr>
<tr>
<td>16</td>
<td>47.74±0.22^Aa</td>
<td>27.64±0.53^Cb</td>
<td>82.81±0.96^Cc</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD (n = 3). Values with uppercase superscript within the same column and values with lowercase superscript within the same row are significantly different (p < 0.05).
due to phenolic oxidation induced by polyphenol oxidase enzymes that form brown pigments called melanin (Charles et al., 2013).

3.5 Microbiological analysis

Fresh-cut fruits with a large cut surface area provide a good environment for promoting the growth of microorganisms (Oms-Oliu et al., 2010), in turn, increasing the deterioration rate of the fruit and shortening its shelf life (Raybaudi-Massilia et al., 2009). Figure 2 shows that the total plate count (TPC) and yeast and mould counts increased significantly ($p < 0.05$) for the coated and uncoated fresh-cut pineapples during 16 days of storage. The TPC and yeast and mould counts were not significantly ($p > 0.05$) different between the alginate-coated samples and the uncoated samples throughout the entire storage time (Figure 2). This is because the alginate-based coating had no antimicrobial effects on the samples. These findings are comparable to that of previous studies on fresh-cut pineapple (Montero-Calderón et al., 2008; Azarakhsh et al., 2014) and kiwifruit (Benítez et al., 2015).

![Figure 2](image_url)

**Figure 2.** The effect of edible coating on the microbiological analysis (A: total plate count; B: yeast and mount count) of fresh-cut pineapple. The error bars represent the error of the mean ($n = 3$). The different uppercase letters indicate significant differences for the same sample during different storage times ($p < 0.05$).

According to the Institute of Food Science and Technology (1999), the acceptance limit for the microbial count of minimally processed fruit and vegetable in a shelf-life study was $6 \log_{10}$ CFU/g. In this study, the uncoated pineapple was the first to reach the microbial acceptance limit, i.e. on the 8th day of storage (Figure 2). The pineapple coated with Aloe vera gel reached the limit on day 14 and showed a significantly ($p < 0.05$) lower amount of TPC and yeast and mould counts compared to the uncoated samples throughout the storage period (Figure 2). This phenomenon is mainly due to the antimicrobial effect of Aloe vera gel. Aloe vera gel is composed of a wide range of constituents such as saponins, acemannan, and anthraquinones that exhibit antimicrobial activity against various microorganisms (Lone et al., 2009). In agreement with the current results, Aloe vera gel-coated kiwifruit (Benítez et al., 2015) and Aloe vera gel-coated strawberry (Sogvar et al., 2016) also showed a reduction in TPC and yeast and mould counts during storage.

3.6 Coating homogeneity and adherence

Figure 3 shows that both Aloe vera gel and alginate formed coatings that were homogenous and had good adherence to the surface of the fresh-cut pineapple over the 16 days of storage. Coatings could easily break during handling and storage if their adherence to the fruit surface is not high enough (Montero-Calderón et al., 2016). The coatings did not detach from the fruit surface throughout the storage time in this study, indicating that both Aloe vera gel and alginate are suitable coatings for fresh-cut pineapple. The pineapple coated with Aloe vera gel had a thicker layer of coating compared to the alginate-coated pineapple. The thickness of the coating depends on several properties, including the composition of the coating formulation, particle size, and wettability (Rojas-Graü et al., 2009). Aloe vera gel consists of approximately 99.5% water and 0.5% solid materials, including vitamins, minerals, enzymes, phenolic compounds, and organic acid (Boudreau and Beland, 2006). Hence, the Aloe vera gel formed a thicker gel-like coating on the sample surface compared to the alginate.

4. Conclusion

Aloe vera gel coating and alginate coating extended the shelf life of the fresh-cut pineapples by 8 days and 6 days, respectively, compared to the uncoated pineapple. The Aloe vera gel coating adhered better to the surface of the fruit whereas the alginate coating contributed to a brighter and more yellownish fruit colour throughout storage. As there was no difference in the headspace gas composition and the firmness of the coated and uncoated pineapples during storage, Aloe vera gel is considered a better coating agent than alginate. The Aloe vera gel coating also extended the postharvest life of the fresh-cut pineapples by up to 14 days along with reduced fluid
loss. For future research, the application of Aloe vera coatings incorporated with natural ingredients such as leaf extract for postharvest life prolongation, quality preservation and nutritional value enhancement of fresh-cut MD2 pineapple or other fruits can be investigated.

Conflict of interest
The authors declare no conflict of interest.

Acknowledgements
The authors thank Universiti Putra Malaysia for providing the funds to conduct this study under Project GP-IPS/2017/9556500.

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