Effects of Fermentation Time and Turning Intervals on the Physicochemical Properties of Rambutan (Nephelium lappaceum L.) Fruit Sweatings

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ABSTRACT

Sweatings, the exudates that leach out from fermenting fruits during rambutan fruit fermentation are considered as a waste by-product and are allowed to be drained off. This could lead to a pollution problem. Besides, it is a waste if the sweatings are possible to be transformed into food products and ingredients. However, prior transformation, the fundamental knowledge of the sweatings should be understood. Hence, the main aim of this study was to investigate the physicochemical properties of sweatings as affected by fermentation time and turning intervals during natural fermentation of rambutan fruits. In this study, peeled rambutan fruit was fermented for 8 days and turned. Different batches of the fruits were turned every 24, 48 or 72 h and sweatings from the process were collected and analyzed. The results showed that fermentation time significantly reduced (p<0.05) the yield, pH and sucrose content of the sweatings by 79-84%, 32-33%, 76.5-80.8%, respectively. Fermentation time also significantly increased (p<0.05) the titratable acidity, total soluble solids, fructose, glucose, total sugar, citric acid, lactic acid, acetic acid and ascorbic acid contents of the sweatings by 5.6-6.0, 1.5-1.6, 2.4-2.6, 2.1-2.5, 1.0-1.1, 5.7-6.5, 2.4-2.6, 2.1-2.5 and 2.6-2.8 folds, respectively. However, turning intervals did not significantly affect (p>0.05) the physicochemical properties of the sweatings. High concentrations of sugars and organic acids allow the sweatings to have a balance of sweet and sour taste and they are suitable to be used in the production of syrup, soft drinks, jam, jelly, marmalade and vinegar.

Keywords: Fruit sweatings; mixing; organic acids; solid-state fermentation; sugars

INTRODUCTION

Rambutan (Nephelium lappaceum L.) is an exotic fruit from the Sapindaceae family (Wall 2006). It is a native of west Malaysia and the island of Sumatra (Indonesia) (Tindall 1994). The fruit is an important commercial crop in Asia and is usually consumed fresh, canned or processed into juice, jam, jelly, marmalade, spread, chips or as rambutan stuffed with a chunk of pineapple and canned in syrup (Morton 1987; Sirisompong et al. 2011). Besides, the fat of rambutan seed has been reported...
to have potential to be used in various sectors of food industry due to its physicochemical properties which are quite similar to those of cocoa butter (Chai et al. 2018a). However, there is always a glut of the fruit during harvest season and much of the fruits are wasted. Studies have shown that fermenting rambutan fruits could transform the fruits into other products and subsequently, reducing wastage (Febrianto et al. 2016, 2014; Luma et al. 2017; Mehdizadeh et al. 2015).

Similar to cocoa bean fermentation, fruit sweatings which are yellowish exudates that leach from fruits, are produced during the early stages of rambutan fruit fermentation. It is the breakdown product of the mucilage (pulp) surrounding the fruit seed. The production of sweatings is most probably caused by the activity of pectolytic enzymes which are secreted by some of the microorganisms involved in the fermentation process (Ansh & Dzogbefia 1990). The sweatings are regarded as waste by-product and are allowed to be drained off during fermentation. This could lead to pollution caused by the necessity of disposing of large quantities of sweatings. Hence, efforts should be carried out in order to prevent the pollution.

During cocoa bean fermentation, 11.8% (v/w) of sweatings from the total fresh weight of cocoa beans (5.3 L from 45 kg cocoa beans) could be collected and it was reported that the sweatings were no longer produced beyond two days of fermentation (Buamah et al. 1997; Leal Jr. et al. 2008). Cocoa sweatings have been reported to contain high concentrations of sugar, pectin and organic acids which caused the sweatings to have a pH of 3.4-3.8 (Adomako 1985). Besides, it has been shown that various products could be derived from the sweatings including wine, jam, jelly, marmalade, vinegar, syrup and soft drinks (Adomako 2006).

Fermentation time and turning intervals have been reported to have significant effects on the quality of fermented cocoa bean (Camu et al. 2007; Emmanuel et al. 2012; Guehi et al. 2010; Passos et al. 1984; Rodriguez-Campos et al. 2012; Romero-Cortes et al. 2013). Mehdizadeh et al. (2015) reported that fermentation time of rambutan seeds greatly affected their quality. Hence, it is rational to deduce that fermentation time and turning intervals could affect the physicochemical properties of rambutan sweatings as well. Besides, it is crucial to understand the fundamental knowledge of the sweatings if they are going to be transformed into various types of products. However, to date, there has been no report on the characteristics of rambutan sweatings during fermentation. Due to a fairly limited information in these areas, the objective of this study was, therefore, to investigate the physicochemical properties of fruit sweatings as affected by fermentation time and turning intervals during natural fermentation of rambutan fruits.

**MATERIALS AND METHODS**

**MATERIALS**

Rambutan Clone R4 fruits were obtained from the University Agricultural Park, UPM, Serdang, Malaysia (latitude 2.995222, longitude 101.712403). The fruits used in this study were those that were free of blemishes and uniform in color and size. Two batches of samples were used in this study. Each batch was analyzed in triplicate.

**RAMBUTAN FRUIT FERMENTATION**

Prior to fermentation, the peels of the rambutan samples were removed and four batches of 7 kg of the fruit per batch were placed in separate perforated open plastic containers (40 cm×28 cm×10 cm) for fermentation. Each perforation, made using a soldering iron, was 1 cm apart and there was a total of 480 holes. A non-perforated plastic container was placed under each container to collect sweatings produced during the fermentation. To create a relatively anaerobic condition, rambutan fruits were fully covered by tap-water-cleaned banana leaves before the set up was transferred to an incubator cupboard (30±2°C) for eight days of fermentation. During the fermentation, one batch of fruits was turned every 24 h, another batch was turned every 48 hours and the third batch was turned every 72 h. Fruits that were fermented without turning were used as the control.

Sweatings from each fermentation batch were collected and measured daily for up to three days only since no sweatings were produced after Day 3 of fermentation. Prior to analysis, the sweatings were first clarified by centrifugation at 4000×g for 20 min (Beckman J2-21M/E, USA) and the supernatant was then vacuum filtered through a Whatman No. 1 filter paper (Kelebek et al. 2009).

**pH, TITRATABLE ACIDITY AND TOTAL SOLUBLE SOLIDS OF SWEATINGS**

The pH, titratable acidity (TA) and total soluble solids (TSS) of the sweatings were determined according to the method described by Salomé et al. (2011). Briefly, 20 mL of sweatings was used to determine the pH by using a calibrated pH meter (Model 430, Corning, NY, USA). To determine the TA of the sweatings, a 10 g sweatings was weighed and titrated with 0.1 M NaOH solution. The volume of titer required to make the pH of the homogenate to pH 8.1 (end-point) was measured. The titratable acidity of rambutan sweatings is defined as % lactic acid using the following equation:

\[
\% \text{ Lactic acid} = \frac{\text{Volume of 0.1 M NaOH (mL) × 0.90}}{10}
\]

A refractometer (0.0-53.0 %, Atago, Japan) was used to determine the TSS of the sweatings. A drop of the sweatings was put on to the surface of the refractometer window that had been cleaned and dried with a tissue paper.
The brix value (total soluble solids) of the sweatings was read by looking in the refractometer through eyepiece. The results of TA and TSS were expressed as % lactic acid and °Brix, respectively.

**SUGAR PROFILE OF SWEATINGS**

The sugar profile and content of the sweatings were obtained using High Performance Liquid Chromatography (HPLC) and analysis was performed using a Waters 2695 Alliance HPLC (Waters Corp., Milford, MA, USA) connected to a Waters 2414 refractive index detector, two Waters 515 HPLC pumps, an auto-sampler and an online degasser. The chromatographic column used for separation was a Purospher® Star NH₂ column (259 × 4.6 mm, particle size of 5 µm from Merck, Darmstadt, Germany) connected to a Purospher® NH₂-18e guard column (4 × 4 mm I.D from Merck, Darmstadt, Germany) and thermostated at 35°C (Hunt et al. 1977). The eluent used in this analysis was degassed HPLC-grade acetonitrile and deionized water (80/20, v/v) and the flow rate was at 1.5 mL/min. Sweatings samples were first filtered through a Sep-Pak C18 cartridge followed by a 0.45 µm membrane filter (Sartorius, Germany) (Augustin & Khor 1986) and a 10 μL sample was injected into the HPLC. Three sugar standards, glucose, fructose and sucrose from Sigma Aldrich (St. Louis, MO, USA) with concentration ranging from 0-8% (w/v), were used to identify and quantify the sugars present in the sweatings.

**ORGANIC ACID AND ASCORBIC ACID CONTENTS OF SWEATINGS**

Organic acid and ascorbic acid analyses of fruit sweatings were carried out by referring to the method described by Medlicott & Thompson (1985) and Sturm et al. (2003) with slight modifications. The HPLC system and software used in this analysis were similar to those used for sugar analysis but with a different detector (Waters 2478 two-channel UV detector). A Purospher® Star RP18 end-capped column (250 × 4.6 mm I.D, 5 µm particle size, from Merck, Darmstadt, Germany) connected to a Purospher® Rp-18e guard column (4 × 4 mm I.D from Merck, Darmstadt, Germany) were used to effect separation, and thermostated at 30°C. Degassed 0.008 M H₂SO₄ solution was used as the mobile phase for separation of organic acids and ascorbic acid in sweatings and the flow rate was 0.5 mL/min (Sturm et al. 2003). Sweatings samples were first filtered through a Sep-Pak C18 cartridge followed by 0.45 µm membrane filters (Sartorius, Germany) and a 10 μL of sample was injected into the HPLC. Detection of organic acids was done at 210 nm. Six organic acids, namely lactic acid (85 % purity, JT. Baker, Central Valley, USA), tartaric acid (99% purity) and citric acid (99% purity) (all from Fisher Scientific, Rochester, NY, USA), malic acid (99% purity, from Sigma Aldrich, St. Louis, MO, USA) and acetic acid (99% purity, from Merck, Darmstadt, Germany) and ascorbic acid (99% purity, from Sigma Aldrich, St. Louis, MO, USA) were used to obtain standard curves.
time and different turning intervals. A total of 1042.75 ± 27.46 mL of sweatings was collected, and this represented 15% of the total weight of rambutan mass used for fermentation. As can be seen, the yield of the sweatings significantly reduced (p<0.05) by 79-84% after three days of fermentation. However, turning intervals did not significantly affect (p>0.05) the yield of the sweatings. Leal Jr. et al. (2008) reported that a total of 11.8 % (v/w) of sweatings was obtained from 45 kg of cocoa bean during fermentation. They also reported that the yield of the cocoa sweatings they studied was reduced by 90% after three days of natural fermentation. Compared to rambutan fermentation, a lower percentage of sweatings was collected in cocoa bean fermentation, probably due to the difference in pulp-to-seed ratio between both fruits. Rambutan fruit has higher pulp content compared to that of cocoa bean (Selamat 1994; Solís-Fuentes et al. 2010), thus, higher percentage of sweatings was drained and collected during fermentation. Since a large quantity of sweatings could be obtained during rambutan fruit fermentation, the sweatings are suitable to be used in the production of soft drinks and marmalade.

Table 1 shows also that fermentation time significantly affected (p<0.05) the pH of the sweatings but with or without turning, did not affect their pH. The initial pH (pH4.18-4.25) of rambutan sweatings decreased by about 32-33% after three days of fermentation, most probably due to the activities of lactic acid bacteria. This finding is supported by Mehdizadeh et al. (2015) who reported that lactic acid bacteria were found when they fermented rambutan seeds.

Similar to pH, the titratable acidity (TA) of rambutan sweatings increased significantly (p<0.05) after three days of fermentation but it was not significantly affected (p>0.05) by different turning intervals (Table 1). The results showed that the TA of the sweatings increased by 5.6-6.0 folds after three days of fermentation, again, likely due to the activities of lactic acid and acetic acid bacteria. This finding is in agreement with Adams et al. (1982) who reported that the TA of cocoa sweatings increased after fermentation, probably due to the acids produced by lactic acid and acetic acid bacteria.

As shown in Table 1, the total soluble solids (TSS) of the sweatings increased by 50-53% after three days of fermentation compared to Day 1 TSS. However, different turning intervals did not significantly (p>0.05) affect the TSS of the sweatings. The increase of TSS in the sweatings after 3 days of fermentation is most probably due to increased release of soluble solids, mainly fructose and glucose, from the fruit pulp. The sugars present in the sweatings represent remaining sugars after microbial activity and the continued increase in sugar content of the sweatings indicates that the rate of sugar utilization by microorganisms (e.g. lactic acid bacteria) was lower than the rate of sugar released. Hansen et al. (1998) explained that the increase in the concentrations of reducing sugars during fermentation is the result of enzymatic reaction promoted by invertase which hydrolyzes sucrose into glucose and fructose. The low pH and high TA and TSS in the sweatings showed that they are acidic and contain a high sugar content. These characteristics should allow the sweatings to be produced into jam as sweet and sour tastes are the core flavors in jam.

Sugar profiling results showed that the sweatings contained no other sugars apart from fructose, glucose and sucrose (Table 2). These sugars are the main sugars in the rambutan pulp as well (Chai et al. 2018b; Lee et al. 2013; Tindall 1994). In general, different turning intervals did not significantly affect (p>0.05) the sugar contents of the sweatings. However, the total sugar of the sweatings increased by about 3.3-14.2% between Day 1 and 3 of fermentation. The fructose and glucose contents of the sweatings increased by about 2.4-2.6 and 2.1-2.5 folds, respectively, after three days of fermentation. However, the concentration of sucrose decreased by about 76.5-80.8% after three days of fermentation, regardless on the turning interval. These findings are similar to the observations made by previous researchers (Afoakwa et al. 2013, 2011; Hashim & Mat Hashim 2013) who worked on cocoa bean fermentation. Hansen et al. (1998) explained that the increase in the concentrations of reducing sugars during fermentation is the result of enzymatic reactions promoted by invertase from the fruit pulp which hydrolyzes sucrose into glucose and fructose. On the other hand, the decline in sucrose content during fermentation is due not only to hydrolysis, but also due to the utilization of the free sugars by the microorganisms to sustain microbial metabolic activity (Dzogbéfia et al. 1999). The high sugar content found in the sweatings is again showing that the sweatings are suitable for production of food products and/or ingredients.

The organic acid and ascorbic acid contents of the rambutan sweatings as affected by fermentation time and different turning intervals are summarized in Table 3. Three main organic acids, namely, citric, lactic and acetic acids, were found in the rambutan sweatings. Different turning intervals did not significantly (p>0.05) affect the concentrations of all the organic acids in rambutan sweatings. However, the concentrations of all the acids increased significantly (p<0.05) after 3 days of fermentation irrespective of turning intervals. Citric acid in the sweatings increased by 5.7-6.5 folds after 3 days of fermentation and is most likely due to citric acid already present in the rambutan pulp (Lee et al. 2013; Tindall 1994). In addition, to a lower volume of sweatings collected on the third day of fermentation, the sweatings had become more concentrated and thus, increasing the citric acid concentration of the sweatings when there was a further leakage of exudates from the pulp. The concentrations of lactic and acetic acids in the sweatings increased by 2.4-2.6 and 2.1-2.5 folds, respectively, after three days of fermentation and indicating that, indeed, fermentation took place. Chai et al. (2018a) had reported that fresh fruits contained neither lactic acid nor acetic acid. Thus, their presence in the sweatings testifies to the activities of lactic acid bacteria and acetic acid bacteria during fermentation. This is supported by Mehdizadeh et
### TABLE 1. Yield, pH, titratable acidity and total soluble solids of rambutan sweatings

<table>
<thead>
<tr>
<th>Turning intervals (h)</th>
<th>Yield (mL)</th>
<th>pH</th>
<th>Titratable acidity (% of lactic acid)</th>
<th>Total soluble solids (°Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
<td>Day 1</td>
</tr>
<tr>
<td>No turning</td>
<td>640.5±</td>
<td>316.0±</td>
<td>99.5±</td>
<td>4.25±</td>
</tr>
<tr>
<td></td>
<td>106.4±</td>
<td>19.8±</td>
<td>3.5±</td>
<td>0.05±</td>
</tr>
<tr>
<td>24</td>
<td>596.5±</td>
<td>334.5±</td>
<td>126.5±</td>
<td>4.24±</td>
</tr>
<tr>
<td></td>
<td>6.4±</td>
<td>9.2±</td>
<td>7.8±</td>
<td>0.07±</td>
</tr>
<tr>
<td>48</td>
<td>612.5±</td>
<td>313.5±</td>
<td>112.0±</td>
<td>4.22±</td>
</tr>
<tr>
<td></td>
<td>26.2±</td>
<td>21.9±</td>
<td>11.3±</td>
<td>0.12±</td>
</tr>
<tr>
<td>72</td>
<td>613.5±</td>
<td>307.5±</td>
<td>98.5±</td>
<td>4.18±</td>
</tr>
<tr>
<td></td>
<td>16.3±</td>
<td>33.2±</td>
<td>6.4±</td>
<td>0.11±</td>
</tr>
</tbody>
</table>

Mean ± standard deviation values with similar small letters within the same column (as affected by turning intervals) are not significantly different (p>0.05). Mean ± standard deviation values with similar capital letters within the same row of the same property (as affected by fermentation time) are not significantly different (p>0.05).

### TABLE 2. Sugars in rambutan sweatings

<table>
<thead>
<tr>
<th>Turning intervals (h)</th>
<th>Sugars in first day sweatings (%)</th>
<th>Sugars in second day sweatings (%)</th>
<th>Sugars in third day sweatings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fructose</td>
<td>Glucose</td>
<td>Sucrose</td>
</tr>
<tr>
<td>No turning</td>
<td>2.42±</td>
<td>2.30±</td>
<td>6.89±</td>
</tr>
<tr>
<td></td>
<td>0.06±</td>
<td>0.07±</td>
<td>0.25±</td>
</tr>
<tr>
<td>24</td>
<td>2.23±</td>
<td>1.95±</td>
<td>6.81±</td>
</tr>
<tr>
<td></td>
<td>0.20±</td>
<td>0.37±</td>
<td>1.00±</td>
</tr>
<tr>
<td>48</td>
<td>2.34±</td>
<td>2.13±</td>
<td>6.35±</td>
</tr>
<tr>
<td></td>
<td>0.22±</td>
<td>0.50±</td>
<td>0.70±</td>
</tr>
<tr>
<td>72</td>
<td>2.42±</td>
<td>2.35±</td>
<td>6.52±</td>
</tr>
<tr>
<td></td>
<td>0.14±</td>
<td>0.25±</td>
<td>0.67±</td>
</tr>
</tbody>
</table>

Mean ± standard deviation values with similar small letters within the same column (as affected by turning intervals) are not significantly different (p>0.05). Mean ± standard deviation values with similar capital letters within the same row and same sugar (as affected by fermentation time) are not significantly different (p>0.05).
### TABLE 3. Organic acids and ascorbic acid in rambutan sweatings

<table>
<thead>
<tr>
<th>Turning intervals (h)</th>
<th>Acids in first day sweatings (g/100 mL)</th>
<th>Acids in second day sweatings (g/100 mL)</th>
<th>Acids in third day sweatings (g/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Citric acid</td>
<td>Lactic acid</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>No turning</td>
<td>0.14±</td>
<td>0.23±</td>
<td>0.14±</td>
</tr>
<tr>
<td>24</td>
<td>0.13±</td>
<td>0.23±</td>
<td>0.12±</td>
</tr>
<tr>
<td>48</td>
<td>0.14±</td>
<td>0.24±</td>
<td>0.14±</td>
</tr>
<tr>
<td>72</td>
<td>0.13±</td>
<td>0.24±</td>
<td>0.14±</td>
</tr>
</tbody>
</table>

Mean ± standard deviation values with similar small letters within the same column (as affected by turning intervals) are not significantly different (p>0.05). Mean ± standard deviation values with similar capital letters within the same row and same acid (as affected by fermentation time) are not significantly different (p>0.05).
al. (2015) who reported that lactic acid bacteria and acetic acid bacteria were found during fermentation of rambutan seeds.

Table 3 also shows that the ascorbic acid content of the sweatings was not significantly affected (p>0.05) by different turning intervals but was significantly (p<0.05) affected by fermentation time, where the content increased by 2.6-2.8 folds after three days of fermentation. Adetuyi and Ibrahim (2014) also found that fermentation also increased the ascorbic acid content of okra seeds they studied which increased by about 436% due to the synthesis of the acid by microorganisms present during the fermentation. The increase in ascorbic acid content in the present study was most probably due to leakage from fruit pulp into the sweatings and possibly due also to microbial synthesis.

CONCLUSION

Rambutan fruits were fermented for eight days at different turning intervals. Fruit sweatings were formed during the initial stages of fermentation for up to three days and they were then collected and analyzed. The results showed that sweatings after 3 days of fermentation were high in sugar, organic acid and ascorbic acid contents. By understanding the characteristics of the sweatings, it can be produced into various types of food products and ingredients instead of being disposed. This could increase the variation of food products derived from rambutan fruit, reduce wastage of the sweatings, and more importantly, pollution could be prevented.

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