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Physicochemical properties of rambutan (*Nephelium lappaceum* L.) seed during natural fermentation of the whole peeled fruit

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Abstract

A novel way to reduce rambutan wastage is to ferment the fruit and valorise the seed post-fermentation into other food products and ingredients. Hence, the objective of this study was to investigate the physicochemical properties of rambutan seed during solid-state fermentation of the fruit. Peeled rambutan fruits were subjected to natural fermentation for ten days at 30°C. The environmental temperature, relative humidity, internal and external temperatures of the fermentation mass were measured daily. After ten days of fermentation, the seeds had higher cut test score (867.5), fermentation index (1.527), and a* value (8.20 for non-dried seeds and 9.93 for dried seeds), and lower L* (51.90 for non-dried seeds and 49.22 for dried seeds) and b* (30.52 for non-dried seeds and 30.12 for dried seeds) values; as compared to the non-fermented seeds (cut test score, 0.0; fermentation index, 0.856; L*, a*, and b* values, 64.52, 2.25, and 42.07 for non-dried seeds, respectively, and 61.03, 3.23 and 36.70 for dried seeds, respectively). During this time, pH, total soluble solids, fructose, glucose, sucrose, citric acid, and tartaric acid contents of the seeds decreased by 46, 44, 59, 61, 100, 85, and 100%, respectively, while the titratable acidity, lactic acid, acetic acid, and ascorbic acid contents of the seeds increased by 5.5, 7.8, 6.0, and 2.2-fold, respectively. Results showed that eight days of fermentation are adequate to produce well-fermented rambutan seeds that could be further processed into a cocoa powder-like product by roasting the fermented fruits in a manner similar to that of cocoa bean roasting.

Introduction

Rambutan (*Nephelium lappaceum* L.) is an exotic fruit from the Sapindaceae family, and native to Malaysia and Indonesia. The fruit pulp contains high contents of sugars and organic acids while its seed has high levels of crude fat (Chai *et al.,* 2018a; 2018c). The fruit is usually consumed fresh, canned, or processed. However, there is often a glut of the fruit during every harvesting season, leading to much waste when supply is greater than demand. Hence, it is possible to ferment the fruit similar to that of cocoa bean fermentation in order to produce a cocoa powder-like product. This could increase the variation of the food product derived from the fruit and subsequently, wastage is reduced. Besides, studies showed that fermentation can lead to beneficial changes in the physicochemical properties of a food product (Chai *et al.,* 2019a; 2019b).

The fermentation process adopted in this study was an imitation of cocoa bean fermentation where both pulp and seed of cocoa bean are fermented prior to drying and roasting (Lima *et al.,* 2011). The sugars, mainly sucrose, fructose, and glucose contained in the rambutan pulp provide nutrients for microorganisms to grow and carry out fermentation (Chai *et al.,* 2018c). Fermentation of rambutan seed alone with very little or without the pulp has been previously reported (Febrianto *et al.,* 2014; 2016; Mehdizadeh *et al.,* 2015; Khairy *et al.*, 2017). In cocoa bean processing, the fermentation of beans begins almost immediately and ends by the 5th or 6th day. It is an essential process to develop appropriate flavours from precursors found in the beans. During this stage, biochemical reactions occur within the beans leading to the formation of the important precursors of the cocoa flavour, colour development, and reduction of bitterness and astringency of the beans (Lima *et al.,* 2011). It has been reported that fermentation time greatly affects the quality of the cocoa beans. This may also be the case for fermentation of rambutan fruit. Rodriguez-Campos *et al.* (2011) found that six days of fermentation were sufficient to produce volatile compounds with flavour notes desirable in cocoa beans, and to avoid the production of compounds with off-flavour notes at the same time.

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Emmanuel *et al.* (2012) reported that cocoa beans fermented for six days had a higher fermentation index; where, according to Romero-Cortes *et al.* (2013), the sufficiently fermented beans should have a fermentation index value equal or higher than 1, and higher amount of well-fermented beans. Mehdizadeh *et al.* (2015) also found that ten days of rambutan seed fermentation could increase the fermentation index of the seeds.

Since fermentation time is a crucial variable in determining the final quality of the product, it is essential to ascertain how much time is needed to ferment the rambutan fruit. Six days required for the fermentation of cocoa beans may or may not be sufficient as the overall size and dimension of rambutan fruits differ from those of cocoa beans. Thus, the objective of this study was to investigate the effect of solid-state fermentation of peeled rambutan fruit on the physicochemical properties of the seed.

Materials and methods

Raw material

Ripe fruits of rambutan Clone R4 were obtained from University Agricultural Park, Universiti Putra Malaysia. The fruits for the study were fully matured, ready for consumption, free of blemishes, and uniform in colour (red) and size. Two batches of samples were used in this study, and each batch was analysed in triplicate.

Fermentation of peeled rambutan fruit

Peeled rambutan fruit fermentation was carried out as described by Chai *et al.* (2018b). Briefly, a perforated plastic container (40 × 28 × 10 cm) was used to ferment 7 kg of peeled rambutan fruits. The depth of the fruit mass was about 8 cm. A non-perforated plastic container was placed under each container that contained the fruit mass to collect the sweatings produced during the fermentation (Chai *et al.,* 2018b). The peeled rambutan fruits were then fully covered by tap-water-cleaned banana leaves to create a relatively anaerobic condition before the set up was transferred to an incubator cupboard (30 ± 2°C) for the fermentation (Chai *et al.,* 2019c). The fruits were allowed to ferment for one, three, five, seven, and ten days in five separate containers. Non-fermented fruits served as the control.

During the fermentation, several measurements were determined daily; environmental temperature, relative humidity, and internal and external temperatures of the fermentation mass. The environmental temperature and relative humidity were determined by using a digital thermo-hygrometer (TFA, Wertheim, Germany). The internal (taken at 5 cm from the surface of the fermentation mass) and external (taken at the surface of fermentation mass) temperatures of the fermentation mass were measured using a thermocouple (Fisher Scientific, Pittsburgh, PA, USA) at five different locations (at the centre and four corners of the fermentation mass) during the course of fermentation, and the temperature was expressed as average temperature of five readings.

Characterisation of seeds after fermentation

Fermented fruits collected after each fermentation period (0, 1, 3, 5, 7, and 10 d) were divided into two portions. The first portion which was used for the determination of cut test, fermentation index, and colour was first dried at 60°C for 48 h (Febrianto *et al.,* 2016). For the second portion of the fermented fruits, the pulp that was attached to the seeds was carefully removed, and the colour, pH, titratable acidity, total soluble solids, sugar, organic acid, and ascorbic acid contents of the seeds were determined.

Measurement of cut test

The method described by Guehi *et al.* (2010) was used to determine the cut test of fermented rambutan seed. Briefly, 100 of the dried fermented seeds were randomly selected and cut lengthwise through the middle using a penknife. Both halves of each seed were examined in full daylight. Observations were made on the colour of the seeds (yellow, partly yellow/partly brown, and fully brown). Yellow seeds would be obtained when fermentation has been terminated prematurely. Fully brown seeds were considered as well-fermented seeds. Results of the cut test were expressed as a percentage. Based on the official standard for cocoa beans, a batch of beans with more than 60% of fully brown beans is considered as high quality product. Cut test score was calculated using Eq. 1(Hii *et al.,* 2011):

$$\text{Cut test score} = (10 \times \%\ \text{brown}) + (5 \times \%\ \text{partly brown}) + (0 \times \%\ \text{yellow}) \quad \text{(Eq. 1)}$$

Determination of fermentation index (FI)

FI was determined following the method described by Nazaruddin *et al.* (2006). Briefly, 0.5 g of ground dried fermented seeds was mixed with 50 mL of 97:3 (v/v) mixture of methanol:concentrated HCl, and the homogenate was allowed to stand in a refrigerator at 8°C for 16 - 19 h before vacuum-filtered. The absorbance of the filtrate was separately read in a UV-Visible spectrophotometer (Shimadzu UV-160A PC, Shimadzu Corporation, Kyoto, Japan) at 460 and 530 nm. The FI of the sample was obtained

by calculating the ratio of the absorbance at 460 and 530 nm as shown in Eq. 2:

$$FI = A_{460}/A_{530} \quad \text{(Eq. 2)}$$

Determination of colour

The colour of control and fermented seeds was measured after they had been ground. Briefly, 20 g of the seeds was ground into powder using a commercial kitchen blender (Model MX-SM1031S, Panasonic, Selangor, Malaysia) for 2 min. Then, the powder was allowed to pass through a 60-mesh sieve (Retsch, Haan, Germany) with a mesh size of 250 µm. Colour analysis was carried out by using a Minolta CR-10 colour reader (Konica Minolta Sensing, INC., Tokyo, Japan), and the L*, a*, and b* values of the powder were recorded.

Determination of pH, titratable acidity, and total soluble solids

The pH, titratable acidity, and total soluble solids of fermented seeds were determined following the method described by Ranganna (1977). The results of titratable acidity and total soluble solids were expressed as % (w/w) lactic acid and °Brix, respectively.

Determination of sugar content

High performance liquid chromatography was used to determine the sugar profile and content of the fermented seeds. 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 were used in this analysis. The chromatographic column used for separation was a Purospher® Star NH_2 column (259 × 4.6 mm, particle size of 5 µm from Merck, Darmstadt, Germany) connected to a Purospher® NH_2-18e guard column (4 × 4 mm I.D from Merck, Darmstadt, Germany), and thermostated at 35°C (Hunt *et al.,* 1977). The eluent used was degassed with 80% acetonitrile in deionised water, and flowed at 1.5 mL/min.

To extract sugar from the seeds, 10 g of ground seed sample was mixed with 100 mL of 85% methanol for 30 min at 80°C in a water bath. The sample was then filtered through a Whatman No. 1 filter paper, and the sample residue was then re-extracted twice as earlier described using 75 mL of 85% methanol. The filtrates were pooled, and the volume was reduced in a rotary vacuum evaporator and finally made up to 10 mL with deionised water. The solution was then filtered through a Sep-Pak C18 cartridge followed by a 0.45 µm membrane filters (Sartorius, Germany), and then, 10 µL of the sample was injected into the HPLC (Hunt *et al.,* 1977). Three sugar standards (glucose, fructose, and sucrose from Sigma Aldrich, St. Louis, MO, USA) with concentrations ranging from 0 - 8% (w/v) were used to identify and quantify the sugars present in the seeds.

Determination of organic acid and ascorbic acid contents

Organic acid and ascorbic acid analyses of fermented seeds were performed by referring to the method reported by Medlicott and Thompson (1985) and Sturm *et al.* (2003) with slight modifications. Organic acids and ascorbic acid in fermented seeds were simultaneously extracted by first making a 50 mL suspension of 10 g of homogenised seed sample with deionised water which was then clarified by centrifugation at 6,000 *g* for 15 min (Beckman J2-21M/E, USA) (Sturm *et al.,* 2003). The extract was filtered through a 0.45 µm membrane filters (Sartorius, Germany), and 10 µL of the sample was injected into the HPLC. 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 Purovspher® Star RP18 end-capped column (250 × 4.6 mm I.D., 5 µm particle size, from Merck, Darmstadt, Germany) was connected to a Purovspher® RP-18e guard column (4 × 4 mm I.D from Merck, Darmstadt, Germany), and thermostated at 30°C. Degassed 0.008 M H_2SO_4 was used as the mobile phase for separation of organic acids and ascorbic acid in fermented seeds, and the flow rate was 0.5 mL/min (Sturm *et al.,* 2003). The detection of organic acids and ascorbic acid was done at 210 nm. Six organic acids, namely lactic acid (85% purity, JT. Baker, Central Valley, USA), tartaric acid, citric acid (99% purity, respectively, Fisher Scientific, Rochester, NY, USA), malic acid (99% purity, Sigma Aldrich, St. Louis, MO, USA), acetic acid (99% purity, Merck, Darmstadt, Germany), and one vitamin (ascorbic acid, 99% purity), were used to obtain the standard curves.

Statistical analysis

The analytical data were analysed by one-way analysis of variance followed by Tukey's test using Minitab v. 16 Statistical Software (Minitab Inc., Coventry, UK). The results were expressed as mean value ± standard deviation. Statistical significance differences were considered at the level of $p < 0.05$.

Results and discussion

Changes in environmental temperature, relative humidity, and internal and external fermentation mass temperatures

The average environmental temperature and relative humidity during ten days of rambutan fruit

fermentation were 28.5°C and 68%, respectively. Figure 1a shows that the daily internal temperature of the fermentation mass gradually increased by 7°C until day 5, attaining a maximum temperature of 35.6°C. Thereafter, a gradual decrease in temperature was observed and the final internal temperature of the mass was 32.1°C. This increment trend can also be observed in cocoa bean fermentation where the temperature of the fermentation mass increases from around 27 to 50°C (Ouattara *et al.,* 2008; Pereira *et al.,* 2012). Ouattara *et al.* (2008) and Pereira *et al.* (2012) reported that the maximum temperature of their cocoa masses was found on the 3rd and 4th day, respectively. Together with acids, heat is produced when the pulp undergoes ethanolic, acetic, and lactic fermentations (Afoakwa et al., 2008). The increase in internal temperature of rambutan fermentation mass is very slight as compared to cocoa bean fermentation due to a lower quantity of fruits (7 kg) that was used in this study. In cocoa bean fermentation, at least 50 - 500 kg of beans are heaped and used, and thus, more heat is generated.

Figure 1a also shows the daily external temperature of fermentation mass for ten days of fermentation. Similar to internal temperature, the external temperature of the fermentation mass increased until day 5 with a maximum temperature of 34.3°C, and then gradually decreased to 31.3°C by the end of the fermentation. The changes in internal and external temperatures of the fermentation mass were not significant due to the low quantity of fruits that were used in this study, causing the fermentation mass to have a shallow depth, and the heat produced during the fermentation easily radiated to the environment. The internal and external temperatures of the fermentation mass shared the same trend due to the release of the heat from the fermentation mass to the environment, and this exothermal process is probably due to the oxidation of ethanol. These conditions and the presence of sugars in the pulp provided a conducive condition for the development of microbial populations in the fruit mass.

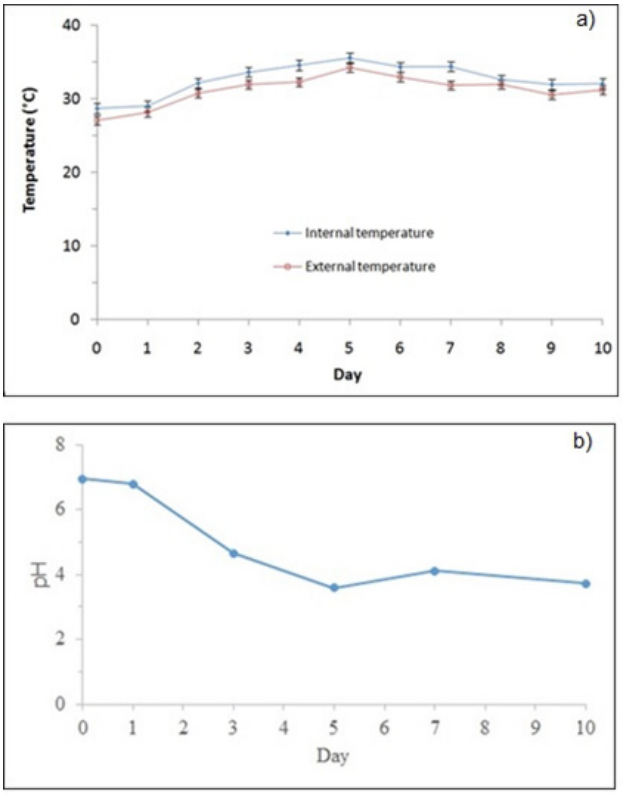

Figure 1. (a) Internal and external temperatures of fermentation mass during solid-state fermentation, and (b) pH trend of rambutan seed during solid-state fermentation.

Properties of rambutan seeds during fermentation

Cut test

The cut test is used as a fermentation indicator for cocoa bean, and was adopted in this study as this test allows the monitoring of fermentation status and determination of the endpoint of rambutan fermentation. Lima *et al.* (2011) stated that fully fermented cocoa beans are brown in colour, and the same yardstick was used for rambutan seeds. Table 1 shows the percentage of surface colour and cut test score of fermented dried seeds. Generally, the quantity of yellow seeds decreased and brownish seeds increased with fermentation time. The cut test score of the fermented seeds increased after ten days of

Table 1. Effect of fermentation time on surface colour, cut test score, and fermentation index of rambutan seeds after fermentation.

Fermentation time (day)	Yellow (%)	Yellow/ Brownish (%)	Brownish (%)	Cut test score	Colour fractions absorbance values		Fermentation Index (Fraction II / Fraction I)
					Fraction I (530 nm)	Fraction II (460 nm)	
0	100.0 ± 0.0^{a}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.050 ± 0.004^{a}	0.042 ± 0.007^{a}	0.856 ± 0.169^{c}
1	100.0 ± 0.0^{a}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.034 ± 0.003^{b}	0.036 ± 0.003^{b}	1.070 ± 0.129^{bc}
3	13.5 ± 2.1^{b}	53.5 ± 6.4^{a}	33 ± 4.2^{c}	597.5 ± 10.6^{c}	0.029 ± 0.001^{c}	0.035 ± 0.002^{b}	1.212 ± 0.082^{abc}
5	0.0 ± 0.0^{c}	45.0 ± 2.8^{ab}	55 ± 2.8^{b}	775 ± 14.1^{b}	0.021 ± 0.003^{d}	0.027 ± 0.001^{c}	1.286 ± 0.136^{abc}
7	0.0 ± 0.0^{c}	33.5 ± 3.5^{bc}	66.5 ± 3.5^{ab}	832.5 ± 17.7^{a}	0.015 ± 0.003^{e}	0.021 ± 0.002^{d}	1.484 ± 0.436^{ab}
10	0.0 ± 0.0^{c}	26.5 ± 3.5^{c}	73.5 ± 3.5^{a}	867.5 ± 17.7^{a}	0.010 ± 0.002^{f}	0.014 ± 0.00^{e}	1.527 ± 0.344^{a}

Mean ± standard deviation values with similar letters within the same column are not significantly different ($p > 0.05$).

fermentation. Control seeds and seeds after one day of fermentation appeared to be 100% yellow with a cut test score of 0. Results showed that there was only a slight difference between the cut test scores of rambutan seeds fermented for seven and ten days, and were not statistically different ($p > 0.05$). This indicates that seven days could be the endpoint of rambutan fruit fermentation. As fermentation progresses, barriers that separate enzymes and substrates are progressively broken down. This will increase the activity of polyphenol oxidase during drying and result in more melanin production, the brown polymer (Lopez, 1983). Based on the official standard for cocoa bean, a batch of beans with more than 60% of fully brown colour beans is considered as a good quality product. Thus, this indicates that resultant seeds after seven days of fermentation are of good quality.

Fermentation index (FI)

FI is one of the established methods to measure the degree of fermentation of cocoa beans (Gourieva and Tserevitinov, 1979; Pettipher, 1986), and was also adopted in this study. Table 1 shows the absorbance values and FI of rambutan seeds after various fermentation times. FI of the seeds increased by 78% after ten days of fermentation. This is probably due to the increase of brown pigment in the seeds resulted from the fermentation and drying processes as the absorbance range used in the FI analysis is sensitive to brown colour. Pettipher (1986) suggested that sufficiently fermented cocoa beans should have an FI value of ≥ 1.

In this study, the FI of the seeds after one day of fermentation achieved a value of 1.07, indicating well-fermented seeds were obtained. These findings are not comparable to the results reported by Mehdizadeh *et al.* (2015) who found that at least four days of fermentation of rambutan seeds were needed to produce rambutan seeds with FI value of ≥ 1. This difference may be due to the drying process adopted in this study prior to the analysis. Similar to the cut test, there was only a slight difference between the FI of rambutan seeds fermented for seven and ten days. This indicates that seven days of fermentation would be sufficient for rambutan seed fermentation.

Colour

In order to determine the colour of the fermented seeds in a more objective manner than the visual assessment of the cut test, the seeds were first ground, and are shown in Figure 2. Colourimetry was used to determine the development in the L*, a*, and b* values of the non-dried and dried fermented seeds, and the values are tabulated in Table 2. As can be seen in Table 2, fermentation time significantly ($p < 0.05$) affected the L*, a*, and b* values of both non-dried and dried fermented seeds. Generally, as fermentation time increased, the samples moved towards lower L* and b* values by 20 and 27%, respectively, for non-dried fermented seeds, and 19 and 17%, respectively, for dried fermented seeds; and higher a* value by 264% for non-dried fermented seeds, and 207% for dried fermented seeds, in which corresponding well with the samples becoming increasingly brown with time. Similar findings were reported by Mehdizadeh *et al.* (2015) who fermented rambutan seeds. Besides, the drying process used in this study was also found to have an influence on the colourimetry measurements. Results showed that the dried fermented rambutan seeds had a lower L* value as compared to non-dried fermented rambutan seeds. Generally, the seed samples became darker (lightness decreased) progressively as fermentation time increased. This is due to the heat produced during fermentation and drying processes, and subsequent browning of the seeds.

Table 2. L*, a*, and b* values of non-dried and dried fermented rambutan seed powder.

Fermentation time (day)	L* value		a* value		b* value	
	Non-dried fermented seeds	Dried fermented seeds	Non-dried fermented seeds	Dried fermented seeds	Non-dried fermented seeds	Dried fermented seeds
0	64.52 ± 1.80^{a}	61.03 ± 0.77^{a}	2.25 ± 0.39^{d}	3.23 ± 0.20^{e}	42.07 ± 1.08^{a}	36.70 ± 0.37^{a}
1	61.96 ± 1.16^{ab}	58.78 ± 0.40^{b}	2.83 ± 0.24^{d}	4.77 ± 1.01^{d}	36.95 ± 4.03^{b}	34.90 ± 0.51^{b}
3	60.88 ± 2.10^{b}	57.65 ± 0.65^{b}	5.05 ± 0.69^{c}	6.25 ± 0.55^{c}	36.38 ± 0.85^{b}	33.92 ± 1.16^{bc}
5	57.33 ± 2.29^{c}	55.03 ± 0.97^{c}	6.08 ± 0.48^{b}	7.50 ± 0.29^{b}	34.62 ± 0.82^{bc}	32.93 ± 0.54^{cd}
7	54.03 ± 1.14^{d}	53.02 ± 1.45^{d}	6.55 ± 0.48^{b}	8.17 ± 0.14^{b}	32.30 ± 1.70^{cd}	31.60 ± 0.47^{d}
10	51.90 ± 1.81^{d}	49.22 ± 1.63^{e}	8.20 ± 0.81^{a}	9.93 ± 0.42^{a}	30.52 ± 0.56^{d}	30.12 ± 1.17^{e}

Mean ± standard deviation values with similar letters within the same column are not significantly different ($p > 0.05$).

pH, titratable acidity, and total soluble solids

The pH, titratable acidity, and total soluble solids of the rambutan seeds after zero, one, three, five, seven, and ten days of fermentation were measured and are tabulated in Table 3. Generally, the pH of the rambutan seeds decreased with fermentation time. A similar reduction trend was reported by Mehdizadeh *et al.* (2015) who fermented rambutan seeds for ten days, and found that the pH of the seeds reduced from around pH 7 to 4. The decrease is probably due to the infusion of organic acids such as lactic acid and acetic acid produced during fermentation. According to Nazaruddin *et al.* (2006) and Selamat (1994), during fermentation, the adhering pulp becomes liquid and under aerobic conditions, microorganisms will produce acetic acid and ethanol. The acetic acid penetrates the cotyledons during fermentation leading to a reduction in pH. Towards the end of the fermentation, an increase in pH was observed (Table 3), possibly due to the evaporation of volatile acids like acetic acid (Afoakwa *et al.,* 2011). pH is one of the major indicators in determining the quality of cocoa beans. Based on the cut test and fermentation index results, seven days of fermentation, are adequate to produce well-fermented rambutan seeds. However, as can be seen in Figure 1b, it is better to ferment the seeds for up to eight days in order to produce fermented seeds with a relatively similar pH with that of the seeds fermented for ten days. This indicates that there is no fluctuation of pH after eight days of fermentation and thus, fermentation process is considered as complete.

The trend of change in pH corresponds well with titratable acidity (Table 3). The titratable acidity of the seeds increased by 5.5-fold after ten days of fermentation. Similar observations were made by Afoakwa *et al.* (2013) and Nazaruddin *et al.* (2006) who fermented cocoa beans, and found that the titratable acidity of the beans increased with fermentation time. The increase in acidity might be due to the metabolism of microorganisms during the

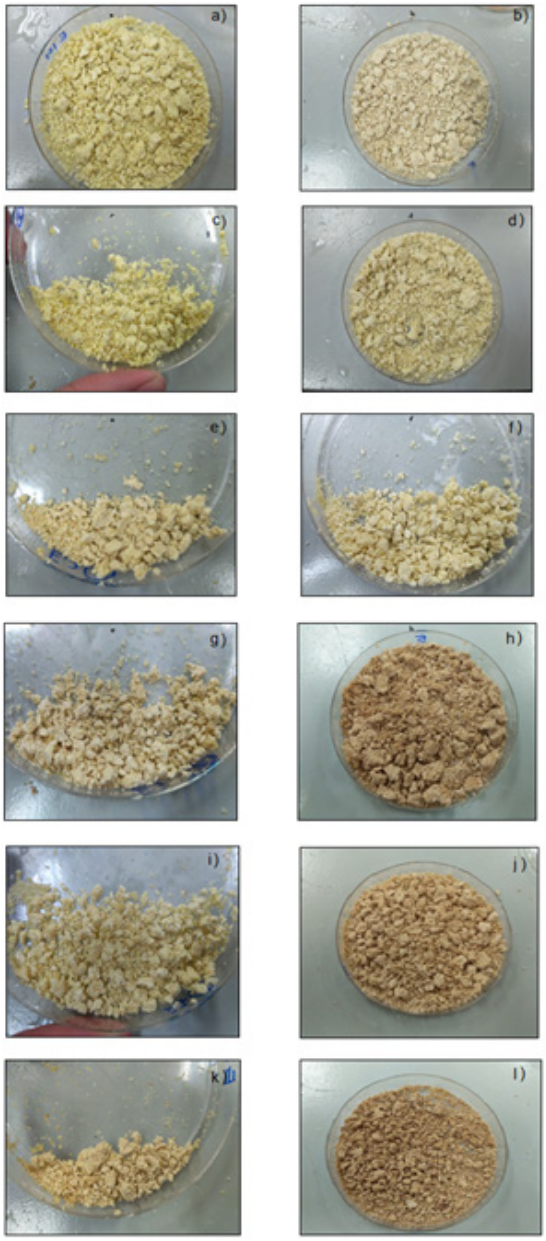

Figure 2. Visual appearance of rambutan seed powder from portion 2 after (a) zero, (c) one, (e) three, (g) five, (i) seven, and (k) ten days of fermentation, and dried rambutan seed powder from portion 1 after (b) zero, (d) one, (f) three, (h) five, (j) seven, and (l) ten days of fermentation.

Table 3. pH, titratable acidity, total soluble solids, sugars, organic acids, and ascorbic acid contents of rambutan seeds during fermentation.

Fermentation time (day)	pH	Titratable acidity (expressed as % lactic acid)	Total soluble solids (°Brix)	Fructose (g/kg)	Glucose (g/kg)	Sucrose (g/kg)	Total sugar (g/kg)	Citric acid (g/kg)	Tartaric acid (g/kg)	Lactic acid (g/kg)	Acetic acid (g/kg)	Ascorbic acid (g/kg)
0	6.94 ± 0.07^{a}	0.10 ± 0.01^{e}	10.17 ± 0.75^{a}	18.7 ± 0.7^{d}	22.1 ± 1.9^{d}	43.2 ± 3.1^{a}	84.0	2.6 ± 0.1^{a}	0.2 ± 0.0^{a}	0.4 ± 0.0^{e}	0.2 ± 0.0^{c}	1.3 ± 0.1^{e}
1	6.78 ± 0.02^{a}	0.13 ± 0.01^{e}	9.00 ± 1.10^{a}	22.8 ± 1.0^{c}	28.1 ± 1.7^{c}	35.6 ± 2.0^{b}	86.5	2.2 ± 0.2^{b}	0.1 ± 0.0^{b}	0.7 ± 0.0^{e}	0.2 ± 0.0^{c}	1.5 ± 0.1^{d}
3	4.65 ± 0.21^{b}	0.23 ± 0.03^{d}	7.33 ± 0.52^{b}	44.3 ± 1.8^{a}	52.1 ± 2.1^{a}	25.8 ± 2.5^{c}	122.2	1.1 ± 0.1^{c}	0.1 ± 0.0^{b}	1.1 ± 0.0^{d}	0.7 ± 0.1^{b}	1.9 ± 0.2^{c}
5	3.58 ± 0.05^{d}	0.30 ± 0.04^{c}	6.33 ± 0.82^{bc}	32.7 ± 11.2^{b}	44.1 ± 5.3^{b}	15.6 ± 0.9^{d}	92.4	0.7 ± 0.0^{d}	0.1 ± 0.0^{b}	1.6 ± 0.0^{c}	0.7 ± 0.0^{b}	2.1 ± 0.2^{c}
7	4.12 ± 0.02^{c}	0.35 ± 0.06^{b}	6.00 ± 1.10^{bc}	10.0 ± 0.6^{e}	10.6 ± 0.4^{e}	2.7 ± 0.2^{e}	23.3	0.4 ± 0.0^{e}	tr	2.5 ± 0.1^{b}	1.1 ± 0.2^{a}	2.4 ± 0.1^{b}
10	3.73 ± 0.06^{d}	0.55 ± 0.04^{a}	5.67 ± 0.52^{c}	7.6 ± 0.7^{f}	8.6 ± 0.4^{e}	ND	16.2	0.4 ± 0.0^{e}	tr	3.1 ± 0.4^{a}	1.2 ± 0.1^{a}	2.9 ± 0.3^{a}

Note: ND = not detected, tr = trace amount (≤ 50 mg/kg). Mean ± standard deviation values with similar letters within the same column are not significantly different ($p > 0.05$).

fermentation process which develops volatile acid (acetic) and non-volatile acids (citric and lactic) in the pulp through sugar degradation and subsequently, diffusing into the cotyledon and causing a gradual increase in acidity of the seeds (Selamat, 1994).

As shown in Table 3, the total soluble solids of the rambutan seeds decreased by 4.5% after ten days of fermentation. Chen *et al.* (2013) who studied the production of longan mead found that the total soluble solids decreased by 52% after fermentation. The decrease in total soluble solids in rambutan seeds is expected as yeasts and lactic acid bacteria consume sugars and organic acids to produce ethanol and lactate during fermentation (Leal *et al.,* 2008).

Sugar composition and content

The sugar contents of the rambutan seeds before and after fermentation are shown in Table 3. Fructose (18.7 g/kg) and glucose (22.1 g/kg) were the main reducing sugars even in the fresh seeds, with glucose being more dominant. Fermentation up to five days caused significant ($p < 0.05$) increase in glucose and fructose contents where the increases were 135 and 136%, respectively. On the other hand, sucrose content declined significantly ($p < 0.05$) from 43.2 g/kg in fresh seeds to not being detected after ten days of fermentation. From day 7 of fermentation onwards, fructose and glucose contents decreased to less than 10 g/kg. Mehdizadeh *et al.* (2015) who fermented rambutan seeds also found that the fructose and glucose contents of the seeds increased at the beginning of the fermentation and then gradually decreased until day 10 of fermentation. Changes in fructose, glucose, and sucrose contents in cocoa bean have been primarily attributed to the action of invertase in the cocoa pulp and beans which hydrolyses sucrose to fructose and glucose (Afoakwa *et al.,* 2013). It is essential to produce reducing sugars during fermentation as these sugars would react with peptides and free amino acids in the Maillard reaction during drying and roasting to produce the acceptable flavour compounds (Afoakwa *et al.,* 2013).

Organic acid and ascorbic acid contents

As shown in Table 3, four types of organic acids (citric, tartaric, lactic, and acetic acids) were found in the fermented seeds. Citric acid was the major organic acid in the fresh seeds. However, its concentration together with that of tartaric acid decreased as fermentation progressed. The decrease in citric acid content is probably due to the acid being metabolised to other acids such as lactic acid (Lopez, 1983). Similar observations can be seen in the work reported by Rodriguez-Campos *et al.* (2011) who found that after eight days of fermentation, the content of citric acid of cocoa bean reduced. Yeasts, *Lactobacillus plantarum, L. collincides,* and *Acetobacter rancens* have been shown to metabolise citric acid in cocoa (Selamat, 1994). The loss of tartaric acid could be resulted from microorganisms-initiated and/or enzyme-catalysed reactions, salt precipitation, and oxidation-reduction reactions (Lamikanra, 1997).

There was a negligible quantity of lactic acid in fresh rambutan seed (an indication of freshness), but the content increased by 7.8-fold after fermentation for ten days. This finding is supported by Mehdizadeh *et al.* (2015) who reported that the lactic acid content in rambutan seeds that they fermented for ten days increased by 5-fold. Similar observations were reported by Camu *et al.* (2007) who reported that lactic acid content of cocoa bean increased after six days of fermentation. Lactic acid is produced either from metabolic processes within the cotyledon during anaerobic process or by the microbial action on the pulp (Lopez, 1983). In the pulp, lactic acid is produced mainly through degradation of reducing sugar by both homo- and hetero-fermentative lactic acid bacteria (Selamat, 1994).

Results also showed that acetic acid increased 5-fold after fermentation for ten days (Table 3). Mehdizadeh *et al.* (2015) on the other hand, found that acetic acid content of rambutan seeds increased by around 8-fold after ten days of fermentation. Similar to rambutan fermentation, Camu *et al.* (2007) reported that the acetic acid content of cocoa bean increased after six days of fermentation. Acetic acid is produced mainly by acetic acid bacteria through oxidation of ethanol in the presence of oxygen. It can also be produced when citrate is metabolised to oxaloacetic acid and acetate by *Lactobacilli* spp. (Selamat, 1994).

Similar to lactic and acetic acids, the ascorbic acid content of rambutan seeds increased significantly ($p < 0.05$) by 2.2-fold at the end of the fermentation. A similar increment trend was found in fermented okra seeds (Adetuyi and Ibrahim, 2014) and fermented tea (Pasha and Reddy, 2005). The increase of ascorbic acid content in fermented food is due to the synthesis of this acid by microorganisms present during the fermentation (Adetuyi and Ibrahim, 2014).

Conclusion

The results from this study showed that seeds

of rambutan fruits that had been fermented for seven and ten days possessed higher quality in terms of cut test, fermentation index, and colour. Based on the pH trend of the fermented seeds, eight days of rambutan fruit fermentation are considered as adequate and complete. Hence, fermenting rambutan fruits for eight days could produce high quality seeds with a high cut test score and fermentation index, acceptable colour, pH, and titratable acidity. After fermentation, the fermented fruits can be subjected to a roasting process similar to that of cocoa bean processing. By doing so, a cocoa powder-like product could be produced. This could increase the variation of rambutan-derived products and thus, wastage of the fruit could be reduced.

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