

Cocoa-like flavor compound development of rambutan seed fat as the effect of fermentation and roasting

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Abstract

Rambutan seed waste has become a noteworthy problem in rambutan canning industry that need to be solved. Previous finding showed that rambutan seed could be utilized by extracting the fat that could be utilized as confectionery fat with improved characteristic by fermentation and roasting treatment. The study to evaluate the cocoa-like flavor compounds development as the effect of these process was carried out. The rambutan seed was fermented for 3, 6, and 9 days followed/unfollowed by roasting process at 150°C for 30 min. The browning index of the powder, the Maillard Reaction Products (MRPs) and the volatile flavor compounds of the rambutan seed fat were analysed. The study found that the fermentation treatment followed by roasting treatment significantly increase the browning index and melanoidin content in powder and fat, respectively. Six and 9 days fermentation followed by roasting possessed highest value of browning index (1.4875 and 1.5485 AU, respectively) and melanoidin content (0.318 and 0.295 AU, respectively). The result also showed that fermentation of rambutan seed followed by roasting process could successfully developed desired pyrazine compounds, in which the contribution of the pyrazine content could be as much as 42.69% of total flavor compound of rambutan seed fat.

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Introduction

Rambutan seed is considered as a waste in rambutan canning manufactures with a noteworthy value as much as 94,500 tonnes/year from Thailand, Indonesia and Malaysia alone (Norlia *et al.*, 2011). This massive value has become an issue that need to be solved. Previous studies showed that the extraction of fat from rambutan seed can be the alternative to utilize rambutan seed, as the fat can be used in candles, soaps and fuel manufacturing (Morton, 1987). Furthermore, research carried out by Solis-Fuentes *et al.* (2010) and Sirisompong *et al.* (2011) showed that edible rambutan fat has the physical and chemical characteristics that make it possible to be applied in the food industry as confectionery ingredient. Febrianto (2013) and Febrianto *et al.* (2014) then reported that fermented and roasted rambutan seed fat have similar characteristic with cocoa butter and potential to be utilized as cocoa butter substitute.

Fermentation and roasting treatments also generate other value added characteristics such as flavor compounds that lead to quality enhancement of food product (Reineccius and Henry, 2006;

Bonvehi and Coll, 2002). In addition, these processes become a compulsory in processing step to produce highly valued product such as cocoa bean due to its contribution to the production of the unique chocolate flavor (Lopez, 1986; Puziah *et al.*, 1998). The development of flavor compounds during fermentation has also been reported to be generated during the fermentation of other material such as soybean, cassava bagasse and tropical agro-industrial substrates (Bramorski *et al.*, 1998; Couto and Sanromán, 2006). Medeiros *et al.* (2001) reported that the fermentation of cassava bagasse could generate fruity flavor due to the occurrence of monoterpene alcohols and isoamyl acetate. Whereas, Larroche *et al.* (1999) also mentioned that soybean fermentation by lactic acid bacteria could induce the development of pyrazine compounds. Pyrazines are known to be important flavor compounds in cocoa that contribute more than 40% of cocoa flavor fraction. They are responsible to provide chocolate, vanilla, roasted and nutty flavor as well as having an effect on bitter and astringency sensation (Lindsay, 1996). However, the duration of fermentation is a crucial factor since it was reported that insufficient as well as excess duration of fermentation could lead to development

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of undesirable flavor (Schwan and Wheals, 2004).

On the other hand, the roasting process is an important step for the development of flavor compound in food product due to the occurrence of the non-enzymatic Maillard browning reaction. The reaction between amino acids and sugars contribute to the development of flavor, aroma and color which then improve the palatability and sensory properties of the food product (Fellows, 2000). In this paper, we evaluated the browning index, maillard reaction and volatile flavor compounds of rambutan seed fat (RSF) as affected by fermentation and roasting process. It is anticipated that the results generated could provide better understanding on maillard reaction and flavor development of RSF.

Materials and Methods

Materials

Rambutan seeds were supplied by a rambutan canning industry at Sungai Petani, Kedah, Malaysia which was collected in September 2011 harvest season. The seeds were by-products of rambutan pulp-canning production. The seeds were still covered by a small amount of rambutan pulp due to the use of mechanical cutter during canning process.

Preparation of rambutan seed fat sample

The rambutan seed fermentation process was carried out immediately after receiving fresh raw materials. The rambutan seeds were transferred into plastic baskets (625 mm × 425 mm × 294 mm) which were lined with banana leaves. After the basket was filled with raw rambutan seeds, the upper part of the basket was then covered with banana leaves. The fermentation process was carried out for 3, 6, and 9 days, with stirring every 3 days. Stirring was done using a wooden spatula. After the fermentation process completed, the rambutan seeds were immediately dried in the oven (Afos Mini Kiln, Hull, England) at 60°C for 36-48 hours until it reached 10-11% of moisture content.

Fermented dried rambutan seeds were then stored in a closed container at room temperature before the screw-pressing process used to obtain fermented rambutan seed fat (F-RSF). For unfermented rambutan seed fat (U-RSF), the rambutan seeds were prepared by oven drying fresh rambutan seeds. For roasted rambutan seed fat (R-RSF) and fermented-roasted rambutan seed fat (FR-RSF), the dried rambutan seeds were roasted at 150°C for 30 minutes in an oven, cooled at room temperature and then stored prior to screw-pressing process. In addition to the fat extraction process, all the samples were

ground into powder and subjected to the analysis of browning index.

Extraction of RSF was carried out using a screw oil expeller Komet DD 85 IG (IBG Monforts Oekotec GmbH & Co. KG, Germany). Prior to screw-pressing process, the dried (unfermented, fermented and fermented-roasted) rambutan seeds were dehusked and heated at 60°C for 30 minutes in an oven. The screw-pressing process resulted a viscous mixture of RSF. The viscous mixture was then filtered in a heated condition (60°C). The RSF collected were then transferred into inert-screw-cap bottle and stored at -4°C prior to analysis.

Browning index

Browning index analysis was determined according to method of Misnawi (2003) based on polyphenol spectrum determination with slight modification. Fifty milliliters of methanolic: hydrochloric acid (37%) (97:3) was used to dilute a known weight of powdered rambutan seed (0.5 g) and the mixture was then cooled in the refrigerator at 8 ± 2°C for 16-18 hours. Filtration using Whatman filter paper no. 1 was done to obtain a clear extract of the solution. Browning index was determined according to the spectral data as absorbance at 420 nm (UV-160A, Shimadzu Corp., Nagakyo-ku, Kyoto, Japan).

Maillard reaction products

Maillard reaction products (MRPs) in rambutan seed fat were analyzed as the formation of melanoidins content. The analysis was done following the method of Delgado-Andrade *et al.* (2010) with slight modification. Briefly, the analysis was performed as follows: 0.5 g RSF was melted in an oven at 65°C (10 min) prior to analysis. The melted RSF was then dissolved using 10 ml iso-octane (2,2,4 trimethylpentane) and vortexed vigorously for 15 s. The solution obtained was then analyzed and measured as absorbance at 420 nm in a UV-160A Shimadzu spectrophotometer (Shimadzu Corp., Nagakyo-Ku, Kyoto, Japan) using 10 mm light path quartz cuvette. The result was expressed as absorbance units (AU).

Solid phase micro extraction (SPME) – Gas chromatography Mass Spectrometry (GCMS) analysis

Analysis of flavor compound was carried out following method of Supelco (1998) using Polydimethylsiloxane/Divinylbenzene/Carboxen on stableflex fiber purchased from Supelco (Supelco, Bellefonte, Pennsylvania, USA). Agilent GC 7890 equipped with a SPME auto-sampler and Agilent mass

spectrometry (MSD 5977) was used in this analysis. HP-5MS ((5%-phenyl) - methylpolysiloxane, 0.25 mm ID, 30 m and 0.25 μ m film) column was used for the analysis. Prior to use, the SPME fiber was pre-conditioned in the injection port of the GC set at 260°C for 1 hour.

The condition of analysis was carried out as follows: The extraction of flavor compound from RSF was done by heating 5 g of RSF samples in 40 mL vial in a heating block at 65°C for 30 min using the headspace extraction method. After that, SPME device was then transferred into the injection port of the GC for desorption process. The injection port of GC was set at 260°C and desorption was done in splitless mode for 5 min. The column was set at an initial temperature of 40°C (5 min), ramped to 230°C at 4°C/min. Ion trap mass spectrometer ($m/z = 30-350$ at 0.6 sec/scan) was used for compound identification. The compound was identified based on the library provided by NIST (National Institute of Standards and Technology). The identified compound were then classified into seven different groups such as carboxylic acids, aldehydes, ketones, alcohols, esters, hydrocarbons and pyrazines and quantified based on its % area of chromatogram based on Watkins *et al.*, (2012).

Statistical analysis

Data analysis including General liner model (GLM), post-hoc analysis using Tukey HSD (Honestly Significant Difference), and Pearson Correlation was performed using Statistical package for social science (SPSS) software version 17.0 (IBM Corporation, Armonk, New York, USA). The statistical analyses were performed at 5% significance level.

Result and Discussion

Browning index

Browning index (BI) is usually used to measure the occurrence of brown-colored compound in the product (Bal *et al.*, 2011). Analysis of BI in rambutan seed showed that untreated rambutan seed also possessed brown-color compound (0.554 AU at 420 nm) (Figure 1). This condition could be due to the natural existence of brown pigment in rambutan seed. However, fermentation and roasting treatment significantly increased the BI of rambutan seed, in which high increase of BI was observed in all the roasted rambutan seed. Fellows (2000) previously mentioned that roasting/baking process could change the physicochemical properties of the product due to the occurrence of Maillard non-enzymatic browning reaction.

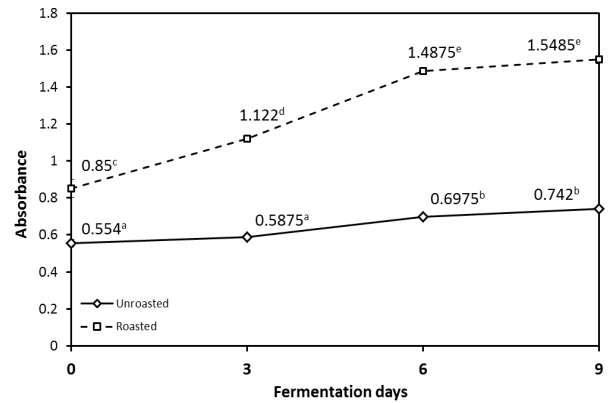


Figure 1. Browning index of rambutan seed under different treatment. Mean (n=3) value with different superscript letters were significantly different (Tukey HSD, $p < 0.05$)

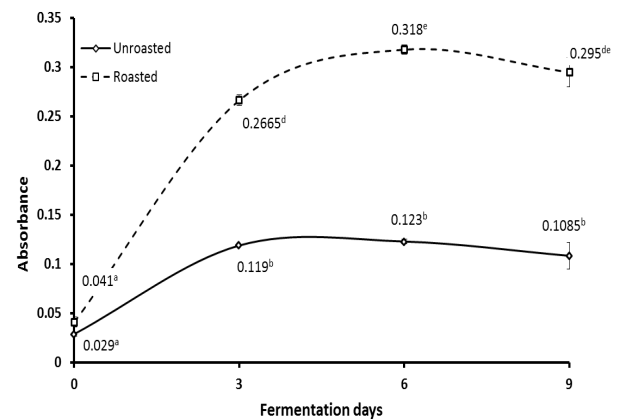


Figure 2. Melanoidin of rambutan seed under different treatment. Mean (n=3) value with different superscript letters were significantly different (Tukey HSD, $p < 0.05$)

Significant differences ($p < 0.05$) of BI between unroasted and roasted fermented samples indicate the intensity of browning reaction that occurred during the roasting process. The highest increase of BI was observed in 6 days and 9 days fermented samples (from 0.7 to 1.49, and 0.74 to 1.55, respectively), whereas the 3 days fermented samples showed smaller increases. Since the Maillard reaction is highly correlated with its precursor compounds, high intensity of BI in fermented seed means that fermentation process could generate Maillard reaction's precursor compound such as reducing sugars and amino acids (Belitz and Grosch, 1999). This is similar to the fermentation process of cocoa beans, where free amino acids, peptides and reducing sugar will be developed and act as precursors of Maillard non-enzymatic browning (Mohr *et al.*, 1976). The formation of Maillard reaction's precursors during fermentation then intensified the development of brown-colored compound in rambutan seed during roasting.

Table 1. Non-pyrazine flavor compounds and major compounds in each categories identified in RSF

| Compounds | U-RSF* | R-RSF* | 3 F-RSF* | 3 FR-RSF* | 6 F-RSF | 6 FR-RSF | 9 F-RSF | 9 FR-RSF |
|--|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Total acids (>13 compounds) | 22.06 | 10.93 | 25.51 | 14.05 | 12.42 | 10.30 | 36.80 | 12.94 |
| - Acetic acid | 16.45 | 9.98 | 22.08 | 13.62 | 11.64 | 9.31 | 35.41 | 12.69 |
| Total aldehydes (>55 compounds) | 24.59 | 18.75 | 0.38 | 5.87 | 1.37 | 4.81 | 0.93 | 9.32 |
| - Pentanal | 2.68 | 9.07 | - | - | - | - | - | - |
| - Hexanal | 11.08 | 2.39 | - | - | - | - | - | - |
| - Nonanal | 3.38 | 2.93 | 0.26 | - | 1.14 | 1.23 | 0.32 | 2.66 |
| - Benzaldehyde | 4.70 | 2.47 | - | 1.17 | - | 2.29 | 0.49 | 1.61 |
| Total ketones (>14 compounds) | 1.80 | 2.65 | 3.13 | 5.03 | 1.23 | 3.21 | 0.91 | 6.05 |
| Total alcohols (>13 compounds) | 17.26 | 7.7 | 40.76 | 22.75 | 30.83 | 17.59 | 14.65 | 12.74 |
| - 2,3-butanediol | 3.51 | - | 36.20 | 17.63 | 17.61 | 10.57 | 12.81 | 6.02 |
| - Phenol alcohol | - | 1.27 | - | 1.54 | 9.41 | 5.50 | - | 2.50 |
| - 1-Octen-3-ol | 7.89 | 1.36 | 2.32 | - | - | - | 0.67 | - |
| - Phenylethyl alcohol | 1.86 | 1.30 | 0.93 | - | 1.57 | 1.26 | 0.64 | 1.41 |
| Total Esters (>20 compounds) | 1.26 | 0.25 | 0.11 | 0.01 | 2.62 | 2.24 | 6.24 | 4.84 |
| - Hexadecanoic acid ethyl ester | 0.03 | 0.01 | - | - | 0.12 | - | 1.22 | 0.77 |
| - Propanoic acid 2-methyl butyl ester | - | - | - | - | 1.69 | - | - | - |
| - 9-octadecenoic acid ethyl ester | - | - | - | - | - | - | 2.33 | 0.16 |
| - Ethyl oleate | - | - | - | - | 0.21 | 0.14 | 0.32 | 1.33 |
| Total hydrocarbons (>19 compounds) | 20.38 | 10.76 | 6.62 | 2.57 | 3.72 | 1.37 | 2.86 | 1.90 |
| - Styrene | 8.23 | 3.27 | 3.89 | 1.89 | 3.10 | 1.13 | 2.22 | 1.08 |
| - Ethyl-benzene | 4.14 | - | - | - | - | - | - | - |
| - 1-methyl-4-(1-methyl ethyl)-benzene | 0.47 | 1.66 | - | - | - | - | - | - |
| Total non-pyrazine compounds | 87.35 | 51.04 | 76.51 | 50.28 | 52.19 | 39.52 | 62.39 | 47.79 |

Data presented is in % area of GC chromatogram

*U-RSF: Unfermented-RSF

*R-RSF: Roasted-RSF

*n F-RSF: n days Fermented-RSF

*n FR-RSF: n days Fermented-Roasted-RSF

Maillard reaction products (MRPs)

Melanoidin content of RSF was measured at 420 nm, which is also used in some researches to measure the degree of browning. Melanoidins are brown-colored compounds formed during the final stage of Maillard reaction that contribute in the discoloration of the product (Nursten, 2005). As shown in Figure 2, sample treated with fermentation followed by roasting have significantly higher absorbance compared to unroasted samples. The highest absorbance observed is in samples that have been fermented for 6 days (0.318 AU). The 6 FR-RSF (0.318 AU) had the value which is not significantly different with 9 FR-RSF (0.295 AU). The lowest absorbance were observed in both roasted and non-roasted unfermented samples (0.029 and 0.041 AU).

Lignert and Eriksson (1980) and Delgado-Andrade *et al.* (2010) mentioned that Maillard reaction occurred spontaneously during roasting and storage through the interaction between reducing sugars and amino groups which resulted in MRPs. As

shown in Figure 2, fermentation treatment followed by roasting obviously gave significantly higher result than the unfermented and unroasted samples. These results affirm that during fermentation treatment, precursors of Maillard reaction are formed and further reacted during roasting process of rambutan seed resulting in intense browning color. It is also convinced by previous report of Lee *et al.* (2001) which mentioned that roasting of fermented cocoa bean resulted in brown and darkened color; however, over roasting condition will result in a decrease of the browning.

Apart from the increase of melanoidin content induced by roasting treatment, an increase of the absorbance was also observed in the unroasted samples. The highest absorbance is exhibited by 6 day fermented-unroasted sample (6 F-RSF) and 9 F-RSF also increased albeit with no significant differences among them. As mentioned by Lertsiri *et al.* (2001), Maillard non-enzymatic reaction could also occur during fermentation due to the occurrence

of reactive amino acid compound and reducing sugar. In addition, browning reaction can also occur during storage (Nursten, 2005), and drying process of product as mentioned by Lopez *et al.* (2007) who found that the browning reaction in hazelnut could happen in the drying process at a temperature range of 30 – 80°C.

Carboxylic acid

The occurrence of carboxylic acid compounds in food products is responsible for several taste perceptions, such as vinegar-like (acetic acid), buttery (2-methylpropanoic acid), pungent, cheesy, soapy and animal-like flavor (Mahajan *et al.*, 2004). Table 1 shows that more than 13 compounds were identified and acetic acid occurred in all RSF samples and was the main constituent of carboxylic acid that contribute to the RSF flavor. Acetic acid contribute from 9.31% to 35.41% from total carboxylic acids (10.30% to 36.80%); in which, the lowest percentage of acetic acid was observed at 6 FR-RSF. The proportion of acetic acid as well as total carboxylic acid fluctuated during the fermentation process, but in general its proportion was decreased after roasting.

Carboxylic acids could be generated during fermentation by the activity of acetic acid bacteria, lactic acid bacteria and yeast. Organic acids such as acetic acid and lactic acid are mostly generated via the pyruvate pathway, and lactic acid via hexose isomerase and phosphoketolase pathway (Jay *et al.*, 2005). In addition, other organic acids such as, oxalic acid, citric, tartaric, succinic acid may also be generated (Jinap *et al.*, 1998). However, these organic acids are non-volatile; thus, acetic acid was most detected due to its volatile nature. Heat treatments such as roasting, frying and boiling has been reported to lead to significant decrease of organic acid content in chestnuts (Ribeiro *et al.*, 2007). As shown in Table 1, roasting treatment resulted in lower total acid percentage, caused by the decrease of acetic acid and the disappearances of minor carboxylic acid compounds. On the other hand, the formation of several acids during roasting could occur from the degradation of carbohydrate as reported previously by Ginz *et al.* (2000).

Aldehydes

Aldehyde is commonly found in tea (more than 55 compounds has been identified) and is the main flavor constituent in peach, almond, apricot, plum and cherry (Maarse, 1991; Doyle *et al.*, 2001). Analysis on flavor compounds resulted in more than 15 aldehyde compounds were present in all RSF samples. However, their compositions differ

in each RSF sample (Table 1). Pentanal, hexanal, nonanal and benzaldehyde were found to have major proportion in the total aldehyde content in RSF (2.68-9.07%, 2.39-11.08%, 0.26-3.38%, and 0.49-4.70%, respectively). Pentanal was reported to possess flavor perception such as strong, acid, pungent and also contribute into chocolate and nut-like flavor (Maarse, 1991). Nonanal has been reported to give floral aroma in elderberry, whereas hexanal is main aroma constituent in grapes giving fruity sensation (Berger, 2007). According to Bonvehi (2005), 4-methyl-2-phenyl-2-pentenal and 5-methyl-2-phenyl-2-hexenal had cocoa sensorial attributes.

Ketones

On the overall flavor compound identified in RSF, ketones contributed less flavor compared to others. The flavor compound is in the range of 0.91% (9 F-RSF) to 6.05% (9 FR-RSF). Basically, saturated ketones give fruity, cheesy and fatty perception, whereas diketone contribute importantly in coffee due to its perception of sweet, buttery and caramel flavor. Similar to aldehydes, ketone concentration can be changed during fermentation due to the occurrence of aldehyde reductase or alcohol dehydrogenase enzymes that provide alteration between ketones and secondary alcohol (Pigeau and Inglis, 2007; Jordan *et al.*, 2011). However, it is also reported that ketones can be formed from microbial-induced lipid oxidation by the activity of lipases and lipoxidase-like activity (Reineccius and Henry, 2006).

Alcohols

The occurrence of alcohol compounds in food product commonly generate sweet, fruity, alcoholic, balsamic and green flavor and sensation. However, it also depends on its molecular structure (Curioni and Bosset, 2002). Result shows that more than 13 alcohol compounds were identified from the RSF samples (Table 1). Alcohol content in RSF samples were mostly contributed by 2,3-butanediol (3.51-36.2%), phenol alcohol (1.27-9.41%), 1-Octen-3-ol (0.67-7.89%), and phenylethyl alcohol (0.64-1.86%); whereas total alcohol contributed was in range of 7.7% up to 40.76% of the total flavor of RSF.

As mentioned previously, the activity of several enzymes such as aldehyde reductase or alcohol dehydrogenase could generate alcohol from aldehyde and ketones, resulting in the formation of primary alcohol and secondary alcohol. 2,3-butanediol has been reported to be generated during fermentation involving *Saccharomyces cerevisiae* by the activity of butanediol dehydrogenase (Ng *et al.*, 2012). However, Buttery *et al.* (1999) reported that 2,3-butanediol not

Table 2. Pyrazine compounds identified in RSF

| Compounds | U-RSF* | R-RSF* | 3 F-RSF* | 3 FR-RSF* | 6 F-RSF | 6 FR-RSF | 9 F-RSF | 9 FR-RSF |
|--|-------------|--------------|-------------|--------------|--------------|--------------|-------------|--------------|
| Methyl pyrazine | - | 1.47 | - | 3.5 | - | 1.09 | - | 2.22 |
| Dimethyl-pyrazine | 1.93 | 12.52 | - | 11.33 | 1.03 | 2.89 | 1.61 | 7.46 |
| Trimethyl-pyrazine | - | - | 0.74 | 7.93 | 3.94 | 9.27 | 6.01 | 10.8 |
| 3-ethyl-2,5-dimethyl-pyrazine | - | 5.29 | - | 2.49 | - | 1.34 | - | 1.45 |
| 2,3-Dimethyl-5-ethylpyrazine | - | 1.59 | - | - | - | - | - | - |
| Tetramethyl-pyrazine | 0.40 | - | 5.41 | 2.39 | 24.06 | 22.96 | 13.8 | 10.93 |
| 2-Acetyl-6-methylpyrazine | - | - | - | - | - | - | - | 0.72 |
| 1-(6-Methyl-2-pyrazinyl)1ethanone | - | - | - | - | - | - | - | 0.24 |
| 5H-5-Methyl-6,7-dihydrocyclopentapyrazine | - | 0.47 | - | 0.1 | - | 0.04 | - | 0.13 |
| 3,5-diethyl-2-methyl-pyrazine | - | 1.41 | - | - | - | - | - | - |
| 2,3,5-Trimethyl-6-ethylpyrazine | - | 0.49 | 0.33 | 0.6 | 0.98 | 2.68 | 0.28 | 1.19 |
| 2-Acetyl-3-ethylpyrazine | - | - | - | - | - | 0.07 | - | - |
| 2,3-dimethyl-5-(1-propenyl)-, (E)-pyrazine | - | - | - | - | - | 0.28 | - | - |
| 2,6-Dimethyl-3-sec-butylpyrazine | - | - | 0.07 | - | - | - | - | - |
| 2-methyl-5-(1-propenyl)-, (E)-pyrazine | - | - | - | - | - | - | - | 0.26 |
| 2,5-dimethyl-3-(2-methylpropyl)-pyrazine | - | - | - | 0.07 | - | 0.05 | - | 0.07 |
| 2,3,5-Trimethyl-6-propylpyrazine | - | - | - | - | - | 0.19 | - | - |
| 2-Isoamyl-6-methylpyrazine | - | 0.59 | - | 0.31 | - | 0.2 | - | 0.37 |
| Benzopyrazine | - | 0.06 | - | - | - | - | - | - |
| 2,3,5-Trimethyl-6-butylpyrazine | - | - | - | - | 0.57 | 0.59 | - | - |
| 2-butyl-3,5-dimethyl-pyrazine | - | 0.09 | - | 0.93 | - | - | - | - |
| 2,5-dimethyl-3-(3-methylbutyl)-pyrazine | - | - | - | - | - | 0.58 | - | - |
| 2,3-Dimethyl-5-isopentylpyrazine | - | 0.1 | - | - | - | 0.15 | - | 0.07 |
| 2,3,5-Trimethyl-6-isopentylpyrazine | - | 0.01 | - | - | 0.09 | 0.31 | - | 0.21 |
| Total pyrazines | 2.33 | 24.09 | 6.55 | 29.65 | 30.76 | 42.69 | 21.7 | 36.12 |

Data presented is in % area of GC chromatogram

(-) means the compound was not detected in the sample.

*U-RSF: Unfermented-RSF

*R-RSF: Roasted-RSF

*n F-RSF: n days Fermented-RSF

*n FR-RSF: n days Fermented-Roasted-RSF

directly important to the flavor contribution. On the other hand, Curioni and Bosset (2002) mentioned that phenylethyl alcohol is an aromatic alcohol which provide floral, rose and honey sensation, whereas 1-Octen-3-ol contributed to undesirable flavor such as green, moldy or meaty flavor.

Esters

Esters cover a wide spectrum of odor and flavoring effects, and they are widely distributed as major constituent in fruit and essential oils. It provide fruity, sweet, floral, honey, and flowery perception in food product. Ester can be generated from the reaction between alcohols or phenols with acids and its derivatives (Maarse, 1991; Reineccius and Henry, 2006; Fraundorfer and Schieberle, 2006). More than 20 ester compounds contributing to flavor of RSF (Table 1) were identified. Despite numerous types of esters, its contribution is the least compared to others. Total esters were observed to contribute in range of 0.01% (3 FR-RSF) to 6.24% (9 F-RSF). The major ester compounds observed were hexadecanoic acid ethyl ester (0.01 to 1.22%), propanoic acid 2-methyl-

butyl ester (1.69%), 9-octadecenoic acid ethyl ester (0.16 to 2.33%), and ethyl oleate (0.14 to 1.33%). Formation of esters during fermentation process could be generated by lipid metabolism in acids and alcohols rich condition that allowed the yeast to carry out esterification reactions, resulting in variety of esters (Reineccius and Henry, 2006).

Hydrocarbons

More than 19 hydrocarbon compounds were identified in rambutan seed fat and observed to be in the range of 1.90% (9 FR-RSF) to 20.38% (U-RSF) (Table 1). Major compounds observed were styrene (1.08-8.23%), ethyl-benzene (4.14%), 1-methyl-4-(1-methyl ethyl)-benzene (0.47-1.66%). Hydrocarbon compounds have been reported to be important flavor constituent in food products. Hydrocarbons such as sesquiterpenes were commonly identified in tea, whereas benzenoid hydrocarbons were identified in cocoa. Benzenoid hydrocarbons generally give perception of green and rose-like flavor (Maarse, 1991). Total hydrocarbon observed in our analysis decreased after the application of fermentation and/

or roasting treatment. The decrease of hydrocarbon during fermentation may have occurred due to the utilization of hydrocarbon compounds as carbon source by microorganism during fermentation (Hommel, 1990; Doshi *et al.*, 2010).

Pyrazines

Pyrazines are strong flavor compounds mostly found in food products which undergo heat treatment induced Maillard reaction. According to Maarse (1991) more than 100 monocyclic pyrazine, 15 bicyclic pyrazines and 12 quinoxaline have been identified from food products, in which most of them (94 compounds), are found in cocoa. Thus, the occurrence of pyrazine in cocoa is often associated with proper processing method of cocoa beans (Frauendorfer and Schieberle, 2006; Puziah *et al.*, 1998). In general, the occurrence of some pyrazine compounds in cocoa is desirable due to its contribution to certain cocoa taste and flavor such as nutty, roasted-nuts, chocolate-like (methylpyrazine); like, caramel, nutty, vanilla, coffee flavor (dimethylpyrazine); cocoa, roasted-nuts, peanut, and coffee flavor (2,3,5-trimethylpyrazine); and fuller vanilla, chocolate, cocoa and coffee flavor (2,3,5,6-tetramethylpyrazine) (Bonvehi and Coll. 2002; Bonvehi, 2005).

There are 23 pyrazine compounds contributing to the flavor of RSF (Table 2). Methylpyrazine (MP), dimethyl-pyrazine (DMP), trimethylpyrazine (TrMP), 3-ethyl-2,5-dimethyl-pyrazine and tetramethyl-pyrazine (TMP) were the major pyrazines which contribute up to 3.5%, 12.52%, 9.27%, 5.29% and 24.06% of total pyrazines, respectively. In unroasted samples, pyrazines contributed in the range of 2.33% (U-RSF) to 30.76% (6 F-RSF) of total RSF flavor. The formation of pyrazine compound during the fermentation process have also been reported previously. Puziah *et al.* (1998) and Besson *et al.* (1997) mentioned that the activity of several microorganisms such as *Bacillus subtilis* and *Bacillus megatrium* could generate the formation of pyrazine compound during soybean fermentation. Roasting process significantly increased pyrazine from 24.09% (R-RSF) to 42.69% (6 FR-RSF) of the total RSF flavor, which made pyrazine the most dominant flavor compound in fermented-roasted rambutan seed fat. In addition to increasing the total pyrazines percentage of RSF, the roasting process also contributed into the development of more numerous and varied pyrazine compounds. However, the pyrazine percentage in roasted sample decreased after 6 days fermentation. This result indicate that sufficient fermentation of rambutan seed effectively generated Maillard reaction precursors such as amino

acid and reducing sugar, which then converted into pyrazine compounds during the roasting process; but, longer time of fermentation will decrease its concentration instead. This result shows that 6 day fermentation of rambutan seed fat is sufficient to produce RSF with high concentration of pyrazines.

Correlation between BI, MRP and pyrazine content

Result of Pearson Correlation analysis carried out between BI, MRP and Pyrazine content show significant positive correlation was found between BI with MRP ($r=0.899$, $p<0.05$), BI with Pyrazine ($r=0.836$, $p<0.05$), and MRP with pyrazine content ($r=0.773$, $p<0.05$). This results show that there was a direct correlation between the performance of roasting with cocoa-like flavor compound development. Research carried out by Asikin *et al.* (2014) mentioned that there was a positive correlation between the browning rate with volatile Maillard compound development in cane brown sugar. On the other hand, Nursten (2005) previously mentioned that the production of flavor compound occurred during the intermediate and final stage of Maillard reaction which also happened simultaneously with the production of color compound of discoloration. The results also show that the more extensive the browning reaction happened during roasting, the more desired aroma compound (pyrazine) was formed which then resembles the flavor of cocoa.

Conclusion

The study found that the fermentation treatment followed by roasting treatment significantly increase the browning index and melanoidin content in rambutan seed powder and fat, respectively. RSF from 6 and 9 days fermentation followed by roasting possessed highest value of browning index and melanoidin content. Analysis on the volatile flavor of rambutan seed fat successfully identified more than 126 compounds from carboxylic acid, aldehyde, ketone, alcohol, hydrocarbon, ester, and pyrazine group. Flavor compounds from carboxylic acid, aldehyde, alcohol and pyrazines groups were observed to have higher contribution on RSF flavor. Analysis on the pyrazine group showed that fermentation of rambutan seed followed by roasting process successfully generated desirable pyrazine compounds in RSF. Highest pyrazines content was observed at 6 FR-RSF followed by 9 FR-RSF. This result indicated that 6 days fermentation of rambutan seed can be considered as optimum due to its high content of pyrazines and low acid concentration.

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