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Identification of flavor compounds in rambutan seed fat and its mixture with cocoa butter determined by SPME-GCMS

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ABSTRACT

Rambutan (Nephelium opossum L) is one of the most important tropical fruits that is originally found in Malaysia, Thailand, Philippines, Vietnam, Borneo and other countries in this region. The industrial processing of this fruit produces seeds and peels as waste materials. The aim of this work was to determine the flavor active compounds of rambutan seed fat (RSF) and its mixtures with cocoa butter (CB) after fermentation 6 days and roasting at 150 °C for 30 min in term of ester, alcohol, hydrocarbon, carboxylic acid, aldehyde, ketone and pyrazine compounds. The result showed total ester flavoring compounds increased in the range of 10.59%–16.49% in the RSF mixtures as compared to CB. Alcoholic flavoring compounds also found in the range of 10.03%–23.15% of the total flavor in CB and RSF mixtures respectively. The most important flavoring compounds, Pyrazine, 2,6-dimethyl (1.42% and 2.01%), Pyrazine, 2-ethyl-6-methyl (0.67% and 0.62%), Pyrazine, trimethyl (5.10% and 5.22%), Pyrazine, tetramethyl (14.34% and 14.72%), 2-Acetyl-3,5-dimethylpyrazine (0.56% and 0.45%), 2-Isoamyl-6-methylpyrazine (0.34% and 0.34%) and Pyrazine, 2-butyl-3,5-dimethyl (0.34% and 0.27%) estimated in CB and RSF mixtures respectively. Rambutan seed fat up to 20% can be used with cocoa butter as a potential ratio of CB and RSF for the application in chocolate manufacturing as a cocoa butter alternative to improve and get the best quality aroma in final products.

1. Introduction

Flavor is a crucial factor to consumer perception accept in chocolate and is influenced, not just by volatile smell compounds, but also by non-volatiles and behavior of the continuous fat phase, influencing the liberation of volatiles into the mouth headspace and taste perception. Precursor composition depends on bean genotype and environmental effects, especially on contents of storage proteins and polyphenols (Afoakwa et al., 2008). The evolution of flavor compounds during fermentation process has been reported to be generated during the fermentation of other material such as soybean, bagasse and tropical agro-industrial substrates (Couto and Sanromán, 2006). Medeiros et al. (2011) showed that the fermentation of cassava bagasse could generate fruity flavor due to the occurrence of isoamyl acetate and monoterpene alcohols. While, Shaligram et al. (2008) also reported that soybean fermentation by lactic acid bacteria could induce the development of pyrazine compounds. Pyrazines, a volatile heterocyclic nitrogen-containing compound, is known to be the major flavor compound that is responsible for forming of the typical roasted rambutan seed fat and cocoa butter flavor. They are also responsible for supply chocolate, vanilla, roasted and nutty flavor besides having an effect on bitter and astringency sensation (Zhu et al., 2010). Nevertheless, the duration of the fermentation process is also a decisive factor since it was also noted that insufficient, as well as excess duration of fermentation, could lead to the development of undesirable flavor (Lefeber et al., 2012). Wherein, Febrianto (2013) noted, the pyrazine group showed that fermentation of rambutan seed followed by roasting process successfully generates a desirable pyrazine compounds in rambutan seed fat. Highest pyrazines content was observed at 6 days fermented-roasted rambutan seed

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fat followed by 9 days fermented-roasted rambutan seed fat (42.69% and 36.12%, respectively).

On the other hand, roasting process is considered as a cooking method that uses dry heat and incurs vital role in the seeds, including Millard reactions, wherein reducing sugars and amino acids are recognized to produce featured seed flavor (Beckett, 2003). Ramli et al. (2006) reported that cocoa butter has nutty, astringent, acidic and bitter notes before roasting. Acidity is further reduced during roasting by reducing the volatile acid concentration (Ramli et al., 2006). However, non-volatile acids such as lactic, succinic, tartaric, citric and oxalic acid are not influenced. Millard reactions are quite important to develop flavor and are critical in roasting where reducing sugars, peptides, and free amino acids will take part. 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 (Voigt et al., 1993). Hydrophobic amino acids such as tyrosine, phenylalanine, alanine, and leucine have been recovered by Voigt et al. (1994). The activities of proteinase in fermentation process are critical contributors that release them where hydrolysis of sucrose resulted in glucose, fructose and reducing sugars (Voigt et al., 1993; Beckett, 2003). Various studies on the rambutan seed fat and cocoa butter properties have been reported without describing its flavor compounds by blended together in certain proportion. Therefore, the aim of this present study to identify the development of flavor compounds of rambutan seed fat and its mixture with cocoa butter. It is expected that the result of this identification could provide the better understanding of aroma development and composition of fat generated from blended rambutan seed fat with cocoa butter.

2. Material and methods

2.1. Materials

CB was purchased from an Indonesian coffee company and the Cocoa Research Institute, Jember, East Java, Indonesia. Meanwhile, raw rambutan (Nepheliumlappaceum L.) seeds were supplied by a rambutan canning industry in Sungai Petani, Kedah, Malaysia.

2.2. Fermentation and roasting of rambutan seeds

The fermentation process was performed on rambutan seeds, which were still covered by small amounts of rambutan pulp. The rambutan seeds were transferred into plastic baskets (625 mm × 425 mm × 294 mm), which were previously lined with banana leaves. After filling in the baskets with raw rambutan seeds, the baskets were covered with banana leaves for 6 days, with mixing every 3 days. On the 6th day of fermentation, the banana leaves cover was opened and the rambutan seeds inside the fermentation container were stirred with a wood spatula. After the fermentation time was sufficient, the dried rambutan seeds were roasted at 150 °C for 30 min by oven-drying (AFOS Mini Kiln, Hull, England). After roasting, the samples were cooled at room temperature and stored until the screw-pressing process for RSF production (Febrianto, 2013).

2.3. RSF extraction

RSF extraction was performed using a KOMET screw oil expeller DD 85 IG (IBG MonfortsOekotec GmbH & Co. KG, Ger-many). Dried rambutan seeds were dehusked and heated at 60 °C for 30 min by oven-drying (AFOS Mini Kiln, Hull, England). The screw-pressing process produced RSF, which was a viscous mixture of rambutan seed powder and RSF (Febrianto, 2013). The separation of RSF from rambutan seed butter was achieved through filtration under a heated condition (60 °C). Afterward, the collected RSF was transferred into inert-screw-cap bottles in Fig. 1.

2.4. Preparation of RSF and cocoa butter mixtures

Mixtures of rambutan seed fat and cocoa butter were prepared following proportions as mentioned in Table 1. The levels were in the range of 0%–100% with the total mixture was 100% (CB + RSF = 1). The mixing process was carried out by adding predetermined proportions (w/w) of cocoa butter and rambutan seed fat. The CB was incorporated into RSF in six proportions, namely, 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100 (w/w) CB to RSF, as CB, M1, M2, M3, M4, and RSF respectively. Then, the mixtures were melted in the oven (AFOS Mini Kiln, Hull, England) at 60 °C for 15–20 min. The melted mixtures were then homogenized using a vortex and transferred into inert-screw-cap bottles and then stored at −4 °C until they were used for analysis

2.5. Solid phase microextraction (SPME)-Gas chromatography-Mass Spectrometry (GCMS) analysis

Approximately 1 g of the samples were placed into the vials and covered tightly. Analysis of flavor compound was implemented following method of Santos et al. (1996) using PDMS/DVB/Carboxen on stable flex fiber purchased from Supelco (Supelco, Bellefonte, Pennsylvania, USA). Agilent GC equipped with an SPME auto - sampler and Agilent mass spectrometry was employed in this analysis. HP-5MS ((5% phenyl)-methylpolysiloxane, 0.25 mm ID, 30 m and 0.25 μm film) column was set for the analysis. Before use, the fiber was pre-conditioned in the injection port of the GC set at 260 °C for 1 h.

In one word, the condition of analysis was implemented as follows: The extraction of flavor compound from RSF and its mixture with CB was done in a heating block at 65 °C for 30 min using the headspace extraction method. Thereafter, SPME device was then transferred to the injection port of the GC for desorption process. The injection port of the 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 s/scan) was used for compound identification.

3. Results and discussion

3.1. Esters compounds

There are a large number of ester compounds contributed to the overall flavor of the rambutan seed fat and cocoa butter. Esters cover a wide spectrum of odor and flavoring effects, and they are widely distributed as a major constituent in fruit and essential oils. It provides fruity, sweet, floral, honey, and flowery perception in food products. Ester can be generated from the reaction between alcohols or phenols with acids and its derivatives (Reineccius and Henry, 2006). Despite numerous types of esters, its contribution is the least compared to others and the blending process between CB and RSF caused a change of these compounds through the production of other types of ester compounds as noted in the M2, M3, and M4 samples. Total esters were observed to contribute in the range of 10.59% (CB) to 16.49% (RSF) of total RSF and its mixture with cocoa butter flavor. The major esters compounds were found in cocoa butter, 1-Methoxy-2-propyl acetate (2.71%), Butanoic acid, butyl ester (1.32%), Formic acid, 1-methylpropyl ester (0.31%), Succinic acid, isohexyl octyl ester (0.53%) and Ethyl

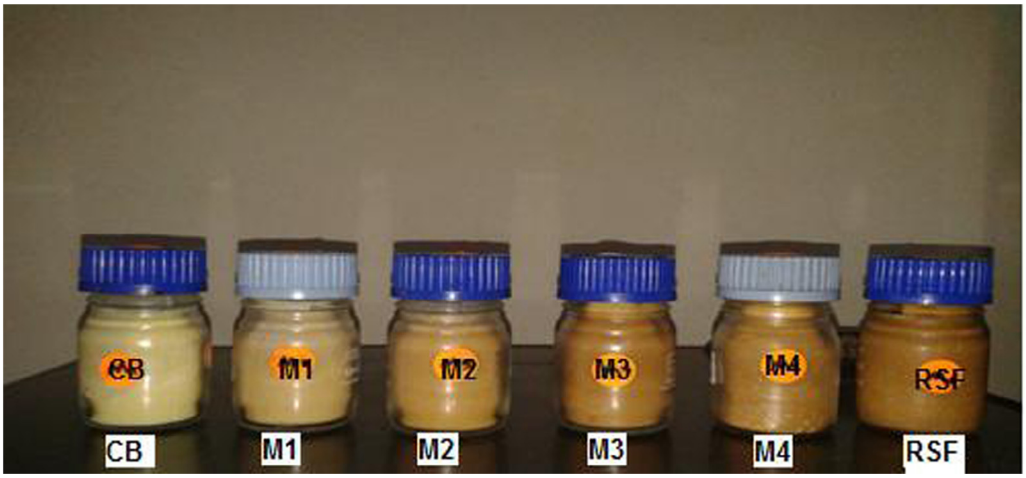

Fig. 1. Photograph of extracted Rambuta seed fat.

Table 1
List of proportions of the RSF and Cocoa Butter.

Mixtures of CB & RSF	CB%[a]	RSF%[b]
Mixture 1 (CB)	100	0
Mixture 2 (M1)	80	20
Mixture 3 (M2)	60	40
Mixture 4 (M3)	40	60
Mixture 5 (M4)	20	80
Mixture 6 (RSF)	0	100

[a] CB% = proportion of cocoa butter.

[b] RSF% = proportion of rambutan seed fat.

(trimethylsilyl) acetate (0.54%). Whereas, the majority ester compound was found in rambutan seed fat, Hexadecanoic acid, ethyl ester (1.30%), 9-Octadecenoic acid, methyl ester (1.34%) and Ethyl Oleate (5.29%). Meanwhile, these ester compounds were also found in cocoa butter in lower proportions than RSF around (0.45%, 1.31%, and 0.76%) respectively, and provide the same ester flavor as mentioned above. In addition, RSF has another ester compound, such as Propanoic acid, 2-methyl-, butyl ester in a range 6.55%, which also provide a fruity, sweet, floral and honey flavor (Table 2). Our results are agreed with Febrianto (2013), found that total ester compounds in fermented-roasted rambutan seed fat were 12.24% and the types of ester compounds in RSF almost same to that observed in our results. The formation of esters during fermentation process can be generated by lipid metabolism in acids and alcohols rich condition that allow the yeast undergoes esterification reactions resulted in a variety of esters (Reineccius and Henry, 2006). Thus, the succession of microorganism and enzyme activity may be responsible for this condition.

Table 2
Ester compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
1-Methoxy-2-propyl acetate	2.71 ± 0.01	2.16 ± 0.01	ND	ND	ND	ND
Butanoic acid, butyl ester	1.32 ± 0.01	1.06 ± 0.01	ND	ND	ND	ND
1-Octyl trifluoroacetate	0.66 ± 0.01	0.53 ± 0.01	ND	ND	ND	ND
Formic acid, 1-methylpropyl ester	0.31 ± 0.02	0.33 ± 0.01	0.36 ± 0.01	0.38 ± 0.01	0.40 ± 0.01	0.43 ± 0.02
Pentadecanoic acid, 14-methyl-, methyl ester	0.40 ± 0.01	0.32 ± 0.01	0.13 ± 0.00	ND	ND	ND
Hexadecanoic acid, ethyl ester	0.45 ± 0.01	0.62 ± 0.01	0.79 ± 0.01	0.96 ± 0.00	1.13 ± 0.01	1.30 ± 0.03
9-Octadecenoic acid, methyl ester	1.31 ± 0.02	1.32 ± 0.01	1.32 ± 0.01	1.33 ± 0.00	1.33 ± 0.00	1.34 ± 0.03
Ethyl Oleate	0.76 ± 0.02	1.67 ± 0.01	2.57 ± 0.01	3.48 ± 0.01	4.38 ± 0.01	5.29 ± 0.03
Succinic acid, isohexyl octyl ester	0.53 ± 0.01	0.50 ± 0.02	ND	ND	ND	ND
Ethyl (trimethylsilyl)acetate	0.54 ± 0.01	0.50 ± 0.01	ND	ND	ND	ND
Heptanoic acid, ethyl ester	0.70 ± 0.01	0.14 ± 0.00	ND	ND	ND	ND
Chloroacetic acid, dodecyl ester	0.40 ± 0.00	0.08 ± 0.00	ND	ND	ND	ND
Propanoic acid, 2-methyl-, butyl ester	ND	1.31 ± 0.00	2.62 ± 0.01	3.93 ± 0.01	5.24 ± 0.01	6.55 ± 0.02
Acetic acid trichloro- nonyl ester	ND	ND	0.66 ± 0.02	ND	ND	ND
3-Hydroxymandelic acid, ethyl ester	ND	ND	0.97 ± 0.00	1.63	ND	ND
Sulfurous acid, dodecyl 2-propyl ester	ND	ND	0.23 ± 0.00	ND	ND	ND
Ethyl 3,3-dimethylbutyrate	ND	ND	0.27 ± 0.01	0.28 ± 0.01	0.37 ± 0.01	0.46 ± 0.01
Sulfurous acid, decyl hexyl ester	ND	ND	0.28 ± 0.01	ND	ND	ND
Propanedioic acid, bis (trimethylsilyl) ester	ND	ND	0.31 ± 0.01	ND	ND	ND
Hexadecanoic acid, methyl ester	ND	ND	0.18 ± 0.01	0.27 ± 0.00	0.36 ± 0.01	0.45 ± 0.01
Dibutyl phthalate	ND	ND	0.11 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.28 ± 0.00
Fumaric acid, heptyl 3-methylbut-3-enyl	ND	ND	ND	ND	0.25 ± 0.00	ND
Ester	ND	ND	ND	ND	0.04 ± 0.00	ND
Heptacosyl acetate	ND	ND	ND	ND	0.31 ± 0.01	ND
13-Tetradecen-1-ol acetate	ND	ND	ND	ND	0.13 ± 0.00	ND
2-propenoic acid 3-(4-methoxyphenyl)- ethyl ester	ND	ND		ND	0.03 ± 0.00	ND
3-Methyl-2-butenoic acid, nonyl ester Others	0.50 ± 0.01	0.47 ± 0.00	0.46 ± 0.01	0.43 ± 0.00	0.41 ± 0.01	0.39 ± 0.02
Total esters	10.59 ± 0.01	11.01 ± 0.01	11.26 ± 0.01	12.86 ± 0.00	14.60 ± 0.01	16.49 ± 0.02

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

3.2. Alcohol compounds

The occurrence of alcohol compounds in food product commonly generates sweet, fruity, alcoholic, balsamic and green flavor and sensation. However, it also depends on its molecular structure (Curioni and Bosset, 2002). With regard to alcohol compounds, our analysis resulted in different alcohol compounds in rambutan seed fat and its mixture with cocoa butter. The major alcohol content in RSF and CB samples was mostly contributed by 2,3-Butanediol (7.94% and 7.06%) and Phenol (0.68% and 0.53%). Whereas, total alcohol contributed in the range of 10.03%–23.15% of the total flavor of CB, M1, M2, M3, M4 and RSF mixture (Table 3). Our results showed that M1 similar to that observed of CB in the alcohol compound as well as RSF sample was very close to the CB in terms of the main alcohols content. While the RSF mixture significantly higher than that observed of CB in total alcoholic compounds due to RSF sample has other alcoholic compounds was absent in CB sample. The 2,3-Butanediol compound not only directly important flavour contribution in RSF, but also there is phenylethyl alcohol (2.48%) is an aromatic alcohol, which provided floral, rose and honey sensation, whereas 1-Octanol compound (1.95%) also contributed in RSF the green, moldy or meaty flavour (Shahidi and Wanasundara, 2008). This shows that RSF sample contains alcoholic flavor stronger than those in the CB. 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. The 2,3-butanediol compound has been reported to be generated during fermentation involving Saccharomyces cerevisiae by the activity of butanediol dehydrogenase (Ng et al., 2012).

3.3. Hydrocarbon compounds

Hydrocarbon compounds of rambutan seed fat and the mixture with cocoa butter flavor are shown in Table 4. Total hydrocarbon compounds were observed to contribute in the range of 6.11% (CB) to 17.65% (RSF) of total RSF and its mixture with cocoa butter flavor. The Major hydrocarbon compounds observed in CB and RSF sample were Cyclotrisiloxane, hexamethyl-(1.91% and 2.04%) and 2-Cyclopenten-1-one, 3,5,5-trimethyl-(0.99% and 3.65%), whereas RSF also has other hydrocarbon compounds, such as Decane, 3,6-dimethyl-(0.80%) and Decane, 3,8-dimethyl-(0.85%), which give the perception of green and rose-like flavour (Maarse, 1991). Thus, the RSF sample has total hydrocarbon content (17.65%) higher than those noted in the CB sample (6.11%). Meanwhile, the total hydrocarbon compound that found in M1 (8.01%) higher than those in CB sample, while maintaining the same major hydrocarbon content, but in higher concentration than those found in the CB. Hydrocarbon compounds have been reported to be an important flavor constituent in food products. Hydrocarbons such as sesquiterpenes were commonly identified in tea. Total hydrocarbons observed in rambutan seed fat decreased after the application of fermentation and roasting treatment (Febrianto, 2013). The decrease of hydrocarbon during fermentation process may have occurred due to the utilization of hydrocarbon compounds as a carbon source by microorganism during fermentation (Doshi et al., 2010). Furthermore, hydrocarbon compounds were identified and observed decreasing trend with extending roasting temperature. The highest concentration of hydrocarbon content found in raw cocoa bean and the lowest in roasted cocoa bean (Zzaman et al., 2014).

3.3.1. Carboxylic acid compounds

Further, 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). Our result presented in Table 5, shows that acetic acid was the main constituent of carboxylic acid that contributes from 0.30%–8.31% and from total carboxylic acids 9.43%–23.14% to the RSF and its mixture with CB flavor. The proportion of acetic acid, as well as total carboxylic acid, fluctuated during the fermentation process, but in general, its proportion was decreased after the roasting process (Febrianto, 2013).

As shown in Table 5, butanoic acid, 3-methyl- (2.68% and 2.50%), Hexanoic acid, 2-methyl-(0.93% and 0.80%), Octanoic Acid (0.33% and 0.31%) and Pentanoic acid (3.30% and 4.95%) were present in fermented-roasted CB and RSF sample. The results reported that RSF has a higher concentration of the carboxylic acid compound than other mixtures, especially CB sample. Whereas M1 was more similar to that of CB in major carboxylic acid compound than M2, M3, M4 respectively.

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). A most 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 side, the formation of several acids during roasting could occur from the degradation of carbohydrate as reported previously by Ginz et al. (2000).

Table 3
Alcohol compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
2,3-Butanediol	7.06 ± 0.01	7.24 ± 0.01	7.41 ± 0.01	7.59 ± 0.01	7.74 ± 0.00	7.94 ± 0.03
Phenol	0.53 ± 0.01	0.56 ± 0.01	0.59 ± 0.01	0.64 ± 0.01	0.65 ± 0.01	0.68 ± 0.01
Cyclohexanol, 3,5-dimethyl-	0.76 ± 0.00	ND	ND	ND	ND	ND
Fluoren-9-ol,3,6-dimethoxy-9-(2-phenylethenyl)-	0.95 ± 0.00	0.76 ± 0.03	0.57 ± 0.01	0.38 ± 0.00	0.19 ± 0.00	ND
Ethanol, 2-(1-methylethoxy)-	ND	0.16 ± 0.04	0.32 ± 0.01	0.47 ± 0.01	0.63 ± 0.01	0.79 ± 0.03
1-Octanol	ND	0.39 ± 0.04	0.78 ± 0.01	1.17 ± 0.01	1.56 ± 0.01	1.95 ± 0.04
1-Pentanol	ND	0.74 ± 0.01	1.48 ± 0.01	2.22 ± 0.01	2.96 ± 0.01	3.70 ± 0.03
1-Hexanol	ND	0.86 ± 0.01	1.71 ± 0.01	2.57 ± 0.01	3.42 ± 0.01	4.28 ± 0.02
1-Hexanol, 2-ethyl-	ND	ND	0.67 ± 0.01	ND	ND	ND
Phenylethyl Alcohol	ND	ND	0.99 ± 0.01	1.49 ± 0.01	1.98 ± 0.01	2.48 ± 0.01
Phenol44′-(1-methylethylidene)bis-	ND	ND	0.69 ± 0.01	ND	ND	ND
1-Heptanol, 6-methyl-	ND	ND	ND	0.20 ± 0.01	ND	ND
2-Naphthalenol, 3-methoxy-	ND	ND	ND	ND	0.04 ± 0.01	0.49 ± 0.03
5-(1-bromo-1-methylethyl)-2-methyl-cyclohexanol	ND	ND	ND	ND	1.78 ± 0.00	ND
1-Heptanol	ND	ND	ND	ND	ND	0.37 ± 0.03
Others	0.73 ± 0.00	0.68 ± 0.01	0.63 ± 0.01	0.57 ± 0.01	0.52 ± 0.00	0.47 ± 0.01
Total alcohols	10.03 ± 0.01	11.39 ± 0.03	15.84 ± 0.01	17.30 ± 0.01	21.47 ± 0.00	23.15 ± 0.03

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

Table 4
Hydrocarbon compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
Cyclotrisiloxane, hexamethyl-	1.91 ± 0.00	1.94 ± 0.00	1.96 ± 0.01	1.99 ± 0.01	2.01 ± 0.01	2.04 ± 0.00
Cyclohexane,1(cyclohexylmethyl) -2-methyl-, trans-	0.35 ± 0.00	0.28 ± 0.01	ND	ND	ND	ND
2-Ethoxyethoxy-trimethylsilane	1.10 ± 0.00	0.88 ± 0.01	0.66 ± 0.01	0.44 ± 0.01	0.22 ± 0.01	ND
2-Cyclopenten-1-one,3,5,5-trimethyl-	0.99 ± 0.00	1.52 ± 0.00	2.05 ± 0.01	2.59 ± 0.01	3.12 ± 0.01	3.65 ± 0.05
Cyclohexane,1-methyl-3-(1-methylethenyl)-	0.31 ± 0.01	0.25 ± 0.01	ND	ND	ND	ND
Dodecane	0.68 ± 0.00	0.60 ± 0.01	0.53 ± 0.00	0.45 ± 0.20	0.38 ± 0.01	0.30 ± 0.04
Benzene,1-methyl-4 (phenylmethyl)-	0.35 ± 0.00	0.28 ± 0.01	0.21 ± 0.01	0.14 ± 0.01	ND	ND
Cyclotetrasiloxane, octamethyl-	ND	ND	0.58 ± 0.00	1.56 ± 0.20	ND	ND
Benzene,1-methyl-4-(1-methylethyl)-	ND	ND	1.26 ± 0.02	ND	ND	ND
Decane, 4-ethyl-	ND	0.71 ± 0.01	1.41 ± 0.02	2.12 ± 0.01	2.82 ± 0.01	3.53 ± 0.02
Decane, 3,6-dimethyl-	ND	0.16 ± 0.01	0.32 ± 0.00	0.48 ± 0.01	0.64 ± 0.01	0.80 ± 0.02
Tridecane	ND	0.15 ± 0.01	0.30 ± 0.01	0.44 ± 0.01	0.59 ± 0.01	0.74 ± 0.01
Tetradecane	ND	0.27 ± 0.01	0.54 ± 0.01	0.81 ± 0.01	1.08 ± 0.02	1.35 ± 0.01
Decane, 3,8-dimethyl-	ND	ND	0.34 ± 0.00	0.51 ± 0.01	0.68 ± 0.01	0.85 ± 0.00
Oxalic acid, hexylneopentyl ester	ND	ND	0.37 ± 0.01	ND	ND	ND
3,6,9,12,15-Pentaoxanonadecan-1-ol	ND	0.40 ± 0.01	0.79 ± 0.01	1.19 ± 0.00	1.58 ± 0.01	1.98 ± 0.02
Undecane	ND	0.08 ± 0.01	0.16 ± 0.01	0.25 ± 0.00	0.33 ± 0.01	0.41 ± 0.02
2,5,8,11,14-Pentaoxahexadecan-16-ol	ND	ND	ND	ND	0.21 ± 0.10	ND
1 4-cyclohexadiene 1-methyl-4-(1-methylethyl)-	ND	ND	ND	ND	0.44 ± 0.00	0.55 ± 0.02
Cyclohexadecane,1,2-diethyl-	ND	ND	ND	ND	0.13 ± 0.00	ND
Octadecane	ND	ND	ND	ND	0.86 ± 0.01	ND
2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,1	ND	ND	ND	0.41 ± 0.01	0.55 ± 0.01	0.69 ± 0.01
Others	0.42 ± 0.00	0.49 ± 0.00	0.56 ± 0.01	0.62 ± 0.01	0.69 ± 0.01	0.76 ± 0.02
Total hydrocarbons	6.11 ± 0.00	8.01 ± 0.00	12.04 ± 0.01	14.00 ± 0.10	16.33 ± 0.03	17.65 ± 0.02

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

Table 5
Carboxylic acid compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
Butanoic acid, 3-methyl-	2.68 ± 0.01	2.64 ± 0.02	2.61 ± 0.02	2.57 ± 0.00	2.54 ± 0.01	2.50 ± 0.01
Hexanoic acid, 2-methyl-	0.93 ± 0.02	0.90 ± 0.01	0.88 ± 0.01	0.85 ± 0.01	0.83 ± 0.01	0.80 ± 0.01
Dodecanoic acid	0.33 ± 0.02	0.44 ± 0.01	0.55 ± 0.02	0.65 ± 0.01	0.76 ± 0.01	0.87 ± 0.00
Octanoic Acid	0.33 ± 0.00	0.33 ± 0.01	0.32 ± 0.00	0.32 ± 0.01	0.31 ± 0.01	0.31 ± 0.00
Tetradecanoic acid	0.56 ± 0.01	0.51 ± 0.01	0.46 ± 0.00	0.42 ± 0.00	0.37 ± 0.01	0.32 ± 0.00
Pentanoic acid	3.30 ± 0.02	3.63 ± 0.01	3.96 ± 0.02	4.29 ± 0.01	4.62 ± 0.01	4.95 ± 0.02
Acetic acid	1.30 ± 0.02	2.70 ± 0.01	4.10 ± 0.01	5.51 ± 0.01	6.91 ± 0.04	8.31 ± 0.00
Heptanoic acid	ND	0.32 ± 0.01	0.64 ± 0.02	0.96 ± 0.01	1.28 ± 0.01	1.60 ± 0.02
Oleic Acid	ND	ND	0.23 ± 0.01	0.89 ± 0.00	ND	ND
n-Hexadecanoic acid	ND	ND	3.12 ± 0.00	1.61 ± 0.02	0.84 ± 0.04	ND
Nonanoic acid	ND	ND	ND	0.07 ± 0.01	ND	ND
2-Butenoic acid,2-methyl-	ND	ND	ND	ND	0.32 ± 0.03	ND
Propanoic acid, 2-methyl-	ND	ND	0.41 ± 0.01	0.61 ± 0.01	0.82 ± 0.01	1.02 ± 0.01
Benzeneacetic acid	ND	ND	ND	ND	ND	0.52 ± 0.02
Octadecanoic acid	ND	ND	ND	ND	ND	0.59 ± 0.00
Others	ND	ND	0.54 ± 0.01	0.81 ± 0.01	1.08 ± 0.03	1.35 ± 0.00
Total acids	9.43 ± 0.02	11.47 ± 0.01	17.82 ± 0.01	19.56 ± 0.00	20.68 ± 0.02	23.14 ± 0.01

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

3.3.2. Aldehyde compounds

The aldehyde compounds are commonly found in tea, and become the main flavor constituent in peach, almond, apricot, plum and cherry (Doyle et al., 2001). Our analysis of flavor compounds resulted in different aldehyde compounds in CB, M1, M2, M3, M4 and RSF sample (Table 6). Benzaldehyde, Nonanal, Pentanal, 1H-Pyrrole-2-carboxaldehyde, 1-methyl, Decanal, Benzeneacetaldehyde, alpha.-ethylidene, 5-Methyl-2-phenyl-2-hexenal and 2-furancarboxaldehyde 5-(hydroxymethyl) were most abundant in CB and RSF sample. Benzaldehyde has been reported to be the main flavor constituent in RSF and CB sample in the range of (0.63% and 0.56%), which gives them a bitter and almond-like perception. Whereas, nonanal in RSF (2.22%) and CB (4.20%) has been reported to give floral aroma, and also 5-Methyl-2-phenyl-2-hexenal in RSF (1.39%) and CB (0.43%) is the main aroma constituent giving fruity sensation (Berger, 2007).

On the other hand, Pentanal in RSF sample (6.30%) was reported to possess flavor perception, such as strong, acid, pungent and also contribute to chocolate&nut-like flavor (Maarse, 1991). Through the results show that RSF sample has a total aldehyde flavor (14.41%) than CB (8.69%). Hence, can be explained the potential of application RSF as confectionery ingredients and alternative of CB (Issara et al., 2014).

When the fermentation was implemented, the proportion of total aldehyde in RSF was decreased, but the proportion of it increased again during the roasting process. During the fermentation process, the decrease of total aldehyde proportion in the RSF may be due to the production of several aldehyde-degrading enzymes such as aldehyde dehydrogenase and aldehyde reductase (from Saccharomyces cerevisiae) (Febrianto, 2013). Wherein, aldehyde reductase could generate alcohol compounds from an aldehyde, and aldehyde dehydrogenase alters the aldehyde into carboxylic acids (Pigeau and Inglis, 2007; Jordan et al., 2011). Furthermore, the occurrence of Maillard non-enzymatic reaction also a possible increase of total aldehyde in roasted samples due to its generation from the oxidation of unsaturated fatty acids during

Table 6
Aldehyde compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
Heptanal	0.48 ± 0.01	0.38 ± 0.01	ND	ND	ND	ND
Benzaldehyde	0.56 ± 0.01	0.57 ± 0.01	0.59 ± 0.00	0.60 ± 0.01	0.62 ± 0.01	0.63 ± 0.01
1H-Pyrrole-2-carboxaldehyde	0.57 ± 0.01	0.46 ± 0.01	0.34 ± 0.00	0.23 ± 0.01	0.11 ± 0.03	ND
Nonanal	4.20 ± 0.02	3.80 ± 0.02	3.41 ± 0.03	3.01 ± 0.01	2.62 ± 0.03	2.22 ± 0.02
1H-Pyrrole-2-carboxaldehyde, 1-methyl-	0.72 ± 0.02	0.78 ± 0.02	0.84 ± 0.01	0.90 ± 0.02	0.96 ± 0.04	1.02 ± 0.04
Decanal	0.67 ± 0.02	0.63 ± 0.03	0.58 ± 0.03	0.54 ± 0.00	0.50 ± 0.01	0.46 ± 0.00
Benzeneacetaldehyde,alpha.-ethylidene-	0.79 ± 0.01	0.85 ± 0.01	0.92 ± 0.00	0.98 ± 0.00	1.05 ± 0.00	1.11 ± 0.01
5-Methyl-2-phenyl-2-hexenal	0.43 ± 0.01	0.62 ± 0.01	0.81 ± 0.01	1.01 ± 0.00	1.20 ± 0.01	1.39 ± 0.05
2-furancarboxaldehyde5-(hydroxymethyl)-	0.25 ± 0.00	0.20 ± 0.01	ND	ND	ND	ND
Pentanal	ND	1.26 ± 0.01	2.52 ± 0.02	3.78 ± 0.01	5.04 ± 0.01	6.30 ± 0.05
Dodecanal	ND	ND	0.22 ± 0.00	0.08 ± 0.01	0.70 ± 0.01	ND
2-furancarboxaldehyde5-(hydroxymethyl)-	ND	ND	ND	ND	ND	0.38 ± 0.00
2-Decenal, (Z)-	ND	ND	0.29 ± 0.00	ND	ND	ND
Tetradecanal	ND	ND	ND	ND	ND	ND
Hexadecanal	ND	ND	0.49 ± 0.01	ND	ND	ND
Others	0.29 ± 0.02	0.41 ± 0.03	0.53 ± 0.02	0.66 ± 0.00	0.78 ± 0.05	0.90 ± 0.02
Total aldehydes	8.96 ± 0.01	9.96 ± 0.02	11.54 ± 0.01	11.79 ± 0.00	13.58 ± 0.03	14.41 ± 0.04

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

the roasting process (Shahidi and Wanasundara, 2008; Berger, 2007).

3.3.3. Ketones compounds

With regards to ketones, major ketone compounds, such as 2-Cyclopenten-1-one, 2-hydroxy-3-methyl (0.37%–0.50%), Ethanone, 1-(1H-pyrrol-2-yl) (0.95%–1.05%), Maltol (2.01%–2.61%), 7-Hydroxy-1-indanone (0.11%–1.00%), Propanone-1, 3-(4-methylpiperazinyl) (1.06%–5.29%) and (3H)-Furanone, 5-heptyldihydro (0.50%–0.53%) were detected in CB, M1, M2, M3, M4 and RSF mixture (Table 7). Basically, saturated ketones give a fruity, cheesy and fatty perception, whereas diketone contribute importantly in coffee due to its perception of sweet, buttery and caramel flavor. However, ketones contributed less compared to other flavor compounds. It was only contributed in the range of 11.20% (CB) to 12.20% (RSF) with similar trend RSF sample to CB in total highest area percent of ketonic compounds found at 11.21% followed by M1 at 11.65%, respectively.

According to Pigeau and Inglis (2007) and Jordan et al. (2011), ketone concentration can be changed during the fermentation process due to the occurrence of aldehyde reductase or alcohol dehydrogenase enzymes that provide alteration between ketones and secondary alcohol. Nevertheless, it is also stated that ketones can be formed from microbial-induced lipid oxidation by the activity of lipases and lipoxidase-like activity (Reineccius and Henry, 2006). On the other hand, the increasing trend of ketonic compounds observed with increased temperature and time during the roasting process of the cocoa bean because of the occurrence of Millard non-enzymatic reaction or also possible to be generated from the oxidation reaction (Zzaman and Yang, 2013).

3.3.4. Pyrazine compounds

The occurrence of some pyrazine compounds in food product is desirable due to its contribution to certain taste and flavor such as musty, nutty, roasted-nuts, chocolate-like (methylpyrazine); roasted meat like, caramel, nutty, vanilla, green, 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). 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

Table 7
Ketone compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
2-Cyclopenten-1-one,2-hydroxy-3-methyl-	0.50 ± 0.03	0.47 ± 0.01	0.45 ± 0.01	0.42 ± 0.01	0.40 ± 0.01	0.37 ± 0.02
Ethanone,1-(1H-pyrrol-2-yl)-	1.05 ± 0.02	1.03 ± 0.02	1.01 ± 0.02	0.99 ± 0.01	0.97 ± 0.01	0.95 ± 0.01
Maltol	2.01 ± 0.02	1.69 ± 0.01	1.85 ± 0.01	1.77 ± 0.01	1.69 ± 0.02	1.61 ± 0.02
7-Hydroxy-1-indanone	1.00 ± 0.01	0.82 ± 0.01	0.64 ± 0.01	0.47 ± 0.01	0.29 ± 0.01	0.11 ± 0.01
Propanone-1,3-(4-methylpiperazinyl)-	5.29 ± 0.01	4.23 ± 0.01	3.17 ± 0.01	2.12 ± 0.01	1.06 ± 0.01	ND
2(3H)-Furanone,5-heptyldihydro-	0.53 ± 0.03	0.52 ± 0.01	0.52 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.02
Pantolactone	ND	0.16 ± 0.01	0.31 ± 0.01	0.47 ± 0.01	0.62 ± 0.01	0.78 ± 0.02
2-Acetyl-5-methylfuran	ND	1.93 ± 0.04	ND	0.19 ± 0.02	0.10 ± 0.01	ND
2,5-Furandione,3,4-dimethyl-	ND	ND	0.36 ± 0.00	ND	ND	ND
Ethanone, 1-cyclopentyl-	ND	ND	0.79 ± 0.00	ND	ND	ND
4(1H)-Pyrimidinone,6-hydroxy-	ND	ND	0.47 ± 0.00	ND	ND	ND
Caffeine	ND	ND	1.61 ± 0.00	0.16 ± 0.02	ND	ND
3-Hydroxy-3-methyl-2-butanone	ND	ND	ND	ND	0.20 ± 0.01	ND
4-dimethylamino-3-methyl-2-butanone	ND	ND	ND	ND	0.09 ± 0.01	ND
1-Pentene	ND	ND	ND	3.34 ± 0.01	4.45 ± 0.01	5.56 ± 0.01
4-Pyranone, 2,3-dihydro-	ND	ND	ND	0.34 ± 0.01	0.45 ± 0.01	0.56 ± 0.01
2(3H)-Furanone,dihydro-5-pentyl-	ND	ND	ND	0.47 ± 0.01	0.62 ± 0.01	0.78 ± 0.01
2-Butanone, 3-(phenylthio)-	ND	ND	ND	ND	ND	0.32 ± 0.02
Others	0.83 ± 0.04	0.80 ± 0.01	0.76 ± 0.00	0.73 ± 0.02	0.69 ± 0.01	0.66 ± 0.01
Total ketones	11.20 ± 0.02	11.65 ± 0.02	11.94 ± 0.00	11.98 ± 0.02	12.14 ± 0.01	12.20 ± 0.01

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

Table 8
Pyrazine compounds identified in RSF and its mixtures with CB.

Compounds	CB	M1	M2	M3	M4	RSF
Pyrazine, 2,6-dimethyl-	1.42 ± 0.02	2.01 ± 0.02	2.60 ± 0.01	3.19 ± 0.00	3.78 ± 0.01	4.37 ± 0.01
Pyrazine, 2-ethyl-6-methyl-	0.67 ± 0.01	0.62 ± 0.01	0.54 ± 0.01	0.53 ± 0.01	0.48 ± 0.01	0.43 ± 0.08
Pyrazine, trimethyl-	5.10 ± 0.02	5.22 ± 0.01	5.34 ± 0.01	5.47 ± 0.01	5.59 ± 0.01	5.71 ± 0.01
Pyrazine, tetramethyl-	14.34 ± 0.31	14.72 ± 0.02	15.10 ± 0.12	15.48 ± 0.05	15.86 ± 0.02	16.24 ± 0.22
2-Acetyl-3,5-dimethylpyrazine	0.56 ± 0.02	0.45 ± 0.01	0.34 ± 0.01	ND	ND	ND
2-Isoamyl-6-methylpyrazine	0.34 ± 0.02	0.34 ± 0.02	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.34 ± 0.02
Pyrazine, 2-butyl-3,5-dimethyl-	0.34 ± 0.01	0.27 ± 0.01	ND	ND	ND	ND
Pyrazine, 3,5-diethyl-2-methyl-	1.60 ± 0.05	1.36 ± 0.01	1.12 ± 0.01	0.88 ± 0.01	0.64 ± 0.02	0.40 ± 0.00
Pyrazine,2,5-dimethyl-3-(3-methylbutyl)-	ND	ND	ND	0.16 ± 0.00	0.38 ± 0.04	0.48 ± 0.00
2,3,5-Trimethyl-6-ethylpyrazine	ND	ND	ND	ND	0.32 ± 0.01	0.40 ± 0.00
Pyrazine, 3-ethyl-2,5-dimethyl-	ND	0.08 ± 0.01	0.16 ± 0.01	0.24 ± 0.01	0.32 ± 0.01	0.40 ± 0.04
2,3-Dimethyl-5-ethylpyrazine	0.79 ± 0.01	0.63 ± 0.01	0.47 ± 0.01	ND	ND	ND
Others	0.78 ± 0.01	0.75 ± 0.00	0.71 ± 0.04	0.68 ± 0.02	0.64 ± 0.01	0.61 ± 0.01
Total pyrazine	25.94 ± 0.10	26.45 ± 0.02	26.72 ± 0.05	26.97 ± 0.01	28.35 ± 0.01	29.38 ± 0.04

The data presented is in% area of GC chromatogram. (CB = Cocoa butter; M1 = Mixture 1; M2 = Mixture 2; M2 = Mixture 3; M4 = Mixture 4; RSF = Rambutan seed fat.)

(94 compounds), are found in cocoa. The total pyrazine estimated in raw cocoa bean (unroasted) lower than a fermented roasted cocoa bean. The total pyrazine concentration significantly ($p < 0.05$) increased as extending roasting time (Zzaman, 2015).

The total number of pyrazine compounds in rambutan seed fat and its mixture with cocoa butter is presented in Table 8. There were different pyrazines detected by Solid phase micro extraction (SPME)- Gas chromatography-Mass spectrophotometry (GC–MS). The most important identified pyrazine in CB and M1 were Pyrazine, 2,6-dimethyl (1.42% and 2.01%), Pyrazine, 2-ethyl-6-methyl (0.67% and 0.62%), Pyrazine, trimethyl (5.10% and 5.22%), Pyrazine, tetramethyl (14.34% and 14.72%), 2-Acetyl-3,5-dimethylpyrazine (0.56% and 0.45%), 2-Isoamyl-6-methylpyrazine (0.34% and 0.34%) and Pyrazine, 2-butyl-3,5-dimethyl (0.34% and 0.27%). Whereas, Our identification of pyrazine compounds in the RSF sample was almost similar to that observed in CB and M1 with different proportion, such as Pyrazine, 2,6-dimethyl, Pyrazine, 2-ethyl-6-methyl, Pyrazine, trimethyl, Pyrazine, tetramethyl and 2-Isoamyl-6-methylpyrazine, which contribute in the range 4.37%, 0.43%, 5.71%, 16.24% and 0.34%, respectively. Besides, the Pyrazine, 3-ethyl-2,5-dimethyl (0.40%), which also provide the roasted nuts, peanut and coffee flavor to the RSF sample.

With regard the formation of pyrazine during the fermentation process, the activity of several microorganisms such as Bacillus subtilis and Bacillus megatrium could generate the formation of pyrazine compound during cocoa fermentation (Afoakwa et al., 2013). As shown in Table 6, the roasting process increased total pyrazine proportion in CB (25.94%) and RSF (29.38%), which made pyrazine flavor the most dominant flavor compound in fermented-roasted cocoa butter and rambutan seed fat. However, the pyrazine percentage in the roasted sample decreased after six days fermentation. This result indicates that sufficient fermentation of cocoa bean and rambutan seed effectively generated Maillard reaction precursors such as amino acid and reducing sugars, which then converted into pyrazine compounds during the roasting process. But, according to Febrianto (2013), he reported that long time of fermentation will decrease the pyrazines concentration instead. Therefore, our results show that six days fermentation of the cocoa bean and rambutan seed is sufficient for producing cocoa butter and rambutan seed fat with a high concentration of pyrazines.

4. Conclusion

From the results obtained, it is useful for a basis to study flavor compounds of rambutan seed fat before use to alternative cocoa butter. Our analysis of the flavor of rambutan seed fat and cocoa butter successfully identified seven kinds of flavor compounds were found in our mixtures including ester, alcohol, hydrocarbon, carboxylic acid, aldehyde, ketone, and pyrazine compound. The highest concentration of ester, alcohol, hydrocarbon, carboxylic acid, aldehyde and ketone flavour were observed to have a higher contribution to the RSF flavour in the range of 16.49%, 23.15%, 17.65%, 23.14%, 14.41% and 12.20%, respectively followed by CB 10.59%, 10.03%, 6.11%, 9.43%, 8.96% and 11.20%, respectively. Our analysis of the pyrazine group showed the highest pyrazines content was observed at RSF followed by CB (29.38% and 25.94%, respectively). Whereas, M1 showed (26.45%) lower pyrazine flavor than the mixtures M2, M3, and M4 with slight higher to those of the pyrazine content in CB, but similar to major pyrazine compound to the CB sample. This result indicated that six days fermentation of rambutan seed and cocoa bean can be considered as optimum due to its high content of pyrazines. In addition, the roasting process of rambutan seed and cocoa bean was able to achieve desirable flavor characteristics. In conclusion, Up to 20% rambutan, seed fat can be used with cocoa butter as a potential ratio of CB and RSF for the application in chocolate manufacturing. The study concluded that rambutan seed fat can be used as a cocoa butter alternative to improve and get the best quality and aroma in products. Further study can be performed for fat crystallization and melting properties to know behavior of rambutan seed fat.

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