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Study on Color and Antioxidant Properties of Rambutan Seed Fat as Cocoa Butter Alternative

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***Abstract*— In this paper, the color (whiteness, L*, a*, b*) and antioxidant properties (radical scavenging activity, total phenolic compound) of rambutan seed fat (RSF) and its mixture with cocoa butter (CB) were investigated. Different proportions were applied in preparing the samples between (RSF) and (CB). The results showed that significant differences among samples in the whiteness, (L*) and (a*) value, whereas (b*) value had no significant differences. With regard antioxidant activity the results showed that total phenolic compound (TPC) of cocoa butter was (47.37±0.02) mg GA/100 g fat, while the other mixtures between (RSF) and (CB) showed (40.49±0.01-11.12±0.02) mg GA/100 g fat. Radical scavenging activity (DPPH) of cocoa butter valued (67.32±0.44) µmol trolox/100 g fat, similar to the mixture M1 (60.16±0.23). Based on the results the study recommended that mixture ratio up to 40% rambutan seed fat (RSF) can benefit as a cocoa butter replacer whereas a higher ratio completely change original cocoa butter characteristics. Thus, there is the possibility of using the (RSF) as replacer of (CB) and could utilize by chocolate products.**

***Keywords*— cocoa butter alternative, color, total phenolic compound, rambutan seed fat, radical scavenging activity.**

I. INTRODUCTION

Among various available types of fats for confectionery ingredient, cocoa butter is one of the most important popular cocoa products [1] and a valuable fat due to its physical and chemical characteristics [2]. Cocoa butter (CB) contains a lot of important fatty acids such as palmitic acid (C_{16}), stearic acid ($C_{18:0}$) and oleic acid ($C_{18:1}$) with a trail amount of lauric acid (C_{12}) and Myristic acid (C_{14}) [3]− [4]. CB is solid at room temperature and almost liquid at body temperature (~37) [5]. Thus, the use of cocoa butter in chocolate based confections has been extensively discussed [6]− [7]. Nowadays; the cocoa butter price is increasing continue due to a few countries cultivated, supplier of CB can be unstable [8]−[9]. So that, researchers have tried to look for alternative fats to replace cocoa butter, but be available and cheap price. Rambutan (*Nephelium lappaceum L.*) is one variety of the tropical fruit in Southeast Asian countries, especially in West Malaysia and Sumatra, they are usually eaten fresh. During processing, rambutan are peeling off and removed seeds which remain as a wasted by- product of the canned fruit industry Fig. 1. There are potential for exploitation of seed by-products [10]. Rambutan seed used as a natural, sustainable source of fat that "waste products" could provide the seed fat in the pharmaceutical and food industry with a new source of edible fat [11]. The latest research carried out by [12]− [13] 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. With regard food industry, Color in many food products including cocoa butter (CB), is a great importance [14].

This factor was estimated through many color measurement systems. Several systems for color appreciation exist and the most important one is the International Commission on Illumination. Among these systems the Hunter L, a, b system [15].These parameters are the degrees of lightness (L), redness (+a), greenness (-a), yellowness (b) and blueness (-b), these systems are one of the most used in food industry [16]. The color of cocoa

butter depends largely on the quality of cocoa beans. Upon harvesting and fermentation, cocoa beans can be normal, fully purple or molded. Cocoa butters obtained from these different types of beans vary according to the quality of the beans. Cocoa butter can also be obtained from the shell and such butter has some ideal characteristics [17].

Fig. 1 Rambutan seed

However, the concord of lipids with cocoa butter is also important side that should be carried out to measure the ability of lipids to be used as cocoa butter alternatives. Natural antioxidant such as polyphenols, carotene, tocopherol and other specific bioactive compounds had prevented lipid oxidation through the inhibiting of oxidizing reactions in the body. The foods such as fruit, vegetable and seeds contain a significant amount of the natural antioxidant component [18]–[19]. The cocoa bean is rich in phenolic and other antioxidant properties. Approximately 12-18% of phenolic compounds are reported in unfermented cocoa bean [20]. Millard non-enzymatic browning reaction products such as melanoidins which reported to possess antioxidant ability [21].The condition of coffee roasting, bread baking, and food fermentation are suitable for Millard reaction products [22]. On the other hand, the fermentation and roasting process which was applied to rambutan seed could improve the antioxidant activity and increase the total phenolic compound of rambutan seed fat (RSF) [23]. The aim of this study was to investigate color, phenolic compounds and radical scavenging activity of rambutan seed fat (RSF) and its mixture with cocoa butter (CB).

II. Materials and Methods

A. Materials

Cocoa butter (CB) was purchased from Indonesian coffee and the cocoa research institute, Jember, East Java, Indonesia. As for the raw rambutan (*Nephelium lappaceum L.*) Seeds were provided by a rambutan canning industry in Sungai Petani, Kedah, Malaysia.

B. Fermentation and roasted rambutan seeds

The rambutan seeds were transferred into plastic baskets (625 mm × 425 mm× 294mm) which were previously lined with banana leaves. After the basket was filled with raw rambutan seed, the upper part was then covered with banana leaves for 6 days. For roasted, the dried rambutan seeds were roasted at 150 °C for 30 minutes using oven-dried (AFOS Mini Kiln, Hull, England). After roasting these samples were cooled at room temperature and stored until the screw-pressing process for rambutan seed fat production [23].

C. Extraction of rambutan seed fat

Extraction of Rambutan seed fat (RSF) was implemented using a KOMET screw oil expeller DD 85 IG (IBG Monforts Oekotec GmbH & Co. KG, Germany). Dried rambutan seeds were de-husked and heated at 60 °C for 30 minutes using oven-dried (AFOS Mini Kiln, Hull, England). The screw-pressing process resulted in rambutan seed fat, which was a viscous mixture of rambutan seeds powder and (RSF). Separation of (RSF) from rambutan seed butter was done through filtration in a heated condition (60 °C). After that collected the fat of rambutan seed (RSF) and transferred into inert-screw-cap bottle [23].

D. Experimental design

Interaction experiments with the mixture between the cocoa butter and rambutan fat (not treated) were conducted according to the Table 1.

TABLE I

Experimental Mixtures Proportion Between Cocoa Butter And Rambutan Fat

Mixtures of CB & RS	CB% [a]	RSF% [b]
Mixture 1	100	0
Mixture 2	80	20
Mixture 3	60	40
Mixture 4	40	60
Mixture 5	20	80
Mixture 6	0	100

[a] CB% = proportion of cocoa butter, [b] RSF% = proportion of rambutan seed fat

E. Color measurement

CIE L* a* b* analysis of the samples was carried out using a Minolta colorimeter (CM3500d, Osaka, Japan). After calibration against white and black glass standards, the following calibration program provided by the software.The analysis result was presented in L* value for lightness, a* value for redness (+), greenness (-) and b* value for yellowness (+) and blueness (-) [24]. Whiteness were identified using the following formula (*Equation 1*):

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

F. Antioxidant activity determination

1) Methanol extracts prepared

Weighed 2.5 g of fat sample and dissolved with 5 ml n-hexane and 5 ml methanol: water mixture (60:40 v/v) after that mixed well using vortex for 1 min. The mixtures were then centrifuged at 3500 RPM for 10 min. An upper layer in which the soluble part of the solution was taken for further used analysis.

2) Total phenolic content

The total phenolic compound was measured based on the Folin and Ciocalteu's assay. The total phenol content was determined according to method with minor adjustment

described by [25]. The methanol extract (0.5 ml) was taken into a flask containing scales and then added to 0.5 ml of Folin- Ciocalteu reagent in that flask and shaken for 3 min. After that 1ml of saturated hydrous solution of sodium carbonate (Na2CO3) was transferred to same flask, after that the solution was consisted of 10 ml with adding distillate water. The solution was incubated at the 25 ^{o}C for 60 min. After the end of incubation the absorbance was read at 725 nm against reagent blank using UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-ku, Kyoto, Japan). A blank sample was prepared with reagent and distillate water. Gallic acid was used as standard to prepare a calibration curve. Per sample was analyzed in triplicate.

3) DPPH radical scavenging activity

The radical scavenging activity of the fat samples was evaluated according to the method of [26]. The analysis was performed based on the activity of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The analysis was implemented based on inhibition of the DPPH radical and the absorbance was read at 517nm using the UV-160A spectrophotometer (Shimadzu Corp., Nagakyo-ku, Kyoto, Japan). The scavenging effect was conducted based on the percentage of DPPH radical scavenging activity. Triplicate measurements were achieved for each sample. The percentage inhibition of DPPH radical was measured using the following equation with observance of DPPH solution:

$$\% \text{ scavenging } DPPH = 1 - \frac{A_s}{A_c} \times 100 \qquad (2)$$

As= Absorbance of sample
Ac= Absorbance of control

G. Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). The Duncan's new multiple range test was used to determine the differences among means at a 5% significance level.

III. Result and Discussion

A. Analysis of color formation

The color of the fat is necessary to determine a product's suitability for a special purpose. The roasting processes are controlled on the basis of color formation because brown pigment develops as browning and caramel reaction progress during roasting periods [27]. The changes in L*, a*, b* values and whiteness of rambutan seed fat (RSF) and cocoa butter (CB) at different proportion were presented in Fig 2. The implemented two-ways ANOVA indicated significantly affected the color values of cocoa butter and rambutan seed fat during roasting and mixing operations. The results showed that high significant differences ($p < 0.05$) affected in the degree of whiteness, where the highest value recorded in CB about 72.23±0.005 and the less value in RSF about 47.20±0.34, this means increasing the proportion of the RSF in the mixture will reduce the degree of the whiteness, and these results were consistent with [23] he founded the range of degrees of whiteness on fermented-roasted samples of RSF for 6 days have lower value varied from 40.31 to 46.51. Therefore, our results indicated that fermentation and roasting treatment of rambutan seed significantly changed the color of RSF to darker brownish color. The occurrence of enzymatic browning and non-enzymatic reaction could develop Brown discoloration [28], [29]−[30].

The L* value shows lightness of the roasted products and the gradual decline in the L* value was observed at increasing the proportion of RSF in the sample Fig, as the highest percentage founded in CB, while the lowest percentage was in the RSF, thus, the L* value significantly dropped from 80.19±0.005 to 58.38±0.127respectively, similar trends were observed for [31]. The brightness were proportionally reduced due to the roasting treatment of the rambutan seed and therefore lead to the degradation of the color pigment [32]−[33]. On the other hand, the denaturation of protein during roasting allow a concentrated amount of oil particles surrounded in protein matrix and low moisture content could be the reasons of lightening of cocoa butter.

The a* value shows redness of the products. The changes in the a* value during roasting in different proportion of the samples were shown in Fig 2. The results showed, that a* value conducted between CB and RSF about (1.05±0.01 - 8.43±0.03**)** respectively. The formation of brown pigments through the non-enzymatic browning and phospholipids degradation could be the reasons of the increasing of a* value. This study noted that the increase in the a* value was associated with a decrease in the L* value during the roasting process.

The b* value shows the yellowness and the variation in the b* value during roasting treatment were shown in Fig 2. As far as yellowing of the cocoa butter was anxious due to the roasting operation reduced the yellowness of the cocoa butter. Therefore, we noticed no significant difference between the cocoa butter and the other mixtures ($p<0.05$). Similar results were also reported for the b* value of roasted hazelnuts [34]. Finally, the heat treatment also affected on the pigments responsible of the yellow color.

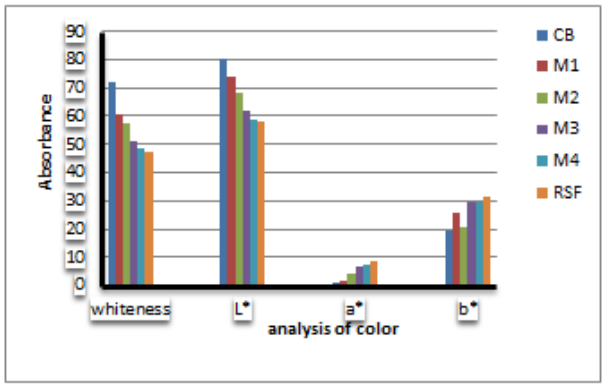

Fig 2 the changes in l*, a*, b* values and whiteness of rambutan seed fat (RSF) and its mixtures with cocoa butter (CB)

B. Total phenolic content (TPC)

Many natural oils have more polar substances that are mostly phenolics. These phenolic compounds have been recognized as natural antioxidant an widely studied by many researchers for their health promoting effects, they may exert high biological activity far beyond their antioxidant properties, e.g., anti-carcinogenic and anti-atherogenic

activities [35]. Roasting process can increase the TPC present in cocoa beans and rambutan seed due to degradation or polymerization products of the seed component during roasting, which reacts with the Folin-Ciocalteu reagent used in the analysis to produce the blue color under an alkaline condition. During the roasting Millard reaction could contribute to the increase of TPC in seeds sample and directly higher absorbance reading at 725 nm [36]. Thus, the transfer of phenolic compounds from seed to oil is of great interest. The results showed that significant increases ($p<0.05$) in the variation of the total phenolic content due to differences in the proportion of the mixture's component of CB and RSF, where the increasing (significant, $p < 0.05$) of TPC was observed in the CB 47.37±0.02 mg/g and the sharp decrease of TPC was observed in RSF 11.12±0.02 mg/g. These differences of the TPC between CB and RSF caused a gradual decline of TPC for the other mixtures (40.49±0.01, 33.75±0.03, 30.33±0.03 and 26.11±0.03) respectively Fig 3. The CB had a higher concentration of polyphenol that found in the pigment cells of the cotyledons than RSF [37], therefore this decline of the phenolic compounds in the mixtures (M1, M2, M3 and M4) due to a decrease in the proportion of CB and an increase of RSF gradually. On the other hand, the appropriate fermentation process should not longer than 6 days, which resulting efficiently increase the total phenolic compounds of RSF [23].

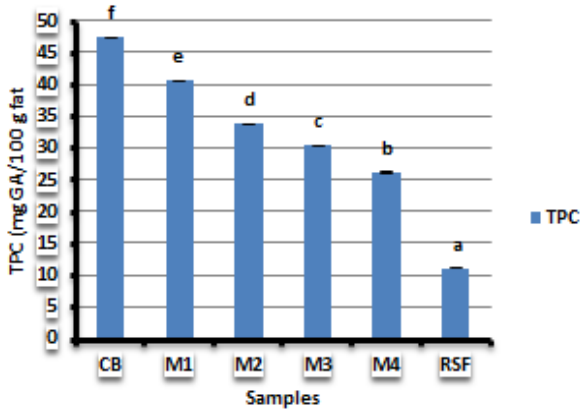

Fig. 3 Total phenolic compound (TPC) of rambutan seed fat (RSF) and its mixture with cocoa butter (CB), M1=80CB/20RSF, M2=60CB/40RSF, M3=40CB/60RSF. Bars represent standard deviation

C. DPPH radical scavenging activity

The result of DPPH radical scavenging activity of RSF and its mixture with CB is presented in Fig. 3. From the result, significant increases ($p < 0.05$) in phenolic compound were observed in the sample of the CB compared with the RSF. Highest antioxidant activity was noticeable at CB (67.32±0.44) and M1 (60.16±0.23) µmol trolox/100 g fat, respectively, whereas the lowest was found in RSF (35.43±1.45) µmol trolox/100 g fat. These results are similar to [38] who mentioned that the antioxidant activity was increased during roasting of cocoa beans in temperature 150 ^{o}C for 10 min.

Cocoa beans are rich in polyphenols and have gained much attention owing to their antioxidant activity [39]. The increase of antioxidant activity in CB due to the fermentation process which include several mechanisms, such as the increase of phenolic compound and the formation of more free hydroxyl groups in bioactive compounds such as phenolic acids, flavonoids and aglycone isoflavone [40]. The antioxidant activity of the M1 was also in a same trend with CB higher value. On the other hand, the antioxidant activity of the M2, M3 and M4 were decreased when the proportion of CB decreased into the mixture. Whereas, RSF has lowest antioxidant activity due to lowest polyphenols in composition, but sometimes the accumulation of brown-colored (Millard reaction) increased the antioxidant activity of the non-phenolic fraction [41].

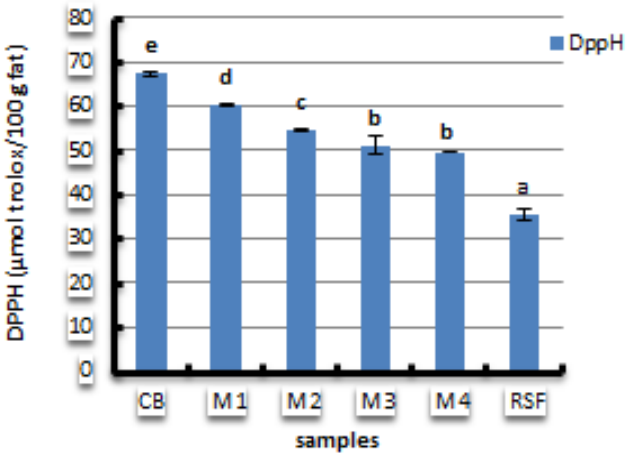

Fig. 4 Changes in the 1,1-diphenyl-2-picrylhydrazyl inhibition of cocoa butter (CB) and its blending with rambutan seed fat (RSF), M1=80CB/20RSF, M2=60CB/40RSF, M3=40CB/60RSF, M4=20CB/80RSF.

IV. Conclusions

The color and antioxidant changes of the mixture between rambutan seed fat (RSF) and cocoa butter (CB) occurred during the roasting process for rambutan seed and cocoa bean using normal oven. The whiteness and the lightness of samples initially increased and then decreased during the proportion of CB became less than 40%. It was observed that the increase in the redness and yellowness of samples with increasing the proportion of RSF. On the other hand, the antioxidant properties decreased with decreasing the total phenol content and increasing RSF proportion in the samples. In general, though the results can be seen that M1 more similar to that of CB comparison with other samples and the RSF can be used as cocoa butter alternatives despite of some differences in physical and chemical properties which allows the mixing with cocoa butter in a small proportion and possibilities of application in different branches of industry.

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