Panelist acceptance level and characterization of physical and chemical properties on dark chocolate bar with addition of kaffir lime (*Citrus hystrix* DC.) leaf essential oil

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Abstract. In this study, panelist acceptance level, physical, and chemical properties of dark chocolate bar enriched with kaffir lime (*Citrus hystrix* DC.) leaf essential oil (0.25%, 0.5%, and 0.75%) were studied. The addition of kaffir lime leaf essential oil in dark chocolate bar is aimed to enrich the content of functional compounds in dark chocolate and is expected to create an Indonesian chocolate signature. The results showed that panelist acceptance level decreased with increasing concentration of kaffir lime leaf essential oil added in parameters of aroma, taste, and overall, thus, the highest panelists acceptance was dark chocolate bar with the addition of 0.25% kaffir lime leaf essential oil. The addition of various formulations of kaffir lime leaf essential oil showed a decrease in hardness significantly and the color differences between dark chocolate with and without addition of kaffir lime leaf essential oil were very small. The addition of 0.25% kaffir lime leaf essential oil increased the moisture content and antioxidant activity in dark chocolate bar significantly. Major compounds detected in GC-MS analysis of dark chocolate bar with the addition of 0.25% kaffir lime leaf essential oil were theobromine, citronellal, caffeine, and vanillin.

1. Introduction
Indonesia is the third biggest cocoa producer in the world, after Ivory Coast and Ghana. Cocoa production in Indonesia has increased from 593,331 tons to 656,817 tons in 2015 and 2016 respectively and estimated to reach 688,345 tons in 2017 [1]. Cocoa contains large concentrations of flavonoids, even greater than tea and wine. Dark chocolate contains considerably higher amounts and also has greater biological effects of flavonoids than milk chocolate because the milk in milk chocolate may slow down the intestinal absorption of flavonoid. Aside from antioxidant, cocoa also contains minerals such as potassium, phosphorus, copper, iron, zinc, and magnesium [2].

*Citrus hystrix* DC., commonly known as kaffir lime or makrut lime, is a common tropical herb in the family Rutaceae found everywhere in Southeast Asia [3]. The production of kaffir lime leaf essential oil in Indonesia in 2011 reached 2-3 million tons and has a stable trend [4]. The main component in kaffir lime leaf essential oil is citronellal. Several studies have revealed that citronellal has antioxidant activity and also antibacterial activity that can inhibit Salmonella and other Enterobacteria [5][6]. Apart from that, the essential oil extracted from kaffir lime has been demonstrated to reduce blood pressure and relieve depression in human studies [7].

The research trend of chocolate products innovation is growing quite rapidly in the community. Several studies have been undertaken to investigate the interaction between cocoa and some herbs and spices such as aniseed, lemon, ginger [8], and cinnamon [9][10] on sensory [11][12], physical [8], and chemical properties [13]. However, the interaction between cocoa and kaffir lime is still poorly
mapped and not well understood. This research aims to study the panelist acceptance level, physical properties, and chemical properties of dark chocolate bar with the addition of kaffir lime leaf essential oil. This study also aimed to enrich the content of functional compounds in dark chocolate and is expected to create an Indonesian signature chocolate.

2. Experimental
2.1 Chocolate preparation
Couverture Dark Chocolate 65% "Easimelt - Aura" from Tulip and kaffir lime (Citrus hystrix DC.) leaf essential oil (CV. Orizho Indonesia, Bantul) were used in this study. Couverture Dark Chocolate was first melted using chocolate melter at 50°C for about 45 minutes until chocolate completely melted. The mixing process of the melted dark chocolate and kaffir lime leaf essential oil (0.25%, 0.5%, 0.75% (v/w); ρ = 0.8507 g/ml at 27°C) was done with a ball mill for 10 minutes with 90 rpm at 50°C. Afterwards, the chocolate mixture was molded into dark chocolate block and cooled in a cooling chamber at 11°C for about 30 minutes. The tempering process was conducted by melted down the dark chocolate block with a temperature less than 45°C. Approximately 3/4 part of the melted dark chocolate was then poured on the marble table, flattened, and inverted by using a scraper and a chocolate knife until the chocolate dough temperature was stabilized at 25°C. Once stabilized, the tempered chocolate was then poured back into the bowl containing 1/4 part of untempered chocolate, then stirred for a few minutes until the temperature stable at ±30°C, and then followed by moulding into chocolate plastic moulds and cooled in a refrigerator at 8°C. After 15 minutes, the moulded chocolates were de-moulding and were wrapped in the aluminum foil and stored at refrigerator temperature until used for further analysis.

2.2 Sensory evaluation
Kaffir lime leaf essential oil-enriched dark chocolates were then evaluated using Hedonic methods [14]. The experimental chocolates were subjected to sensory evaluation by a group of panelists, comprising 30 random panelist members varying in age between 20-22 years. Five attributes (color, aroma, taste, appearance, overall acceptability) were evaluated. Three samples of chocolate with different codes were presented in a single serving plate with crackers and mineral water for rinsing between samples. Panelists were asked for not comparing the samples with other samples. The sensory properties were presented on a five point scoring scale 1) Dislike, 2) Dislike moderately, 3) Neutral, 4) Like moderately, 5) Like.

2.3 Physical and chemical analyses
Color measurements of dark chocolates were conducted by using Chromameter CR-400, Konica Minolta Optic inc. [15]. hue was calculated based on the formula: hue = tan⁻¹ b*/a* [16]. Total Color Difference (TCD*) was calculated based on the values of L*, a*, and b* obtained by the formula: TCD* = [(L treatment - L control)² + (a treatment - a control)² + (b treatment - b control)²]¹/² [15]. The hardness (texture measurement) of dark chocolate samples (3 x 2 cm, depth 1 cm) were measured using Zwick Universal Testing Machine SA/0.5 [15] at pre-load 0.02 N, pre-load speed 50 mm/min, and test speed 10 mm/min. Moisture content of chocolate was determined using Moisture Meter CA-200 Mitsubishi Chemical Analytech by Karl Fischer titration method [17]. The measurement for antioxidant activity were investigated using DPPH (1,1-diphenyl-2-picrylhydrazyl) method [18]. GC-MS analysis was conducted based on [19] with slight modification to identify the active compounds and citronellal content in the sample. The control and the best formula samples were extracted by dissolving samples in methanol solvent, and melted in water bath at 110°C for about 2 minutes, mixed by vortex, and then centrifuged for 2 minutes until separated. One μL of the sample was injected into the Gas Chromatography Mass Spectrometry (GC-MS) Agilent Technologies Model 6890N (GC) 5973 inert (MS). Component separation was performed with helium as carrier gas, sample injected in split mode. The initial oven temperature was programmed at 60°C, kept constant for 3 minutes, with an increase in temperature at a rate of 20°C/min until it reached 100°C. 7°C/min until 240°C, kept constant for 5 minutes. The total program time was 30 minutes. Components of the samples were identified by comparing each peak in the chromatogram with the WILEY 7 database.
2.4 Statistical analysis
Data analysis was performed by SPSS Statistics 20 using One-Way Analysis of Variance (ANOVA) followed by DMRT (Duncan's Multiple Range Test) with a significance level of $\alpha = 5\%$. From the sensory, color, and texture test results, the best formula will be obtained. Afterward, Independent-Samples T Test was used to evaluate moisture content and antioxidant activity results for the best formula with a significance level of $\alpha = 5\%$.

3. Results and Discussion
3.1 Panelist acceptance level (sensory properties)
Table 1 shows that the variation of kaffir lime leaf essential oil addition in dark chocolate gave no significant effect on panelist acceptance in color and appearance parameters, however, all samples received score above 4, suggesting a good acceptability for the color and appearance parameters. The appearance parameter, particularly the glossiness of the samples, is greatly influenced by the tempering process. Tempering process is one of the important steps in producing chocolate as it is affecting the quality of chocolate which were the glossiness of the chocolate, good snap, and chocolate shelf life [21]. There were significant differences at panelist acceptance between F1 sample and the other samples (F2 and F3) in the parameter of aroma and taste. F1 received the highest score from the panelist in both aroma and taste parameters among the samples. F1 sample scored closed to "like moderately" in aroma parameter, while the other samples were both scored "neutral". This is allegedly due to the increased of kaffir lime leaf essential oil concentration that strengthen the aroma of kaffir lime leaf, thus defeating the aroma of the dark chocolate itself, and affecting the panelist acceptance of the samples. Citronellal, the major compound of kaffir lime leaf essential oil, consisted of terpenoid mixture that can give a special scent on kaffir lime leaf essential oil [20]. In the taste parameter, F1 also scored near "like moderately", while F2 scored near "neutral" and F3 "neutral". Panelists tend to dislike the sample with higher concentration of kaffir lime leaf essential oil addition. This might be due to the higher concentration of kaffir lime leaf essential oil addition gave the more bitter taste in the sample. Kaffir lime leaf essential oil has a strong bitter taste as it is consisted of esters, phenols, carbonyls, terpenes, and others that have bitter taste [22]. The result in overall parameter showed significant differences between F1 and the other samples (F2 and F3). F1 received the highest panelist acceptance and was scored "like moderately", while F2 and F3 were "neutral". From the five parameters tested, the F1 sample has the highest acceptance level. Therefore, it can be concluded that the best formula based on the panelists acceptance level is dark chocolate with 0.25% kaffir lime leaf essential oil addition.

3.2 Color measurement
This study was also supported by the result of color measurement. There were significant differences between dark chocolate control and the other three formulas in °Hue value (Table 1). Thus, the addition of kaffir lime leaf essential oil to dark chocolate gave statistically significant effect on °Hue of dark chocolate. However, °Hue value in each sample showed the same color range, which is in red color range [23]. The color difference level of Total Color Difference (TCD*) value obtained between control and F1, F2, F3 were very small, medium, and small, respectively [24]. The result of color measurement showed that F1 (0.25% kaffir lime leaf essential oil addition) has the slightest difference with the control. Therefore, F1 was also selected as the best formula based on the color measurement.

3.3 Texture measurement (hardness)
Table 1 shows that increasing concentration of kaffir lime leaf essential oil caused significant reduction in F max (hardness) in dark chocolate. The decrease in F max may caused by the addition of essential oil which in the form of liquid. Small changes in composition can give effects on texture in chocolate products [17]. Besides, the addition of the kaffir lime leaf essential oil can increase the particle size of dark chocolate. Increase in particle size resulted in linear decreases in textural properties (hardness) in dark chocolate [25]. According to [17], a good chocolate product is a chocolate that has a good snap. The best formula from the texture measurement was determined by selecting the formula with the biggest F max value among the formulations, thus F1 was selected.
Referring to the selected formula on the color and texture measurements, as well as the selected formula from the panelist acceptance level evaluation, F1 (dark chocolate with 0.25% kaffir lime leaf addition) was selected as the best formula. The best formula and control were then tested for the following chemical properties (moisture content, antioxidant activity, and GC-MS) evaluation.

Table 1. Panelist acceptance level, color, and texture measurement of the dark chocolate bars

|                  | Control  | F1 (0.25%) | F2 (0.5%) | F3 (0.75%) |
|------------------|----------|------------|-----------|------------|
| Color acceptabilitya | -        | 4.50±0.68  | 4.63±0.77 | 4.57±0.73  |
| Aroma            | -        | 3.93±0.94  | 3.03±1.30 | 3.03±1.40  |
| Taste            | -        | 3.90±1.13  | 2.80±1.06 | 3.03±1.38  |
| Appearance       | -        | 4.43±0.86  | 4.57±0.77 | 4.53±0.97  |
| Overall          | -        | 4.00±0.95  | 3.07±1.11 | 3.10±1.40  |
| Color Measurement |          |            |           |            |
| L*               | 29.09±0.45 | 29.17±0.39 | 28.42±0.27 | 28.78±0.61 |
| a*               | 9.29±0.17  | 9.26±0.18  | 8.98±0.07  | 8.94±0.27  |
| b*               | 6.92±0.18  | 6.66±0.06  | 6.14±0.35  | 6.32±0.27  |
| °Hue             | 36.68±0.23 | 35.74±0.29 | 34.34±0.56 | 35.25±0.34 |
| TCD              | -        | 0.27       | 1.08      | 0.76       |
| Texture Measurement (Hardness) | | | |
| F max (N)        | 85.99±2.34 | 73.52±0.71 | 72.28±4.08 | 64.54±0.99 |

a Different letters in the same row indicate significant differences (α < 0.05)

3.4 Moisture content

There was a significant difference between dark chocolate control and F1. The result of the moisture content test in this study was contradicting with the similar research that has been done. In the research by [26] there was a significant decrease in moisture content in 0.25% and 0.5% of cinnamon essential oil and a significant increase at 0.75% compared to the control. In the research by [27] noted that with the addition of various essential oils (lime leaf, bay leaf, and lemongrass leaf) into edible film of fish gelatin decrease the moisture content of the edible film. This contradicting result with the similar previous researches is suspected due to very small water vapor migration during the cooling process of the dark chocolate block after the mixing process. The occurrence of heat shock (from extreme temperature changes) from the ball mill process to cooling process in the cooling chamber causes condensation. The dew is allegedly reentered into the chocolate due to being retained by the plastic used as a cover during the cooling process of dark chocolate blocks. According to [28], temperature, solid fat content, crystal structure, and composition of the chocolate are some of the factors affecting moisture migration in chocolate. The result showed that the addition of kaffir lime leaf essential oil can increase the moisture content of dark chocolate significantly, however, it was still good and acceptable, because the results were still below 2%. According to [17], 2% moisture can give a poor shelf life as well as an inferior texture on the chocolate.

Table 2. Moisture content and antioxidant activity of the dark chocolate bars

|                  | Control  | F1 (0.25%) |
|------------------|----------|------------|
| Moisture content (%) | 1.19±0.00 | 1.74±0.06 |
| Antioxidant activity (%) | 57.73±1.46 | 80.41±1.46 |

a Different letters in the same row indicate significant differences (α < 0.05)

3.5 Antioxidant activity

Antioxidants have caught the attention of biochemists and health experts for its ability to protect the body against damage by reactive oxygen, thus avoiding some degenerative diseases. Table 2 shows a significant increase in antioxidant activity by the addition of 0.25% of kaffir lime leaf essential oil to dark chocolate. Citronellal, one of the main active compounds in kaffir lime leaf essential oil, has been known of its high antioxidant activity. From the past studies, Kaffir lime leaf essential oil is known to
has antioxidant activity of 17.65 μmol TE/g [5], while the total antioxidant capacity of dark chocolate is 227 μmol TE/g [29]. Aside from citronellal content, kaffir lime leaf essential oil also contained other compounds that play a role in increasing antioxidant activity in F1 sample, such as citronellyl acetate, geranyl acetate, and β-citronellol.

3.6 Active compounds and citronellal contents

In this study, theobromine has been detected as the major compound in both control and F1 (figure not shown) with relative content 59.27% (in control) and 48.31% (in F1). In dark chocolate bar control, vanillin, caffeine and squalene were also detected with relative content 12.78%, 13.77%, and 14.18%, respectively. While F1 was consisted of theobromine (48.31%), citronellal (18.35%), caffeine (12.81%), vanillin (11.58%), palmitic acid (3.36%), and citronellyl acetate (0.82%). Theobromine is the main compound in cocoa and gave distinct bitter taste in cocoa products along with a small contribution from caffeine. It is used as a vasodilator, diuretic, and cardiac stimulant [30]. Caffeine had effects on cardiovascular parameters and mood including feelings of alertness. Caffeine was reported to increase self-alertness and improve attention and psychomotor performance [31]. Citronellal was found in GC-MS test results in dark chocolate with the addition of 0.25% kaffir lime leaf essential oil. Citronellal is a monoterpene that has fungicidal ability, antifungal [32], antibacterial, and bactericidal activity [33], as well as depressant and antinociceptive activity and potentially useful in treating inflammatory and damage caused by oxidant agents [34]. The presence of squalene in dark chocolate control is suspected to be due to the use of vegetable emulsifier in the form of soybean oil in dark chocolate couverture used. Interestingly, squalene was not found in the dark chocolate with the addition of 0.25% kaffir lime leaf essential oil. This is allegedly due to the advanced process that uses heat. [35] reported that squalene is thermolabile due to its unsaturated linear chain. Squalene is commonly found in many plant oils such as olive oil, soybean oil, and sunflower oil. According to [36], squalene acts as an anticancer, has antihypertensive effects, and cardioprotective activity. Vanillin commonly used as a flavoring agent in chocolate. Citronellyl acetate was derived from the kaffir lime leaf essential oil, plays a role in creating a distinctive aroma on the kaffir lime leaf essential oil. It has antinociceptive, fungicidal, bactericidal, and potentially antihepatoma activity [37]. As the relative content of palmitic acid in this study showed a relatively small percentage, which is 3.53%, palmitic acid detected in this study is believed to be fatty acids derived from cocoa fat. [38] reported that the content of palmitic acid which exceeds 20% is strong evidence of the addition of coconut oil. However, palmitic acid was only detected in F1 allegedly due to the impurities from the ball mill process performed on the dark chocolate bar with the addition of kaffir lime leaf essential oil, since cooking oil was used in cleaning process of the ball mill. The small remnants of cooking oil is possibly become the impurities that causing the detection of palmitic acid in F1.

4. Conclusion

Dark chocolate bar with 0.25% kaffir lime leaf essential oil addition has the highest panelist acceptance. There were significant difference between dark chocolate control and the other formulas (0.25%, 0.5%, and 0.75%) at °Hue value. The increasing concentration of kaffir lime leaf essential oil caused significant reduction in F max (hardness) in dark chocolate. Moisture content and antioxidant activity in dark chocolate bar were significantly increased by the addition of the best formula (0.25% kaffir lime leaf essential oil) in comparison to the control. Theobromine, citronellal, caffeine, and vanillin were detected as major compounds in GC-MS analysis of dark chocolate bar with the addition of 0.25% kaffir lime leaf essential oil.

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