International Journal on Advanced Science Engineering Information Technology

Vol.11 (2021) No. 1
ISSN: 2088-5334

The Effect of Clove Leaves Essential Oil Addition on Physicochemical and Sensory Characteristics of Milk Chocolate Bar

Danar Praseptiangga[a,*], Agitya Renanda Qomaruzzaman[a], Godras Jati Manuhara[a]

[a]*Department of Food Science and Technology, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, 57126, Central Java, Indonesia*
*Corresponding author: *dpraseptiangga@staff.uns.ac.id*

***Abstract*—As one of the most prominent cocoa and clove producers globally, Indonesia needs to develop a chocolate signature with unique characteristics due to the international cravings and high consumption of chocolate compared to other confectionery products. There are three main types of chocolate-based on their compositions: dark chocolate, milk chocolate, and white chocolate. However, milk chocolate has been discovered to be a much-preferred type due to its taste and flavor, but its innovative development is limited despite consumers' interest. Moreover, clove leaves have been less utilized despite their ability to be further processed to the essential oil, which possesses a potency of health benefits such as antioxidant activity due to its main bioactive compounds and its usefulness a flavor in food. This study was, therefore, aimed to determine the effects of adding several concentrations of clove leaves essential oil at 0.1% (F1), 0.2% (F2), and 0.3% (F3) on the physicochemical and sensory characteristics of milk chocolate bars as well as to define the best formula to achieve the optimum product. The results showed different trends at each concentration, while the best formula was recorded at the addition of 0.2% (F2) clove leaves essential oil to the milk chocolate bar, and the panelists further confirmed this.**

***Keywords*— Cocoa; milk chocolate bar; clove leaves essential oil; sensory characteristics; physicochemical characteristics.**

Manuscript received 25 Jul. 2020; revised 25 Nov. 2020; accepted 8 Jan. 2021. Date of publication 28 Feb. 2021.

I. INTRODUCTION

Chocolate is the most famous confectionery product available in the world, and its popularity is associated with the bioactive compounds which potentially provide beneficial health benefits reported to be contained in its main ingredient, cocoa (*Theobroma cacao* L.) [1], [2]. Moreover, the cocoa processed into chocolate or powder has certain polyphenolic compounds mostly composed of flavonoids reported to contain antioxidants [1], [2].

Meanwhile, milk chocolate is one of the favored derived products compared to dark and white products due to its sweet taste, texture, and soft flavor [3]. Consumers prefer the absence of bitter taste but the milk and sugar added could decrease the effectiveness of bioactive compounds contained in the chocolate [4]. Milk chocolate is a complex rheological system made by mixing cocoa, milk powder, and sugar as solid particles that are dispersed in the fat phase (cocoa butter) [5], [6], [7], [8]. It is formulated to be softer than dark chocolate and has a creamier taste, making it a much-preferred type of chocolate, particularly by Indonesian consumers [7].

There has been an increase in bioactive substances investigations in recent times, mainly due to their use in food processing and their potential health benefits [9], [10]. Meanwhile, the use of medicinal plants and spices in the formulations of functional food for traditional enhancement of health or healing diseases has been reported to be promising [10]. There is also growing interest in utilizing essential oils that are derived from plants and spices for food and pharmaceutical applications. Essential oils are volatile hydrophobic liquids extracted from plants comprised of various groups of compounds such as phenols, aldehydes, ketones, mono, and sesquiterpenes that are reported to be responsible for various biological activities.

The essential oils extracted from the medicinal plants and/or spices such as cinnamon, clove, basil, oregano, and thyme show significant antioxidant activities due to major constituents' presence such as *cinnamaldehyde, carvacrol, eugenol*, or *thymol*, etc. The antioxidant potential of essential oils mainly depends on their chemical compositions, and phenolic compounds and other secondary metabolites are responsible for the substantial antioxidant activity of essential oils [11], [12], [13].

Clove (*Syzygium aromaticum*), which is one of the spices native to Maluku islands in east Indonesia, has been applied as food preservatives and medicinal plants for centuries mainly due to its antioxidant and antimicrobial activities [14], [15]. It is an essential oil producer plant containing 45-90% *Eugenol* as the main bioactive compound [15], [16], [17], [18]. Indonesia is the largest clove producer with a total of 98,700 tons, which is 42% of the world's production in 2013 [19]. However, there is less utilization of clove leaves, which are currently considered waste, while flowers and buds are considered the most commercial part. The leaves' essential oil has two main components, which are 75.04%-77.54% *Eugenol* and 17.04-19.53% β-Caryophyllene [20]. Eugenol has been reported to be anti-inflammatory and antioxidant and can be a flavor in food due to its distinctive aroma and a hot and spicy taste [17].

Despite the interests of consumers towards chocolate products continue to increase; the innovative development of milk chocolate is still limited, and there is still a little information of which reported the addition of essential oils from spices on milk chocolate bars that may contribute to enhance their functional properties, particularly concerning health benefits. The potential of clove leaves essential oil as one of the Indonesian spices was, therefore, studied by adding it into milk chocolate to develop Indonesian chocolate signature innovative products. Thus, this study aims to evaluate the effect of clove leaves essential oil incorporation on milk chocolate bars' physicochemical and sensory properties and determine the best formula to attain the optimum product.

II. Materials And Methods

A. Materials

The main material used in producing milk chocolate bar was milk chocolate block couverture provided by the Indonesia Coffee and Cocoa Research Institute (Jember, Indonesia). The essential oil of clove leaves (*Syzygium aromaticum*) was distilled from the third generation of Mollucas (Maluku Island, Indonesia) *Afo* variety cloves and purchased at CV. Orizho Indonesia (Bantul, Indonesia). All the chemicals and solvents used were of analytical grade.

B. Milk Chocolate Bars Preparation

Clove leaves essential oil were added to milk chocolate bars by modifying the methods applied in previous studies [7],[8]. This involved adding the clove leaves essential oil to the melted milk chocolate couverture at 0%, which was used as the Control/C, 0.1% (Formula 1/F1), 0.2% (Formula 2/F2), and 0.3% (Formula 3/F3) during the refining-conching process. The ball mill was set at a speed of 90 rpm and stopped when the chocolate particles have been uniformly mixed to produce a homogeneous mixture.

Approximately 1/3 of the chocolate mixture melted at 45ºC was poured on the marble table, flattened, and inverted by using a scrapper and a chocolate knife at room temperature of 26ºC to achieve stability dough at 25ºC. The dough tempering produced after the stability was poured back to the remaining 2/3 untampered dough, stirred for a few minutes until the dough temperature was stable at ± 30ºC, and shaped into chocolate acrylic molds. They were further de-molded, packaged with aluminum foil, and stored at the refrigerator temperature when needed for further analysis.

C. Sensory and Physicochemical Characterization

The hedonic method used in previous studies was used to analyze the samples [7], [8], [21]. This involved presenting sensory attributes on a 5-hedonic scale using 1 – 5 range to represent dislike, rather dislike, neither like nor dislike, rather like, and like, respectively.

The samples' color attributes were analyzed using a chromameter Konica Minolta CR-400 with the CIE L*a*b* method according to [21]. The L*, a*, and b* values were used in determining the hue angle and the total color difference (TCD). Moreover, the analysis of the texture was conducted using a universal testing machine (UTM) [3]. It is essential that a room temperature of 26-28°C was used in analyzing all these attributes.

Karl Fischer method and a Moisture Meter CA-200 Mitsubishi Chemical Analytech was used in determining the moisture content, and the results are presented as the percentage (%) of water in the sample (w/w) [22]. The evaluation of the total phenolic and antioxidant activity (2,2-Diphenyl-1-picrylhydrazyl (DPPH) IC_{50}) was initiated with maceration extraction of milk chocolate bars, which was conducted by modifying the methods applied [1]. This involved the dissolution of 1g of a shredded chocolate bar in 20 ml of 80% methanol. The extract was heated and stirred at 48-50°C for an hour, after which it was filtered. The total phenolic was measured using the Folin Ciocalteu method by modifying previous studies [1],[21] through the addition of 2% Na_2CO_3 incubated for 10 minutes and 0.5 ml Folin Ciocalteau reagent (1:1) incubated for 30 minutes. Moreover, a UV-Vis spectrophotometer was used to measure the absorbance at λ=750 nm while the determination of the antioxidant activity was through IC_{50} method with some modifications [1], [21].

GC-MS (Gas Chromatography-Mass Spectrometry) analysis was also conducted using an Agilent 6890N instrument equipped with Agilent J&W HP-5 fused-silica capillary column with length of 30 m and an inner diameter of 0.32 mm and a film thickness of 0.25 µm coupled with a 5973 inert mass selective detector spectrometer (Agilent Technologies, USA). Methanol was used as the solvent for chocolate extraction and the method applied for the methanolic extract was according to [23] with modifications. This involved shredding one block of the chocolate bar after which they were added to 13 ml methanol, heated at 110°C for 2 minutes, vortexed for 5 minutes, and the mixture was centrifuged at 5000 rpm for another 5 minutes.

Moreover, the GC-MS injection method slightly modified the methods used in [24] and [21]. The process involved the injection of the methanolic extract (1 µl) with a split ratio of 1/10 and helium, a carrier gas used at 1.5 mL per minute. The inlet temperature was 290°C, MS quad temperature was 150°C, while the MS source temperature was 230°C. Meanwhile, the oven temperature was kept at 50°C for 5 minutes, raised 10°C/minute to 280°C, and held for 5 minutes. The samples' active compounds were identified by comparing the mass spectra of each peak with those of authentic samples in a mass spectra library. The result was expressed as content (%) based on the area percentage obtained.

D. Data Analysis

One-way ANOVA with a significance level of α = 5% was used in analyzing the data. Meanwhile, Duncan multiple range tests were applied for further analysis in the case of significant differences in the results.

III. RESULTS AND DISCUSSION

A. Sensory Characteristics

Human panelists' sensory perception related to the thresholds in determining attributes and the variance in individual sensory response experimental design were used to measure the food products' sensory characteristics and acceptability. Table 1 shows the variation in the clove leaves' concentrations do not affect the panelists' preference for a particular color

TABLE I
SENSORY CHARACTERISTICS OF MILK CHOCOLATE BAR WITH ADDITION OF CLOVE LEAVES ESSENTIAL OIL

Sample	Parameter				
	Color	Aroma	Taste	Appearance	Overall
F1 (0.1%)	$4.73^{a}\pm0.45$	$4.43^{a}\pm0.77$	$4.63^{a}\pm0.72$	$4.07^{a}\pm0.91$	$4.43^{a}\pm0.68$
F2 (0.2%)	$4.67^{a}\pm0.48$	$4.30^{ab}\pm0.79$	$4.37^{a}\pm0.67$	$4.13^{a}\pm0.63$	$4.10^{ab}\pm0.80$
F3 (0.3%)	$4.63^{a}\pm0.61$	$3.90^{b}\pm0.88$	$3.07^{b}\pm0.87$	$4.47^{a}\pm0.63$	$3.87^{b}\pm0.86$

This means the color is an essential sensory attribute and one factor influencing any product's appearance. It is also associated with food quality, with its brightness in some foods being a benchmark for quality [25]. The milk chocolate bar has a light brown color compared to the dark brown usually found with dark chocolate due to paste dominance. A dairy component is also attached to the bar's sweetness and creaminess [7]. Furthermore, the interaction between reducing sugars and the amino acids contained in milk causes browning or Maillard reactions, and this also contributes to the formation of the color [26]. The addition of essential oils had no significant effect on the color attribute of the milk chocolate bar [8], [27].

The aroma is also one of the factors to measure food product quality. The aroma of chocolate is due to the very complex chemical reactions including amino acids, sugars, and compounds of flavonoids during roasting as well as the degradation of the Strecker amino acid derived from aldehyde volatiles [3], [7]. There was a decrease in panelist's preference for milk chocolate bars along with the concentration of essential oil added. Table 1 shows that the preference for aroma at 0.1% or F1 with 4.43 ± 0.77 was not significantly different from 0.2% or F2 with 4.30 ± 0.79, which confused their aroma by the panelists. Meanwhile, the 0.3% or F3 sample had the lowest preference with 3.90 ± 0.88 compared to the other two, and this was allegedly due to the dominance of the aroma for the essential oil over the existing chocolate aroma. However, the essential oil has a distinctive spicy, sharp, and specific odor of cloves [15], [17].

The taste of a food product is one of the flavor-forming components, the aroma, and an important factor affecting consumers' acceptance of food. Table 1 shows the milk chocolate bar containing 0.1% essential oil had the highest value with 4.63±0.72 followed by 0.2% with 4.37±0.67 but without any significant difference as observed from the values. Meanwhile, the sample containing 0.3% had the lowest value of 3.07±0.87 and is significantly different from the others. This, therefore, means there was a decrease in panelist's preferences as the concentration of the essential oil increased. This is like the findings of previous studies [7], [8], and the decline is believed due to the stronger pungent flavor of the essential oil. The panelists were not favored, and its anesthetic effect causes numbness on the tongue when consumed too much [28].

Appearance is another element with great influence on the level of consumers' preference for chocolate. Most of those believed to be good usually have a light brown to dark brown uniform and a shiny or glossy surface [8], [26]. However, the milk chocolate bar's appearance is closely related to its brightness or glossiness, which is an optical phenomenon associated with the ability of a surface to reflect the light that hits it. The results showed appearance is not an important parameter to determine the quality of the chocolate [29]. This was confirmed by the information on Table 1 where no significant difference was observed with the preference of the panelists based on appearance. Meanwhile, the tempering process is believed to have more influence on the milk chocolate bar's appearance. Over-tempering caused a significant increase in the product's hardness, stickiness with reduced gloss, and surface darkening while under-tempering induced fat bloom with consequential quality defects on texture, color, and surface gloss [8], [30]. Therefore, chocolate's shiny or glossy surface is an indicator of the tempering process's success, thereby indicating its quality [26].

The overall parameters, including the sensory attributes of color, aroma, taste, and appearance, were also jointly measured, and the panelists accepted all the three formula of milk chocolate bars developed in this study. However, a decrease in their preference for the overall parameters with an increase in the clove concentration leaves essential oil added. This was observed because there was no significant difference with the color and appearance but a decrease in line with the increase in concentration was recorded with aroma and taste. The overall values obtained for F1, F2, and F3 were $4.43^{a} \pm 0.68$, $4.10^{ab} \pm 0.80$, and $3.87^{b} \pm 0.86$, respectively. The milk chocolate bars were strongly influenced by the two parameters of taste and aroma, which is reflected in the overall results.

B. Physicochemical Characteristics

The measurement of color in this study was intended to provide objective information on the difference in milk chocolate bars due to the addition of clove leaves essential oil. The L* value indicates the sample's brightness level, which ranges from black to white (0-100) [31]. The samples with essential oil were observed to have significantly different lightness from each other, but 0.2% or F2 was not substantially varied from the control treatment. Moreover, the a* value indicates the chromatic color of red (a* positive, +60) to green (a* negative, -60) while b* value indicates the chromatic color of yellow (b* positive, +60) to blue (b* negative, -60) [31]. However, all the samples were observed to have positive a* and b*, which means the milk chocolate bars color with and without the essential oil ranged between red and yellow. Table 2 shows the hue angle of the samples are between 18 and 54, thereby indicating the product color tends to be red [8]. Furthermore, the total color difference value (TCD) was calculated to determine the color difference between the treatment samples and control as well as the visibility of the variation. The TCD value, based on [31], can be <0.5 (invisible), 0.5-1.5 (slightly noticeable), 1.5-3.0 (noticeable), 3.0-6.0 (well visible), or >6.0 (great difference).

TABLE II
COLOUR AND TEXTURE MEASUREMENT OF MILK CHOCOLATE BAR WITH THE ADDITION OF CLOVE LEAVES ESSENTIAL OIL

Sample	L*	a*	b*	°Hue	TCD	Effect	Hardness (N)
C (0%)	$32.46^{a}\pm0.25$	$15.91^{a}\pm0.06$	$14.58^{a}\pm0.15$	$42.51^{a}\pm0.23$	-	-	$27.71^{a}\pm0.33$
F1 (0.1%)	$34.52^{c}\pm0.22$	$15.53^{a}\pm0.44$	$13.38^{a}\pm0.70$	$40.72^{a}\pm0.96$	2.41	Noticeable	$26.38^{b}\pm0.35$
F2 (0.2%)	$32.84^{a}\pm0.44$	$15.54^{a}\pm0.47$	$13.59^{a}\pm0.94$	$41.14^{a}\pm1.07$	1.12	Slightly Noticeable	$24.07^{c}\pm0.23$
F3 (0.3%)	$33.62^{b}\pm0.26$	$15.45^{a}\pm0.13$	$13.28^{a}\pm0.49$	$41.26^{a}\pm0.81$	1.80	Noticeable	$25.54^{d}\pm0.69$

According to the information in Table 2, the addition of clove leaves essential oil presents a noticeable effect on the samples' color difference (F1 and F3) and a slightly noticeable effect for F2. Due to the closeness of the TCD values of the treated samples to the threshold value of 1.5, there was only a small difference that the panelists were unable to find a substantial difference in the color during the sensory evaluation.

The texture is another important component in determining the quality of chocolate products. A smaller and better distribution of particles in chocolate usually leads to higher hardness [26], [30]. Table 2, therefore, shows a significant difference regarding hardness was induced by the addition of clove leaves essential oil to the milk chocolate bar and the sample. The highest value was recorded in control with $27.71^{a} \pm 0.33$N followed by F1 with $26.38^{b} \pm 0.35$N, and F3 with $25.54^{d} \pm 0.69$N while a slight decrease was observed in the F2 with $24.07^{c} \pm 0.23$N allegedly due to an uneven distribution of particles thought to be caused by a non-uniform tempering profile. It is important to note that the chocolate was tempered using hand with the combination of time and temperature found to be the most important part of the process to trigger crystals' formation. Moreover, there was the influence of the refining, conching, and tempering on the milk chocolate bar. This is observed from several activities such as cooling, heating, and inverting the chocolate to ensure the formation of fat crystal β_2 (V form) with resistance to the changes in temperature [26], [30]. It has also been previously reported that under-tempering increases chocolate softness while over-tempering increases its hardness [8]. Meanwhile, an optimized low residence time (<6 minutes) was used in this study, and it is impossible to equal the crystal size and polymorphous size distribution produced with the use of a fully mature stabilized tempering method usually takes 12 minutes - 2 hours. This decline in hardness is related to the increase in the chocolate moisture content due to the addition of the essential oil. This water binds with the sugar to produce a chocolate with a gritty texture, reduced hardness, and high viscosity when melted [3].

Moisture content is one of the critical parameters in chocolate products and is required to be avoided as much as possible due to its ability to reduce quality. It can soften the texture [32] and accelerate the appearance of blooming on the chocolate's surface and these reduce its shelf life [26]. Table 3 shows an increase in the water content of milk chocolate bars with the lowest recorded in the control sample with $2.14^{a} \pm 0.03$% while F1 had $2.30^{b} \pm 0.01$%, F2 had $2.48^{c} \pm 0.08$%, and F3 had $2.51^{c} \pm 0.01$%.

TABLE III
MOISTURE CONTENT, TOTAL PHENOLIC CONTENT, AND ANTIOXIDANT ACTIVITY OF MILK CHOCOLATE BAR WITH ADDITION OF CLOVE LEAVES ESSENTIAL OIL

Sample	Moisture content (%)	Total phenolic (%)	IC_{50} (ppm)
C (0%)	$2.14^{a}\pm0.03$	$1.86^{a}\pm0.26$	$1434.31^{a}\pm30.20$
F1 (0.1%)	$2.30^{b}\pm0.01$	$2.16^{b}\pm0.27$	$1422.04^{a}\pm67.49$
F2 (0.2%)	$2.48^{c}\pm0.08$	$2.53^{c}\pm0.76$	$1157.90^{b}\pm27.83$
F3 (0.3%)	$2.51^{c}\pm0.01$	$2.61^{c}\pm0.66$	$1029.73^{c}\pm24.37$

Table 3 shows that the control and F1 are significantly different from others. In general, the water content increased along with the concentration of the oil added. This was assumed to be due to the hydrophilic properties of the essential oil [33] and the presence of its volatile polar components [34]. An increase in moisture content was also found in the milk chocolate bar with an addition of cinnamon essential oil [8], with the value observed to have been increasing with the concentration, even though the variation was not significant. Moreover, the storage and transfer of chocolate also could contribute to the increase in the moisture content. This is in line with the explanations of Beckett [3] that several factors contribute to this increase and one of them is the transfer of the product from a cold to room temperature with high humidity. The condensation process causes the formation of condensed water on the chocolate bar's surface, which leads to an increment in the moisture content. Furthermore, milk as one of the main ingredients contains lactose crystals which are hygroscopic natural sugar and easily bind with water [26]. This is generally believed to be the reason for the higher moisture content in milk chocolate bars compared to dark bars [3].

Phenolic compounds have great potential in preventing diseases and serving as antioxidants [35]. The total content of phenolic compounds was determined in this study using the Folin-Ciocalteu method because it is easy, rapid, and most used in cocoa and chocolate studies [1], [21]. Table 3 shows the total phenolic content increases with the concentration of essential oil. The control sample had the lowest at 1.86% ± 0.26 followed by F1 at 2.16% ± 0.27, F2 at 2.53% ± 0.76, and F3 at 2.61% ± 0.66. The control and F1 samples were observed to be significantly different from others and the trend was assumed to be due to the presence of eugenol, which is the main phenolic compound [36] in clove essential oil.

The antioxidant activity test conducted on the milk chocolate bar is presented as IC_{50} value as shown in Table 3 and it shows the quantity of extract concentration required to inhibit 50% oxidation. There was a decrease in IC_{50} as the essential oil was added, indicating the milk chocolate bar's stronger antioxidant activity. As the concentration of the essential oil increased, there was a need for the chocolate concentration to scavenge the 50% free radicals of DPPH. This, therefore, means the control sample has the highest IC_{50} value of 1434.31^{a} ± 30.20 ppm which is not significantly different from the 1422.04^{a} ± 67.49 ppm recorded for F1 and this was followed by 1157.90^{b} ± 27.83 ppm for F2 while the lowest was 1029.73^{c} ± 24.37 at F3. The active compounds such as *eugenol* and *β-caryophyllene* in the clove essential oil which are classified as super-antioxidant and antioxidant, respectively, were observed to have caused the increment [37],[38].

Moreover, flavonoids and flavanols such as catechin and epicatechin are polyphenols in cocoa [3], and the methylxanthines are theobromine and caffeine in cocoa, are also antioxidants [39]. According to Molyneux's classification [40], a super potent antioxidant has IC_{50} <50 ppm, strong is 50-100 ppm, the middle is 10-150 ppm, weak is 150-200 ppm, and super weak is >200 ppm. This, therefore, means all the samples are super weak antioxidants. Meanwhile, the values are not constant and have been observed to be varying based on several factors such as the condition, processing, or assay method of the materials [3],[21]. However, this study's findings are in line with the total phenolic and GC-MS evaluations in previous studies [1], [21], which demonstrated the antioxidant activity to be positively correlated with the total phenolic content.

The active compounds in the milk chocolate bar after the essential oil has been added were determined using GC-MS analysis, and Table 4 shows an increase in the percentage of *eugenol* was in line with a higher concentration of the essential oil. Table 4 shows the relative content in F1 was 28.95% while F2 had 53.267% and F3 62.77%. Eugenol is a phenolic compound with several pharmacological activities, roles in the redox status, and applications in food [17]. It has also been reported to have several health benefits by serving as anti-inflammatory, antioxidant, antimicrobial, and anesthetic agents [17],[41]. This means the increase in the concentration of *eugenol* is expected to improve the milk chocolate bars' functionality for human health.

Further studies are, however, needed to understand its functionality comprehensively. β-Caryophyllene, which is another main compound found in clove essential oil, is a natural bicyclic sesquiterpenoid. It has been demonstrated to possess great potential to be applied for several pathological conditions. In vitro and in vivo (preclinical), many studies suggested the treatment with β-caryophyllene was able to improve the phenotype of animals used in modeling different inflammatory pathologies such as nervous system diseases [42]. The same trend with eugenol was observed to have increased with the concentration of essential oil with the relative content in F1 recorded to be 9.64% while F2 had 18.60%, and F3 25.06%.

TABLE IV
GC-MS DETECTION OF MILK CHOCOLATE BAR WITH ADDITION OF CLOVE LEAVES ESSENTIAL OIL

No.	Retention time	Compound	Content (%)			
			C	F1	F2	F3
1	13.95	Eugenol	-	28.95	53.26	62.77
2	14.54	Vanillin	47.01	16.69	7.87	2.05
3	14.82	β-caryophyllene	-	9.64	18.60	25.06
4	19.71	Caffeine	25.36	16.60	8.99	6.66
5	20.22	Theobromine	Trace	9.74	3.83	Trace
6	20.48	Pentadecanoic acid, methyl ester	7.36	5.26	1.77	1.20
7	21.10	Ethyl palmitate	Trace	1.75	0.61	0.51
8	22.11	10-octadecanoic acid, methyl ester	-	0.99	Trace	Trace
9	22.34	Heptadecanoic acid, methyl ester	Trace	1.06	Trace	Trace
10	22.66	Linoleic acid, ethyl ester	Trace	0.919	Trace	Trace
11	22.71	Ethyl oleate	10.39	2.10	2.00	1.76
12	29.67	Beta-tocopherol	9.88	6.11	3.06	Trace

Note: "Trace" shows the proportion of compounds is very small due to the dominance of other compounds (Similarity index used at least 80%)

Moreover, Table 4 also shows some compounds containing cacao and its processing products, such as vanillin and methylxanthines. According to Franco et al. [39], caffeine, theobromine, and theophylline are methylxanthines found in cocoa, and the major ones are theobromine and caffeine, which are in lower concentrations [3],[39]. The GC-MS analysis showed the samples treated with essential oil contained methylxanthine, vanillin, tocopherol, and *eugenol* as *antioxidants*. Furthermore, pharmacological studies have also shown evidence of *eugenol's* clinical potential in treating diseases associated with oxidative stress and inflammatory response [17], which means its inclusion in chocolate bars has potential health benefits.

The best milk chocolate bar formula was determined using a model weighting test [43] and the parameters considered include the sensory attributes, physiochemical properties, including a color test in the form of TCD (Total Color Difference), hardness (N), moisture content (%), total phenolic content (%), and antioxidant activity IC_{50} (ppm), as well as test results of active compounds. Meanwhile, not all the compounds detected in the GC-MS analysis were used to only focus on *Eugenol* and *β-Caryophyllene*. Each of these parameters was provided with a weight from 0-1 according to the effect on the ample quality. However, they were all found to have the same effects, which means they all had 1 as the weight value. The results showed F2 had the highest total score with 0.5757 followed by F3 with 0.5469, and F1 with 0.4615. This, therefore, means F2, which is the treatment with 0.2% of clove leaves essential oil, was the best formula to produce a milk chocolate bar.

IV. CONCLUSIONS

This study evaluated the effect of adding clove leaves essential oils on milk chocolate bars' physicochemical properties and sensory characteristics by measuring panelist's preferences. Sensory analysis showed a decrease in the preference for taste and aroma due to the addition of the essential oil and the overall parameters of the milk chocolate bars were highly influenced by these two parameters. Meanwhile, color and appearance were observed not to have any significant difference as observed with the small variation noticeable in the color analysis's TCD values. The physicochemical characterization showed that the decline in hardness is related to the increase in chocolate moisture content due to the essential oil added. Moreover, an increase was also discovered in the total phenolic content and antioxidant activity based on the increment in the active compound concentration derived from clove leaves essential oil. Based on all aspects of the tests, the best formula was found to be F2, which involves adding 0.2% of clove leaves essential oil to the milk chocolate bar to develop an Indonesian chocolate signature.

ACKNOWLEDGMENT

The authors appreciate and express sincere gratitude to the Ministry of Research and Technology (Ristek-BRIN), Republic of Indonesia, for the partially supported research grant scheme of World Class Research and Universitas Sebelas Maret PNBP research grant.

REFERENCES

[1] D.R.A. Muhammad, D. Praseptiangga, D. Van de Walle and K. Dewettinck, "Interaction between natural antioxidants derived from cinnamon and cocoa in binary and complex mixtures," *Food Chemistry*, vol. 231, pp.356-364, 2017.

[2] D. Praseptiangga., Y. Nabila and D.R.A. Muhammad, "Kajian Tingkat Penerimaan Panelis pada Dark Chocolate Bar dengan Penambahan Bubuk Kayu Manis (*Cinnamomum burmannii*)," *Caraka Tani: Journal of Sustainable Agriculture*, vol. 33, pp.78-88, 2018.

[3] S.T. Beckett, "*The Science of Chocolate 2nd edition*," RSC, Cambridge, 2008.

[4] B. Llang and R.W. Hartel, "Effect of Milk Powders in Milk Chocolate," *Journal of Dairy Science*, vol. 87(1), pp.20-31, 2004.

[5] B. Pajin, L. Dokić, D. Zarić, D. Šoronja-Simović, I. Lončarević and I. Nikolić," Crystallization and Rheological Properties of Soya Milk Chocolate Produced in a Ball Mill," *Journal of Food Engineering*, vol. 114(1), pp.70-74, 2013.

[6] V. Glicerina, F. Balestra, M.D. Rosa and S. Romani, "Effect of Manufacturing Process on the Microstructural and Rheological Properties of Milk Chocolate," *Journal of Food Engineering*, vol. 145, pp.45-50, 2015.

[7] I.N. Rasuluntari, D.R.A. Muhammad and D. Praseptiangga,"Panellist acceptance level on milk chocolate with cinnamon (*Cinnamomum burmannii*) powder addition," *Nusantara Bioscience*, vol. 8(2), pp.297-300, 2016.

[8] A. Ilmi, D. Praseptiangga and D.R.A. Muhammad, "Sensory attributes and preliminary characterization of milk chocolate bar enriched with cinnamon essential oil," In *IOP Conference Series: Materials Science and Engineering* (Vol. 193, No. 1, p. 012031), IOP Publishing, 2017.

[9] D. Granato, J.S. Santos, L.G. Maciel and D.S. Nunes," Chemical perspective and criticism on selected analytical methods used to estimate the total content of phenolic compounds in food matrices," *TrAC Trends Anal. Chem.*, vol. 80, pp.266-279, 2016.

[10] D.R.A. Muhammad, V. Lemarcq, E. Alderweireldt, P. Vanoverberghe, D. Praseptiangga, J.G. Juvinal and K. Dewettinck, "Antioxidant activity and quality attributes of white chocolate incorporated with *Cinnamomum burmannii* Blume essential oil," *Journal of Food Science Technology*, vol. 57, pp.1731-1739, 2020.

[11] A.P. Mishra, H.P. Devkota, M. Nigam, C.O. Adetunji, N. Srivastava, S. Saklani, I. Shukla, L. Azmi, M.A. Shariati, H.D.M. Coutinho and A.M. Khaneghah, "Combination of essential oils in dairy products: A review of their functions and potential benefits," *LWT*, vol. 133(110116), pp.1-10, 2020.

[12] S. Bhavaniramya, S. Vishnupriya, M.S. Al-Aboody, R. Vijayakumar and D. Baskaran, "Role of essential oils in food safety: Antimicrobial and antioxidant applications," *Grain & Oil Science and Technology*, vol. 2, pp.49-55, 2019.

[13] M. Radünz, M.L.M. da Trindade, T.M. Camargo, A.L. Radünz, C.D. Borges, E.A. Gandra and E. Helbig, "Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum*, L.) essential oil," *Food Chemistry*, vol. 276, pp.180-186, 2019.

[14] G.P. Kamatou, I. Vermaak and A.M. Viljoen, "Eugenol -- from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule," *Molecules*, vol 17(6), pp.6953-6981, 2012.

[15] D.F. Cortés-Rojas, C.R. Fernandes de Souza, and W. Pereira Oliveira, "Clove (Syzygium aromaticum): a precious spice," *Asian Pac J Trop Biomed*, vol. 4(2), pp.90-96, 2014.

[16] P. Zhang, E. Zhang, M. Xiao, C. Chen, and W. Xu, "Study of anti-inflammatory activities of α-d-glucosylated eugenol," *Archives of Pharmacal Research*, vol. 36(1), pp.109-115, 2013.

[17] J.N. Barboza, C.S.M.B. Filho, R.O. Silva. J.V.R. Medeiros and D. Pergentino de Sousa, "An Overview on the Anti-inflammatory Potential and Antioxidant Profile of Eugenol," *Oxidative Medicine and Cellular Longevity*, vol. 2018, pp.1-9, Article ID 3957262, 9 pages, 2018.

[18] J.A. Pino, R. Marbot, J Aguero and V. Fuentes, "Essential Oil from Buds and Leaves of Clove *(Syzygium aromaticum* (L.) Merr. et Perry) Grown in Cuba," *Journal of Essential Oil Research*, vol. 13 (July/August), pp.278-279, 2001.

[19] Food and Agriculture Organization 2016," Value of Agricultural Production: Clove," http://faostat3.fao.org/download/Q/QV/E. Accessed 19 Desember 2019.

[20] G. Razafirmamonnjison, M. Jahiel, T. Duclos, P. Ramanoelina, F. Fawbush and P. Danthu, "Bud, leaf, and stem essential oil composition of Syzygium aromaticum from Indonesia, Madagascar and Zanzibar," *Int. J. Basic Appl. Sci.*, vol. 3(3), pp.224-233, 2014.

[21] D. Praseptiangga, S.E. Invicta and L.U. Khasanah, "Sensory and physicochemical characteristics of dark chocolate bar with addition of cinnamon (*Cinnamomum burmannii*) bark oleoresin microcapsule," *Journal of Food Science Technology,* vol. 56, pp.4323-4332, 2019.

[22] S. Nielsen, "*Food Analysis (Fourth Edition),"* Springer, New York, 2010.

[23] M.D.R. Brunetto, Y.D. Cayama, L. Gutiérrez, S.C. Roa, Y.C. Méndez, M. Gallignani, A. Zambrano, A. Gómez, and G. Ramos, "Headspace gas chromatography-mass spectrometry determination of alkylpyrazines in cocoa liquor samples," *Food Chemistry*, vol. 112, pp.253-257, 2009.

[24] C. Proestos and M. Komaitis, " analysis of naturally occurring phenolic compounds in aromatic plants by RP-HPLC coupled to diode array detector (DAD) and GC-MS after silylation," *Foods*, vol. 2(1), pp.90-99. 2013.

[25] R.V. Vazquez, L. Hewson, I. Fisk, D.H. Vila, F.J.H. Mira, I.M. Vicario and J.Hort, "Colour influences sensory perception and liking of orange juice," *Flavour*, vol. 3(1), 2014.

[26] E.A. Afoakwa, "*Chocolate Science and Technology (Second Edition),"* Wiley Blackwell, West Sussex, 2016.

[27] M.I. Dwijatmoko, D. Praseptiangga and D.R.A. Muhammad," Effect of Cinnamon Essential Oils Addition in the Sensory Attributes of Dark Chocolate," *Nusantara Bioscience*, vol. 8(2), pp.301-305, 2016.

[28] C.K. Park, K. Kim, S.J. Jung, M.J. Kim, D.K. Ahn, S.D. Hong, J.S. Kim and S.B. Oh," Molecular Mechanism for Local Anesthetic Action of Eugenol in the Rat Trigeminal System," *International Association for the Study of Pain*, vol. 144, 2009.

[29] V. Briones, J.M. Aguilera, and C. Brown, "Effect of Surface Thopography on Color and Gloss of Chocolate Samples*," Journal of Food Engineering*, vol. 77, pp.776-783, 2006.

[30] E.O. Afoakwa, A. Paterson and M. Fowler, "Factors influencing rheological and textural qualities in chocolate - a review," *Trends Food Sci. Technol*, vol. 18, pp.290-298, 2007.

[31] C.M.G.C. Renard and J.F. Maingonnat, "*Thermal processing of fruit and fruit juices, In: Sun DW (ed) Thermal food processing: new technologies and quality issues, (Second Edition),"* CRC, Boca Raton, 2012.
[32] E.V. Goncalves and S.C.S Lannes," Chocolate Rheology," *Cienc. Tecnol Aliment Campinas*, Vol. 30(4), pp.845-851, 2010.
[33] B.R.R. Rao, "Hydrosol and Water-Soluble Essential Oil of Aromatics Plants: Future Economic Products," *Indian Perfum*, vol. 56, pp.29-33, 2012.
[34] K. Tomi, E. Sakaguchi, S. Ueda, Y. Matsumura, and T. Hayashi, "Physiological and Physicological Effects of Rose 'Wishing' Flowers and Their Hydrosol on the Human Autonomic System and Mood State," *The Holticulture Journal*, vol. 86(10), pp.105-112, 2017.
[35] J. Dai and R.J. Mumper, "Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties," *Molecules*, vol. 15(10), pp.7313-7352, 2010.
[36] M.N.I. Bhuiyan, J. Begum, N.C. Nandi, and F. Akter, "Constituent of the Essential Oil from leaves and buds of Clove (*Syzigium caryophyllatum (L.)* Alston)," *African Plant Science*, vol. 4(11), pp.451-454, 2010.
[37] M.R.C. Raja, V. Srinivasan, S. Selvaraj and S.K. Mahapatra, "Versatile and Sinergistic Potential of Eugenol: A Review," *Pharmaceutica Analytica Acta*, vol. 6(5), 2015.
[38] J. Legault and A. Pichette," Potentiating Effect of β-Caryophyllene on Anti-Cancer Activity of α-Humulane, Isocaryophyllene, and Paclitaxel," *Journal of Pharmacy and Pharmacology*, vol. 59, pp.1643-1647, 2007.
[39] R. Franco, A.O. Astibia and E. Martinez-Pinilla, "Health Benefit of Methyxanthines in Cacao and Chocolate," *Nutrients*, vol. 5, pp.4159-4173, 2013.
[40] P. Molyneux, "The Use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity," Songklanakarin *J of Sci and Technol*, vol. 26, pp.211-219, 2004.
[41] R.S. Bendre, J.D. Rajput, S.D. Bagul and P.S. Karandikar, "Outlooks on Medicinal Properties of Eugenol and Its Synthetic Derivatives," *Natural Product Chemistry and Research*, vol. 4(3), 2016.
[42] F.Francomano, A. Caruso, A. Barbarossa, A. Fazio, C. La Torre, J. Ceramella, R. Mallamaci, C. Saturnino, D. Iacopetta and M. Stefania Sinicropi, "β-Caryophyllene: A Sesquiterpene with Countless Biological Properties," *Appl. Sci.*, vol. 9, 5420, 2019.
[43] W.G. Sullivan, E.M. Wicks, C.P. Koelling," Engineering Economy, "*Pearson* Higher Edu Inc, New Jersey, 2015.