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Evaluation of alkalization process in the making of chocolate substitute from durian seed waste

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Abstract

Previous studies have investigated fermented jackfruit seed and carob flour as potential cocoa powder substitutes, but none have been able to completely replace cocoa powder in food products. The utilization of fermented durian seed as a cocoa powder substitute has shown promise, with the ability to fully substitute cocoa powder in baked brownie production while maintaining good consumer acceptance, although the color was noticeably lighter. In the current research, various fermentation media and alkalization processes were employed with the aim of enhancing the sensory characteristics of the durian seed powder. The fermentation was conducted at room temperature for 7 days under anaerobic conditions, using different ratios of ripe durian pulp and okara (1:0, 1:1, and 0:1) added with 3% *Saccharomyces cerevisiae*. The alkalization process was conducted by using either 3% NaOH, 3% K_2CO_3 or without alkalization. All the alkalized and non-alkalized fermented durian seeds were dried, roasted at 180°C for 40 min, ground, and sieved at 200 mesh. From the sensory analysis, the optimal fermentation medium was 100% ripe durian pulp with the alkalization process. NaOH, as the alkali solution, can enhance the aroma and color of the fermented durian seed powder. The alkalized fermented durian seed that was fermented using 100% ripe durian pulp and alkalization process with 3% NaOH exhibited a chocolate aroma that was not significantly different from that of classic and dark commercial cocoa powders with Chroma value of 20.07±0.38 and °Hue of 67.72±0.45, which fall into the color range of yellow red. The alkalization process was able to enhance the similarity between the cocoa powder substitute made from durian seed and commercial cocoa powder, demonstrating the potential for utilizing durian seed waste as a cocoa powder substitute.

1. Introduction

The demand for cocoa products has continued to increase; however, the production of cocoa was affected by climate change as well as pest infestation. This condition increased cocoa prices (Loullis and Pinakoulaki, 2018; Badan Pusat Statistik, 2023). Cocoa powder substitutes are alternative substances that can partially or completely replace cocoa powder to obtain a similar flavor and aroma of chocolate, especially in the absence of cocoa powder (Medeiros and Lannes, 2009). Several materials have been used as cocoa substitutes, such as fermented jackfruit seed powder and carob flour. However often the substitute cannot be used to completely substitute cocoa powder as it imparts either a sour or astringent aftertaste (Rosa *et al*., 2015; Papa Spada *et al*., 2018; Akdeniz *et al*., 2021). Therefore, it has been a challenge to search for another alternative that can provide a similar flavor and aroma to the cacao bean.

Prior studies have investigated the use of fermented durian seed powder as a cocoa powder substitute, demonstrating its ability to replicate the chocolate aroma and its potential for complete substitution in baked goods with favorable consumer acceptance (Wijayahena and Jayaweera, 2020; Felicia, 2021). However, the durian seed-based cocoa substitute lacks the characteristic brown color of chocolate, which is produced through the Maillard reaction involving protein and reducing sugars (Taş and Gökmen, 2016). This research project aimed to enhance the brown color of the durian seed-derived cocoa substitute by increasing the protein content and

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employing alkalization techniques

Alkalization or Dutch processing is known to enhance the color of cocoa bean after fermentation. This step can also reduce the bitterness and astringency of the cocoa substitute, producing a better cocoa substitute. The extent of alkalization is impacted by the strength, type, and conditions of the alkali solution used (García *et al*., 2020). The most frequently used alkalis during the alkalization process are NaOH and K_2CO_3. Utilizing NaOH is recommended to generate dark-colored cocoa, while K_2CO_3 produces light and reddish-colored cocoa without modifying the flavor profile. This research will explore the use of various alkali sources and concentrations to alkalize durian seed, with the aim of imparting more aroma and color characteristics similar to those of cocoa in the fermented durian seed product.

To further enhance the similarity, okara, a protein-rich substrate, will be incorporated as a fermentation medium in this research. Raw durian seed exhibits a lower protein content compared to cacao beans (Wijayahena and Jayaweera, 2020; Natania and Wijaya, 2022). Okara, a solid byproduct from tofu production, is abundant in protein (20.93%), fiber (21.43%), crude fat (10.31%), and calcium (0.72%). The inclusion of okara is anticipated to facilitate the Maillard reaction during fermentation and the initial roasting stages (Faisal *et al*., 2016).

2. Materials and methods

2.1 Materials

The materials required in this study were durian seed and durian pulp from "Rumah Durian Harum Indonesia", okara from "Susu Kacang Sehat 88 Indonesia", yeast "Fermipan", commercial classic and dark cocoa powder "Oh! Java", distilled water "Amidis", hexane, H_2SO_4 (c), H_2O_2 (35%) "Smart Lab Pro Analysis", NaOH, K_2CO_3, boric acid, selenium, K_2SO_4, bromocresol green-methyl red indicator, and pH buffer 4 and 7 "Merck Pro Analysis".

2.2 Alkalized fermented durian seed powder

The proximate analysis of the okara, raw durian seed, and pulp was done before the main treatment. The okara and durian pulp were sterilized by using an autoclave for 15 min at 121℃ before the fermentation process (Chan *et al*., 2019). The durian seed was cleaned of the remaining pulp and washed. Continued with boiling process at 90℃ for 60 min to remove the mucus, peeled off, and cut into a small size (1×1×1 cm). The fermentation of durian seed was based on the study done by Wijayahena and Jayaweera (2020), with several modifications. The durian seed was added with the fermentation medium (okara or durian pulp or ratio of durian/okara) in a ratio of 2:1 along with the addition of commercial baker yeast (3% w/w). The fermentation was done under anaerobic conditions at room temperature for 7 days. Afterwards, the seeds were cleaned and subjected to an alkalization process.

The alkalization process was conducted by submerging the cleaned seed with either 3% NaOH or 3% K_2CO_3 and heating by using a water bath at 80-85℃ for 1 h (Rodríguez *et al*., 2009; Alasti *et al*., 2020). Afterwards, all the durian seeds were dried at 50℃ for 48 h followed by the roasting process at 180℃ for 40 min, ground, and sieved through 200 mesh.

2.3 Experimental design

The experimental design was a completely randomized two-factorial design with two factors, which were the fermentation medium (ripe durian pulp: okara = 1:0, 1:1, and 0:1) and the alkalization process (no alkalization, alkalization with 3% NaOH, and alkalization with 3% K_2CO_3), with three replications being two replicates. The data were subjected to statistical analysis using IBM SPSS Statistics 25 software.

2.4 Method of analysis

The proximate analysis was conducted on raw durian seed, ripe durian pulp, and okara to determine moisture content (AOAC Official Method 925.09), ash content (AOAC Official Method 942.05), fat content (AOAC Official Method 930.09), and protein content (AOAC Official Method 2001.11) Carbohydrate content was calculated by difference. The resulting fermented durian seed was further analyzed for pH, color, and sensory properties(Li *et al*., 2013; Wijayahena and Jayaweera, 2020; Natania and Wijaya, 2022).

2.5 Sensory analysis

The sensory evaluation was conducted to assess the color and chocolate aroma intensity of the alkalized fermented durian seed product. The assessment was done using scoring tests and multiple paired comparison tests to compare the degree of similarity between the durian seed substitute and commercial classic and dark cocoa powders. The scoring test was ranging from a score of 1 indicates less brown (or no browning, such as the initial color of a fresh product) and a score of 6 indicates a deeper, more significant brown color (indicating a higher degree of change, such as oxidation). Multiple paired comparison test use.

Multiple Comparison Test was conducted to evaluate the relative chocolate aroma intensity of the

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sample compared with a commercial reference product. In multiple comparison test, panelists were instructed to compare the chocolate aroma of each test sample directly with the reference and record their perception using a 7-point relative intensity scale of 1 (Extremely less chocolate aroma/color than the reference), 4 (Comparable to the reference) and 7 (Extremely more chocolate aroma/color than the reference). The sensory evaluation was conducted with 30 semi-trained panelists, who were selected based on their sensitivity and ability to describe flavor. These panelists underwent training using scoring and scalar tests to ensure they had consistent perceptions during the evaluation process (Natania and Milkha, 2018; Natania and Wijaya, 2022).

2.6 pH analysis

The pH analysis was conducted by grinding 5 g of durian seed in 100 mL of water in a beaker. A calibrated pH meter was then submerged in the sample, and the pH value was recorded.

2.7 Color

Color analysis was conducted using a chromameter instrument. The color measurement was performed according to the Hunter L*, a*, b* color system. The chromameter was first calibrated, and the color of the steeping water was recorded by the device. The final analysis yielded the L*, a*, and b* color values.

3. Results and discussion

3.1 Chemical characteristics of durian seed, ripe durian pulp, and okara

The proximate analysis was done on the peeled durian seed. Table 1 shows the durian seed proximate composition. The durian seed that was used in this research contains approximately 36.18±0.81% carbohydrate and 3.06±0.24% protein, similar to previous research (Natania and Wijaya, 2022). The common cacao bean has higher protein up to 21.6±0.83% and slightly lower carbohydrate content of approximately 15.5±0.63% (Bertazzo *et al*., 2013; Nguyen and Nguyen, 2017). The carbohydrates were composed of mono-, oligo-, and polysaccharides, with the highest percentage contributed by cellulose and starch. The glucose content of the cacao beans from various geographical locations varied between 0.42-2.76% (Bertazzo *et al*., 2013). In comparison, durian seed carbohydrate contains approximately 45% of glucose (Amid *et al*., 2012). By using the previous information, the amount of glucose in durian seed that was used in this study was higher compared to the amount of glucose present in the cacao beans (Abdullah *et al*., 2021; Hasanuddin, 2021).

Carbohydrates and proteins contribute to the flavor development in chocolate processing. During the fermentation process, the microbes present will use carbohydrates as an energy source and carbon to be converted into smaller reducing sugar compounds (Assi-Clair *et al*., 2019). The protein will be broken down into free amino acids that act as precursor compounds of chocolate aroma, flavor, and bioactive amines (Junior *et al*., 2021). The reducing sugar and amino acid will later undergo a Maillard reaction in the drying and roasting process, which then contributes to the intense cocoa aroma and color (Bertazzo *et al*., 2013; García *et al*., 2020).

The protein content of the durian seed was lower compared to the cacao beans. According to Abdullah (2022), the amino acids that act as the special precursors for the cocoa aroma formation are hydrophobic amino acids such as alanine, tyrosine, valine, isoleucine, leucine, and phenylalanine. These amino acids are also present among the major amino acids that make up the durian seed protein. The amount of each amino acid in the durian seed protein was as follows: alanine (5.24-6.14%), tyrosine (1.63-2.19%), valine (4.5-5.50%), isoleucine (3.30-4.07%), leucine (30.9-37.3%), and phenylalanine (3.11-9.04%) (Amid *et al*. (2012). The amino acid will be degraded during the roasting process, forming aldehydes, ketones, pyrazines, and furans, which influence the chocolate aroma. From the preliminary study, the chocolate substitute made from durian seed has a pleasant aroma like mild cocoa powder.

The proximate composition of the ripe durian pulp used in this research can be seen in Table 1. Originally, cacao pulp was used as the substrate in the fermentation of the cacao bean. Cacao pulp has similar proximate characteristics, which are high moisture (87.06%) and carbohydrate (11.03%), and it is low in protein (0.31%), lipid (0.12%), and ash (1.48%) (Nguyen and Nguyen,

Table 1. Chemical characteristics of raw durian seed, durian pulp, and okara.

Parameter	Raw durian seed	Ripe durian pulp	Okara
Moisture content (%, wb)	59.19±0.55	76.62±1.22	80.31±0.14
Protein content (%, wb)	3.06±0.24	2.70±0.06	6.09±0.05
Ash content (%, wb)	1.22±0.04	0.21±0.02	0.16±0.00
Fat content (%, wb)	0.35±0.07	1.53±0.03	1.41±0.13
Carbohydrate (by difference) (%, wb)	36.18±0.81	18.92±1.27	12.03±0.32

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2017). These compositions are quite similar to the ripe durian pulp used in this research.

The yeast and lactic acid bacteria from natural sources will convert sugar to ethanol and lactic acid. The metabolism of yeast and lactic acid bacteria will also increase the acidity and temperature of the medium. This condition will then induce the protein degradation into amino acids, which become the aroma precursor compounds. The microorganism that is present in the natural fermentation of durian pulp is lactic acid bacteria (Abdullah *et al*., 2021). Due to the similarity of the content of ripe durian pulp with cacao pulp, durian pulp will have the potency to be used as the fermentation medium.

The proximate composition of the Okara can be seen in Table 1. Okara, a soybean byproduct, is rich in carbohydrates and high in protein content. The protein content in okara is rich in phenylalanine. Fermentation of okara with *S. cerevisiae* produced 2-phenylethanol due to the degradation of several amino acids, which are valine, isoleucine, leucine, and phenylalanine (Vong and Liu, 2017). According to Chetschik *et al*. (2017), 2-phenylethanol is one of the key odorants of chocolate aroma that is characterized by a flowery and sweet scent. It is produced by the Ehrlich degradation of phenylalanine in a medium with the presence of yeast.

3.2 pH of fermented durian seed powder

There was an interaction between the type of medium and the alkalization process toward the pH ($p \leq 0.05$). Figure 1 shows that the fermented durian seed powder had a higher pH when okara was added to the fermentation medium, especially using NaOH as an alkali. NaOH was a strong base, thus providing higher alkalinity compared to potassium carbonate (García *et al*., 2020). Later during fermentation, according to Hasanuddin (2021), the microorganism present in the natural fermentation, the lactic acid bacteria (LAB), will metabolize sugar and produce lactic acid, which results in decreasing pH. The pH values of the fermented durian seed varied between 5.15 to 7.22 for durian seed without alkalization and 10-11 for the fermented durian seed with alkalization. Based on the pH value, cocoa powder with a pH of 5-6 was categorized as natural cocoa, pH 6-7.2 as lightly alkalized, pH 7.2-7.6 as moderately alkalized, and pH over 7.6 as strongly alkalized (Alasti *et al*., 2020). The fermented durian seed powder falls in the category of a strongly alkalized product. The presence of sugar in okara was lower compared to ripe durian pulp (8.12-14.05% wb), however, according to Chan *et al*. (2019), okara contains a small number of sucrose (1.042±0.016% db) compared to durian pulp, thus the pH of durian seed using fermented durian pulp will be lower than the one using okara which can be seen from the relatively lower pH of durian seed fermented with increasing amounts of durian pulp.

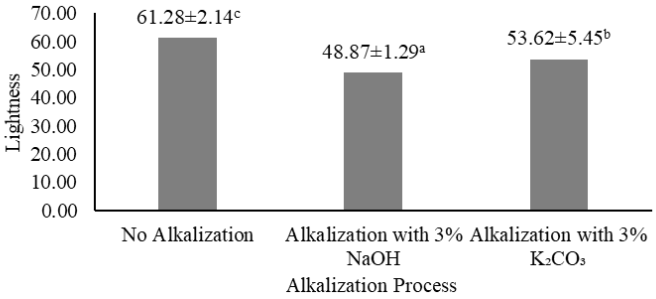

Figure 1. Effect of alkalization process on fermented durian seed powder lightness. Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

3.3 Color of fermented durian seed powder

The color analysis revealed that durian seed powder exhibited a yellow-red hue (Table 2). In contrast, the classic and dark commercial cocoa powders had a °Hue and chroma values of 50.08±0.11 and 14.87±0.08, and 42.62±0.09 and 11.52±0.06, respectively, indicating a red color range. The statistical analysis demonstrated that the alkalization process significantly affected the hue range, with a lower value corresponding to a more reddish tone. Additionally, the use of okara as a medium resulted in a decreased chroma value, which was

Table 2. °Hue of non-alkalized and alkalized fermented durian seed powder.

Fermentation medium	Alkalization process	°Hue	°Chroma value	Scoring color
Durian:okara (1:0)	No alkalization	76.42±1.13	20.06±1.64^{b}	2.37±0.93^{b}
	Alkalization with 3% NaOH	67.72±0.45	20.07±0.38^{b}	5.77±0.57^{f}
	Alkalization with 3% K_2CO_3	66.26±1.49	16.43±0.41^{a}	4.20±0.71^{d}
Durian:okara (1:1)	No alkalization	76.24±1.67	19.83±1.04^{b}	2.13±0.82^{ab}
	Alkalization with 3% NaOH	66.36±0.73	21.84±0.17^{b}	4.97±0.49^{e}
	Alkalization with 3% K_2CO_3	59.74±1.63	16.41±0.28^{a}	3.77±0.90^{c}
Durian:okara (0:1)	No alkalization	67.08±3.73	15.46±2.47^{a}	1.90±0.84^{a}
	Alkalization with 3% NaOH	60.31±0.37	21.76±0.31^{b}	4.90±0.66^{e}
	Alkalization with 3% K_2CO_3	55.11±2.81	16.94±1.07^{a}	4.57±0.73^{de}

Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

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attributed to the higher availability of amino acids that could participate in the Maillard reaction, thereby producing more brown-colored compounds. The greater the amount of ripe durian used, the more yellow the resulting fermented durian seed powder became. The pigment in ripe durian pulp was carotenoids, with the major carotenoids being α-carotene and β-carotene, and the minor carotenoids being lutein and zeaxanthin. Lutein and zeaxanthin are polar carotenoids, which likely contributed to the yellowish hue of the powder (Taş and Gökmen, 2016). The greater the amount of ripe durian used, the yellower the resulting fermented durian seed powder obtained. The pigment in ripe durian pulp was carotenoids, with the major carotenoids being α-carotene and β-carotene, and the minor carotenoids being lutein and zeaxanthin. Lutein and zeaxanthin are polar carotenoids, which likely contributed to the yellowish hue of the powder (Wisutiamonkul *et al*., 2015).

According to Rodríguez *et al*. (2009), cocoa color is also influenced by the activity of the polyphenol oxidase enzyme, which exhibits optimal performance at pH 8.0. PPO oxidizes polyphenolic compounds, leading to the formation of a brown pigment called melanoidins. As the pH increases, the conversion of polyphenolic substances to melanoidins continues, with higher pH resulting in a darker color. Since PPO enzyme is naturally present in durian seed, the increasing pH will stimulate the PPO enzyme to actively oxidize the phenolic compounds into o-quinones, which have a brown hue (Malini *et al*., 2016).

Another indicator of the increased darkness seen in this experiment was the lightness index. From the statistical analysis, there was a significant effect ($p \leq 0.05$) of the alkalization process on the color of the fermented durian seed. Table 2 showed that the fermented durian seed that had undergone the alkalization process had a significantly ($p \leq 0.05$) lower lightness value, and alkalization with 3% NaOH had a lower ($p \leq 0.05$) lightness value compared to 3% K_2CO_3. A similar result was obtained by García *et al*. (2021), where darker cocoa powder was obtained through alkalization with NaOH compared to K_2CO_3. NaOH and K_2CO_3 have different basic strengths, which makes the Maillard reaction during the alkalization process subject to different pH levels. The alkalized durian seed showed a 48.87±1.29 in lightness, which is close to the lightness value of the commercial classic and dark cocoa powder (approximately 40.19±0.01 and 37.38±0.00, respectively). This showed a promising result that the cocoa substitute showed to have a darker hue after alkalization treatment.

3.4 Sensory analysis of the fermented durian seed powder color

A multiple comparison test (MCT) was conducted to compare the degree of color similarity of the fermented durian seed powder with classic and dark commercial cocoa powder. As shown in Figure 2, the fermented durian seed powder had a statistically significantly lighter brown color compared to both types of commercial cocoa powder. The fermented durian seed powder with 100% ripe durian pulp had an MCT value of 3.23±0.77, which was categorized as "slightly less brown". Commercial cocoa powder exhibited a darker color than both alkalized and non-alkalized durian seed powder. This difference may be attributed to the higher protein content of cacao beans compared to durian seeds, which could provide more free amino acids for the Maillard reaction. The greater availability of free amino acids in the presence of reducing sugars may allow the Maillard reaction to proceed for a longer duration, resulting in the production of more melanoidins and a darker brown color (Nguyen and Nguyen, 2017).

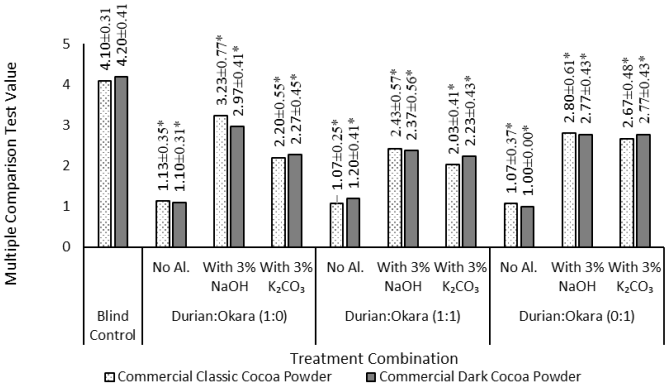

Figure 2. Effect of fermentation medium and alkalization process towards the degree of color similarity with classic and dark cocoa powder.

* Statistically significantly difference ($p \leq 0.05$) compared to control.

The classic and dark commercial cocoa powders exhibited slightly to moderately alkaline pH levels, indicating that they had undergone an alkalization process during their production, which contributed to the darkening of their color. (García *et al*., 2020). Based on the color sensory analysis, ripe durian pulp and okara with a ratio of 1:0 followed by an alkalization process using 3% NaOH can produce the closest darker color in the scoring result and showed to be comparable to the commercial cocoa powder based on the MCT result.

3.5 Sensory analysis of the fermented durian seed powder aroma

According to Figure 3, the alkalization process appears to impact the aroma of the cocoa substitute. Panelists perceived a higher aroma scoring value,

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indicating a stronger chocolate aroma, for the alkalized samples. This aligns with the findings of Huang and Barringer, who reported that the alkalization process increases the volatile compounds in cocoa, particularly for samples that underwent alkalization prior to roasting, thereby enhancing the cocoa aroma intensity. Statistical analysis further revealed a significant effect of the individual alkalization process on the chocolate aroma scoring value.

The aroma of chocolate is enhanced during the roasting process through the Maillard reaction. The production of dicarbonyl compounds that react with amino acids to form Strecker aldehydes and aminoketones is increased in a basic pH environment. These intermediates are then converted to alkyl pyrazines, which are key cocoa aroma compounds, through dimerization. Therefore, a higher pH accelerates the formation of alkyl pyrazine precursors in the initial stage of the Maillard reaction, ultimately leading to increased amounts of these important cocoa odorants in the final product. Alkalized cocoa nibs prior to roasting exhibit a higher pH, which accelerates the Maillard reaction and consequently increases the production of alkyl pyrazines, the key odorant compounds responsible for the cocoa aroma (Huang and Barringer, 2010).

The research done by Sioriki *et al*. (2021) The research by Sioriki et al. also demonstrated an increase in ketones following the alkalization process. This increase may be attributed to the accelerated oxidation of alcohols to ketones during alkalization. Additionally, Figure 3 revealed that alkalization using NaOH resulted in a significantly higher chocolate aroma score compared to alkalization with K_2CO_3. As mentioned previously, a basic pH accelerates the formation of alkyl pyrazines, which are key compounds in the Maillard reaction (Huang and Barringer, 2010). Alkalization using NaOH generates a higher pH compared to K_2CO_3, implying that the fermented durian seed treated with NaOH had a more alkaline environment, thereby promoting the Maillard reaction at an elevated rate.

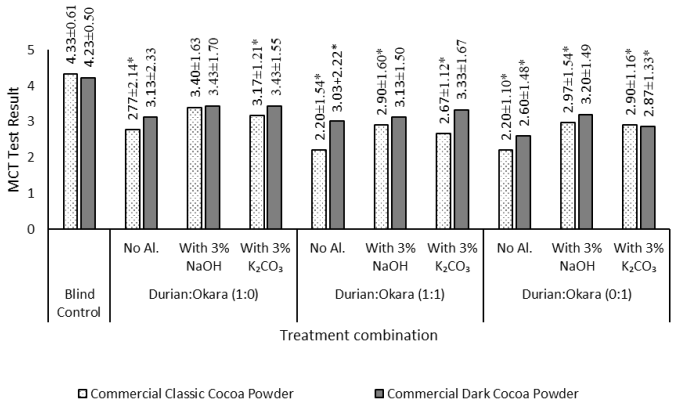

Figure 3. Effect of fermentation medium and alkalization process towards the degree of chocolate aroma similarity with classic and dark cocoa powder.
* statistically significantly difference ($p \leq 0.05$) compared to control.

A comparative analysis was conducted to evaluate the similarity in chocolate aroma profiles between fermented durian seed powder and commercial classic and dark cocoa powder. Based on the MCT results presented in Figure 2, several durian seed powder samples exhibited aroma characteristics comparable to the commercial chocolate controls. As previously discussed, the alkalization process enhanced the chocolate aroma attributes, as reflected in the sensory scoring data. The optimal treatment combination was durian seed fermented with a 1:0 ratio of ripe durian pulp to okara, followed by a 3% NaOH alkalization process. This treatment yielded the highest sensory score and demonstrated no statistically significant difference in chocolate aroma when compared to both commercial cocoa powder references.

4. Conclusion

The research indicated that alkalization can enhance the similarity between chocolate substitutes produced from fermented durian seed and commercial chocolate powder. Fermentation with 100% ripe durian pulp proved to be the most suitable medium for durian seed fermentation in terms of aroma and resemblance to commercial chocolate powder. However, the addition of Okara did not contribute to improved aroma and flavor of the durian seed-based chocolate substitute. Furthermore, alkalization with NaOH was effective in darkening the color and enhancing the chocolate aroma of the fermented durian seed powder. The alkalized fermented durian seed powder, produced using 100% ripe durian pulp and 3% NaOH, exhibited a chocolate aroma highly comparable to classic and dark commercial cocoa powder, as evidenced by its Chroma value of 20.07±0.38 and °Hue of 67.72±0.45, which fell within the yellow-red color range.

Conflict of interest

The authors declare no conflict of interest.

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