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Development and Characterization of New Vegan Ice Cream from Tempeh Milk Enriched with Moringa Leaf Flour

Fransisca[1#], Rike Tri Kumala Dewi[1], Clarissa Christie Harimas[1]

[1] *Food Business Technology, Universitas Prasetiya Mulya, BSD, Tangerang, Banten, 15339, Indonesia*

[#]*Corresponding author: E-mail: fransisca.wijaya@prasetiyamulya.ac.id*

Abstract— **Vegan ice cream is among the currently trending plant-based products, but not all can meet nutritional needs, specifically protein and calcium, which are often found in milk. An effective strategy to address this problem is by adding moringa leaf flour (MLF) and tempeh milk, which are rich in protein and calcium. Therefore, this study aims to develop high-calcium vegan ice cream from tempeh milk enriched with MLF. The experimental method was divided into characterization of MLF and tempeh milk, physical characterization and sensory evaluation of ice cream with Check All That Apply (CATA) method, and chemical characterization for the most preferred recipe. The selected MLF concentrations included 4%, 5%, 6%, and 7%, where ice cream with a higher concentration had harder and stickier texture, as well as a higher melting time, and a smaller overrun value than counterparts developed using less MLF. Sensory evaluation was conducted with 50 untrained panelists, while the must-not-have properties of MLF ice cream were a dark green color, a solid appearance, a bitter taste and aftertaste, greeny flavor, and a hard texture. The most preferred formulation was ice cream containing 4% MLF, with characteristics such as 68.9% water, 0.59% ash, 2.38% protein, 5.43% fat, 22.70% carbohydrate, calcium 93.13 mg/100 g, and iron 1.02 mg/100 g. The best formula did not meet the regulation of calcium and protein source because of the sensory acceptance limitation.**

Keywords— **Calcium, Moringa leaf flour, Protein, Tempeh milk, Vegan ice cream**

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I. INTRODUCTION

Indonesia is sixth among the countries with highest prevalence of malnutrition in Southeast Asia and 17.7 million or 6.5% of the national population is recorded as sufferers [1]. A systematic investigation by Agustina et al. [2] including 18 studies on 5395 Indonesian adults (>18 years old) found that calcium and protein intake was significantly below the recommended levels, suggesting a serious inadequacy in dietary macro and micronutrient consumption among this population. Another similar investigation on the adolescent population conducted by Sandjaja et al. [3], Arini et al. [4], and Sari et al. [5] detected serious problems of protein and calcium deficiency. A report from UNICEF [6] shows 27.7% prevalence of stunting in children under 5 years old in Indonesia, which is considered a very high number because World Health Organization (WHO) has set a maximum global stunting prevalence target of 20% by 2025. These results show that Indonesians really require access to nutritious food sources suitable for consumption and consistent with daily nutritional needs, specifically protein and calcium.

In Indonesia, the majority of products with high protein and calcium content are classified as animal-based, including milk, yogurt, and cheese. However, there is a non-negligible proportion of the population experiencing intolerance or allergic reactions to animal-based products [7]. Lactose intolerance is a quite common type, where consumption of lactose can trigger symptoms such as stomach pain, abdominal distension, vomiting, flatulence, and diarrhea [8]. A study by Hegar and Widodo [9] on children aged 3-15 years old found a prevalence of lactose intolerance reaching 56.2%. In connection with health problems such as allergies or intolerance (15%) and increasing public awareness of the importance of health (53%), there is a rise in demand for plant-based food products. Data show that 73% of 11,657 respondents have consumed non-animal products several times a week. A plant-based category with the highest demand is alternative milk products, where 86% of respondents have tried plant-based milk as an innovation [10]. This shows significant opportunities for innovation in plant-based products consumed as substitutes for animal products to meet nutritional needs, including protein and calcium.

An example of the possible innovative food products commonly developed is ice cream, which has experienced significant growth and increased gradually from 2017 to 2021. Sales of ice cream in Indonesia in 2021 only reached US$425 million [11]. Plant-based ice cream can be prepared from tempeh milk, which is selected as a better alternative to soy milk because of the higher digestibility level caused by the fermentation process during the production [12]. Tempeh milk contains fat, protein, and fiber that provide important health benefits [13], with isoflavones content offering antioxidant effects and anti-cancer potentials [14] . However, it does not have high levels of protein and calcium compared to cow's milk.

The ingredients that have the potential to be a source of protein and calcium include moringa leaves. Processed moringa leaf flour (MLF) contains approximately 22.42-30.3% protein [15-17] and high levels of minerals, specifically calcium of around 1443 - 2000 mg/100 g [15], [18] as well as other nutrients important for body health. Gopalakrishnan et al. [19] reported MLF with content of iron, magnesium, and vitamin C capable of providing additional health benefits. Several studies using MLF as protein and calcium fortification in products including biscuits [20], wet noodles [21], bread [15], and yogurt [22] have shown the increase of nutrients. MLF can be combined with tempeh milk in preparing food products that are lactose-free and suitable for vegans. Therefore, the combination of MLF rich in protein and calcium with tempeh milk which contains a high level of digestibility as well as beneficial fat, protein, vitamin B12, and isoflavone, is a good choice in preparing healthy and nutritious ice cream to meet the needs of vegan and non-vegan consumers.

Tempeh milk and MLF ice cream is particularly important for vegan consumers avoiding all animal-derived products, such as dairy. Vegans require plant-based alternatives that fully meet the nutritional needs, compared to vegetarians capable of consuming dairy products. This ice cream does not contain any animal-derived ingredients and can be enjoyed as a healthy plant-based option by non-vegan and vegetarian consumers.

Ice cream evaluated in this study was prepared from MLF and tempeh milk. Optimization was conducted by formulating MLF due to being the most contributing ingredients to ice cream flavor. The purpose of the optimization was to obtain the best formula for an ice cream with basic ingredients of tempeh milk and MLF that were accepted by consumers. In addition, physical and chemical characteristics, nutrient contents, and sensory qualities were analyzed.

II. MATERIALS AND METHODS

A. *Materials*

Materials used in this study were tempeh, MLF (PopGreen Store in Bandung), coconut milk, xanthan gum, and guar gum. Others included soy lecithin, sugar, vanilla extract, and commercial vegan ice cream matcha flavor (Cream Fiction).

B. *Methods*

This study was divided into several steps, namely tempeh milk production and raw material characterization (tempeh milk and MLF). Other steps included physical characterization of ice cream, sensory evaluation, and chemical characterization of the best formula.

Tempeh milk production

The processing of tempeh milk followed the method from Triastuti et. al. with modification [23]. Tempeh was steamed for 25 minutes, then blended with hot water (70ºC) in a ratio of 1:3 (tempeh:water) until smooth, and the mixture was filtered by cheesecloth.

Raw material characterization

Tempeh milk and MLF were tested for pH, protein, folic acid, iron, and calcium content. Other tests conducted on tempeh milk included the evaluation of soluble solid content and proximate analysis of water, ash, protein, fat, and carbohydrate. The proximate testing for water and ash content through gravimetry was carried out with oven heating and furnace [24]. Protein and fat were determined by using Kjeldahl method and Soxhlet extraction [25], respectively, while carbohydrate content was calculated by difference.

Formulation and physical characterization of MLF ice cream

The formula used for ice cream production are comprehensively presented in **Table 1.** The production process started with blending all dry ingredients, followed by the addition of wet ingredients into the blender around 2 minutes. The mixture was cooked until 72ºC for 30 s, then frozen for 10 minutes, transferred into ice cream maker, and churned for 2 hours. Physical characteristics were analyzed for all samples, except that overrun was not measured for the commercial (sample K). The physical characteristics tests included the evaluation of overrun value [26] (except for sample K), melting rate [27], viscosity [28], and hardness as well as adhesiveness with modification [23], which were carried out in triplicate. The commercial ice cream (sample K) was only used for the comparison of physical characteristics. The list and images of ice cream samples for physical characterization are presented in **Table 2** and in **Figure 1**, respectively.

TABLE 1
MLF ICE CREAM RECIPE

Component	Quantity
Moringa leaf flour	4%, 5%, 6%, 7%
Tempeh milk : coconut milk	2 : 1
Sugar	17.5%
Xanthan Gum	0.125%
Guar Gum	0.375%
Soy lecithin	1%
Vanilla extract	0.3%

TABLE 2
LIST OF SAMPLES FOR PHYSICAL CHARACTERIZATION

Sample	Product code
TéLor ice cream with 4% (w/b) MLF	A
TéLor ice cream with 5% (w/b) MLF	B
TéLor ice cream with 6% (w/b) MLF	C
TéLor ice cream with 7% (w/b) MLF	D
Commercial vegan ice cream (for physical comparison only)	K

K A B C D

Fig. 1**.** The appearance of ice cream products (left to right) K: commercial, A: 4% (w/b) MLF, B: 5% (w/b) MLF, C: 6% (w/b) MLF, D: 7% (w/b) MLF

Sensory testing and chemical characterization of the selected formulas

Sensory testing was carried out for four ice cream formulas (ABCD), except the commercial product, using Check All That Apply (CATA) method which started by conducting Focus Group Discussion (FGD) to generate the attributes of MLF ice cream. FGD was conducted by five consumers who like ice cream or other food products with matcha flavor and have an understanding of general sensory characteristics. Furthermore, CATA test was conducted with 50 untrained panelists (22 males and 28 females), aged between 20 and 35 years, who were regular consumers of vegan ice cream with a minimum consumption frequency of once per month and a preference for matcha flavor. Each panelist received the set of samples (except K) presented in **Figure 1** and mineral water was given as a palette cleanser. Before sensory evaluation, respondents completed a consent form declaring no known allergies to the ingredients, absence of health issues, and voluntarily participation in this test.

During CATA analysis, panelists were firstly asked questions regarding the ideal sensory attributes that should be present in a vegan ice cream product by ticking the attributes before tasting the samples. Subsequently, the panelists were asked to rate the preference for the samples on a 7-point scale from very dislike (1) to very like (7) and assess the attributes present in each sample by ticking after tasting. The list of attributes used is comprehensively presented in **Table 3**. The most preferred product from sensory test results was analyzed through proximate tests, as well as the evaluation of saturated fat, sodium, total sugar, cholesterol, calcium, iron, and total solids in two repetitions.

TABLE 3
LIST OF ATTRIBUTES

Attributes		
Appear-ance (Ap)	light green color	pale/soft green color
	dark green color	deep/intense green color
	solid	compact, dense and firm appearance
	soft	light, smooth and easily deformable
	gritty	uneven, coarse or grainy particles on the surface of the products
Aroma (Ar)	leafy	green, fresh, vegetal aroma
Taste/ Flavor (T/F)	bitter	bitter taste
	sweet	sweet taste
	pungent	sharp/spicy/irritating oral sensation during mastication
	sour	sour taste
	greeny	raw, leafy or grassy taste
Mouth-feel/ Texture (M/Te)	creamy	smooth, rich and lubricating oral sensation
	sandy	coarse sensation in the mouth
	soft	smooth sensation in the mouth
	hard	force to bite or scoop
Aftertaste (Af)	sweet	a lingering sweet taste in the mouth after swallowing
	bitter	a lingering bitter after swallowing
	pungent	a sharp/spicy throat sensation after swallowing
	astringent	a residual drying or puckering mouthfeel
	sandy	a lingering grainy or particulate sensation on the tongue
	sticking to the throat	throat discomfort or lingering mouthfeel

Data analysis

CATA data were analyzed with XL STAT Software version 2022.4.1.1373 (Lumivero, New York, NY, USA) including Cochran's Q test and *correspondence analysis.* Additionally, analysis of variance (ANOVA) test was conducted for the examination of preference data.

III. RESULT AND DISCUSSION

Characterization of tempeh milk and MLF

Table 4 presents the results of tempeh milk characterization. A total of 100 mL of tempeh milk was found to contain 4.05 mg calcium, but iron and folic acid were undetectable by limit of detection for 50 mcg/100 mL. This was attributed to soaking and boiling soybean which could cause a significant loss of folic acid during tempeh preparation [29]. Additionally, the steaming process of tempeh led to another loss of folic acid [30]. Water content in tempeh milk used was significantly high and fat content was very low. These characteristics could reduce the quality of ice cream, making the crystals extremely big and less creamy. To overcome this problem, stabilizers and emulsifiers were used to enhance the mouthfeel [31].

TABLE 4
CHARACTERIZATION OF TEMPEH MILK AND MLF

Parameter	Unit	Tempeh milk	Moringa leaf flour
Water content	%	98.14	not evaluated
Ash	%	0.13	not evaluated
Protein	%	0.68	27.13
Fat	%	0.67	not evaluated
Carbohydrate	%	0.38	not evaluated
pH	-	6.24	5.65
Dissolved solids	% brix	2%	not evaluated
Folic acid	mcg	not detected	not detected
Iron (Fe)	mg/100 g	not detected	12.62
Calcium (Ca)	mg/100 g	4.05	2317.65

Figure 2 shows the applied MLF, while **Table 5** presents the high level of protein and calcium content. Compared to other plant-based sources, protein found in MLF was higher than in Chickpea (20-25%) [32] and Cowpea (23%) [33], yet lower than soybean (37%) [34]. However, calcium in MLF in this study exceeded the level examined by Yun et. al. [35] which was only 1932±22.23 mg/100 g. Calcium content in MLF was higher than in cow's milk (113-134 mg/100 g) [36] and soy milk (25 mg/100 g). The observations show that MLF is a potential material to increase the accessibility of calcium [37].

Fig. 2. MLF

Characterization of ice cream

Physical characteristics of ice cream samples

Table 5 shows the results of the physical characterization of ice cream. There has been a trend caused by the different percentages of MLF used in every ice cream sample. A high percentage of MLF tends to generate poor ice cream physical characteristics, such as being very hard and low in overrun.

Melting rate, viscosity, and overrun.

Melting rate is a significant consumer sensation parameter that influences flavor release and mouthfeel [38]. Ice crystals are melted because of heat transfer from the heated air that envelops the product into ice cream. Firstly, ice melts at the outermost layer of ice cream, leading to a localized chilling effect in the immediate vicinity of the melting ice [39].

TABLE 5
PHYSICAL CHARACTERIZATION OF ICE CREAM

Sample	Melting Rate (g/min)	Viscosity (cP)	Overrun (%)	Hardness (N)	Adhesiveness (N)
A	0.364 ± 0.014^a	10426.67 ± 323.32^b	40.72 ± 7.46^a	9.364 ± 0.540^c	-0.506 ± 0.137^a
B	0.345 ± 0.018^a	9880.00 ± 160.00^c	27.63 ± 0.48^b	12.489 ± 2.202^b	-0.720 ± 0.136^b
C	0.339 ± 0.010^a	10713.33 ± 431.90^b	18.57 ± 1.95^c	13.722 ± 0.840^b	-0.840 ± 0.100^b
D	0.320 ± 0.015^a	11366.67 ± 375.41^a	16.23 ± 1.78^c	21.165 ± 2.064^a	-1.170 ± 0.139^c
K	0.369 ± 0.037^a	5746.67 ± 230.07^d	-	8.410 ± 2.023^c	-0.401 ± 0.173^a

Note: $^{a-d}$ different superscripts in one column represent significant differences between samples (P≤0.05). K: Competitor, A: 4% (w/b) MLF, B: 5% (w/b) MLF, C: 6% (w/b) MLF, D: 7% (w/b) MLF

The melting rate results showed that all products were not significantly different ($p \leq 0.05$). However, a trend showed a decrease in the melting rate with increasing MLF. This supported the previous studies stating that the more flour was used in ice cream dough, the slower the melting rate [26], [27]. The increase was attributed to fat globules in MLF, which could slow down heat transfer through ice cream and stabilize air bubbles in the structure, reducing the melting rate [40]. In this study, fat content in MLF was not evaluated, but a previous investigation reported MLF with a relatively high fat content of 10.42% [41]. Compared to the melting rate, viscosity tends to increase with higher concentrations of MLF, as shown in sample D containing the highest viscosity. These results were consistent with the report by Abdeldaiem et al. [40] that the more flour was used in ice cream, the more viscous ice cream dough became. This could be caused by protein in MLF with ability to bind water, making the dough thicker, and the addition of solids tended increase viscosity [21], [22], [42]. Increased dry matter content may facilitate the formation of additional hydrophilic groups, which bond free water in ice cream, thereby increasing the viscosity and delaying the melting of the mixture [43]. The large value of viscosity of ice cream dough impacts overrun value because the more viscous the dough, the harder air is incorporated during mixing [44]. Overrun refers to the amount of air incorporated into ice cream. A high overrun value means that ice cream has more spaces of air within it. A viscous or thick dough causes difficulty for air to enter during churning. The overrun of ice cream tends to lower as viscosity increases because of high MLF concentration. The overrun value of ice cream has not reached satisfactory levels because the ideal level is between 75-100% [26]. There are two possible strategies to increase the overrun value, namely by adding more fat or reducing the stabilizer. The addition of fat enables the formation of more bubbles in ice cream to insert more air, generating a higher overrun value [45]. Meanwhile, reducing the stabilizer can decrease viscosity to enable easier incorporation of air into ice cream [46].

Hardness and Adhesiveness.
The hardness of all ice cream was significantly different, except between samples A and K. Product A showed no significant difference in hardness compared to commercial product K, indicating similar texture characteristics. The hardness of ice cream increases along with increasing concentrations of MLF. A greater volume of a solid dispersed phase in a composite material leads to increased resistance to an applied force. This is attributed to the polar group of the moringa leaf protein interacting strongly with the stabilizer gum used [46]. Additionally, the hardness of ice cream is impacted by the overrun value. The high MLF content can increase density and lower overrun value to make ice cream harder. Adhesiveness of ice cream rises due to a gelatinization of starch and a stronger protein bond, generating a stickier texture [46].

Sensory analysis
Panellists in FGD identified 21 sensory characteristics, as presented in **Table 6**. To complie ice cream profilling, correspondence analysis and the Cochran's Q test were conducted [47]. The p-values generated by the Cochran's Q test for ice cream (**Table 6**) showed the comparison between each product sample and sensory characteristic. An observed characteristic is considered significantly different from the others when the p-value is less than the 5% significance level [48]. Based on the results of the Cochran's Q test, the characteristics that were not significantly different included leafy aroma, sweet taste, pungent taste, creamy mouthfeel, sweet aftertaste, pungent aftertaste, astringent aftertaste, and sticking to the throat aftertaste. Being insignificantly different implied that all of the characteristics were present in the entire samples and panellists did not perceive differences between the samples. The leafy aroma and the sweet taste were perceived by more than 40% of panellists and not considered significantly different, suggesting that the two characteristics were present in all of the samples. Meanwhile, the attributes perceived to be significantly different were light and dark green color, solid appearance, soft appearance, gritty appearance, bitter taste, sour taste, greeny flavor, sandy mouthfeel, soft texture, hard texture, and bitter aftertaste.

TABLE 6
COCHRAN'S Q TEST OF MLF ICE CREAM

Attributes	p-values	A	B	C	D
Light green color	**<0.0001**	0.600 (c)	0.280 (b)	0.200 (ab)	0.020 (a)
Dark green color	**<0.0001**	0.360 (a)	0.720 (b)	0.800 (bc)	0.980 (c)
Solid appearance	**<0.0001**	0.320 (a)	0.580 (b)	0.660 (b)	0.740 (b)
Soft appearance	**<0.0001**	0.420 (b)	0.420 (b)	0.200 (a)	0.080 (a)
Gritty appearance	**0.04**	0.360 (a)	0.320 (a)	0.360 (a)	0.540 (a)
Leafy aroma	0.179	0.440 (a)	0.580 (a)	0.560 (a)	0.620 (a)
Bitter taste	**0.018**	0.320 (a)	0.340 (a)	0.500 (a)	0.540 (a)
Sweet taste	0.44	0.780 (a)	0.740 (a)	0.660 (a)	0.680 (a)
Pungent taste	0.072	0.080 (a)	0.120 (a)	0.020 (a)	0.140 (a)
Sour Taste	**0.017**	0.080 (a)	0.120 (ab)	0.240 (b)	0.220 (ab)
Greeny/leafy flavor	**0.03**	0.580 (a)	0.640 (ab)	0.620 (ab)	0.800 (b)
Creamy mouthfeel	0.225	0.540 (a)	0.500 (a)	0.380 (a)	0.400 (a)
Sandy mouthfeel	**0.004**	0.180 (a)	0.360 (ab)	0.440 (b)	0.460 (b)
Soft texture	**<0.0001**	0.700 (bc)	0.740 (c)	0.480 (ab)	0.300 (a)

Hard texture	**0.002**	0.160 (ab)	0.140 (a)	0.360 (bc)	0.380 (c)
Sweet aftertaste	0.094	0.520 (a)	0.380 (a)	0.360 (a)	0.300 (a)
Bitter aftertaste	**0.002**	0.300 (a)	0.300 (a)	0.420 (ab)	0.580 (b)
Pungent aftertaste	0.075	0.180 (a)	0.100 (a)	0.040 (a)	0.100 (a)
Astringent aftertaste	0.31	0.280 (a)	0.400 (a)	0.280 (a)	0.380 (a)
Sandy aftertaste	0.288	0.160 (a)	0.280 (a)	0.280 (a)	0.280 (a)
Sticking to the throat aftertaste	0.742	0.500 (a)	0.500 (a)	0.460 (a)	0.560 (a)

a-d different superscripts in one row represent significant difference between samples (p≤0.05)

Sensory profile of MLF ice cream

Sensory characteristics of ice cream samples were visualized using multiple graphical representations. As presented in **Figure 3** and **4**, any of MLF-based ice cream products did not have a sensory profile that closely matched the ideal product. According to panelist evaluations, the ideal vegan ice cream is characterized by a sweet aftertaste, soft appearance, and smooth texture (**Figure 4).** These results indicate that the sensory attributes expected of vegan ice cream are comparable to those of regular dairy ice cream. The results are related to previous studies, such as Gorman et al. describing regular dairy-based ice cream with creamy, smooth, soft, and sweet characteristics that strongly influenced consumer preference [49]. Similarly, the study by Sadek and Usman compared various plant-based ice creams incorporating MLF and found that dairy-based formulations remained the most preferred [50]. These formulations are primarily associated with sensory characteristics such as creamy, fatty, milky, and soft textures that current consumers still demand in plant-based alternatives.

Detailed sensory attribute ratings for each MLF-based ice cream sample are summarized in **Table 7**. Among the samples tested, product A had the closest profile to the ideal, but showed less desirable characteristics, such as pungent aftertaste and greeny flavors. Compared to the other three samples with higher MLF content, product A had milder intensities of undesirable characteristics. Product D containing the highest proportion of MLF was perceived to have a hard texture, corresponding with the results of physical texture analysis, which showed the highest hardness value in this sample.

Increasing the concentration of MLF appears to intensify several negative sensory characteristics, such as bitter, sandy, and pungent aftertastes. These observations are consistent with the report by Gorman et al. [49] that plant-based ice creams often develop nutty, beany, and vegetal off-flavors, along with rough or sandy textures capable of reducing general acceptability. Although nutty and beany characteristics were not prominent in this study, the presence of bitter, greeny, and pungent aftertaste were evident and might contribute to the lower sensory preference scores. The results supported the report by Sadek and Usman [50] that bitter and greeny sensory characteristics negatively influenced the acceptability of moringa-based ice cream products.

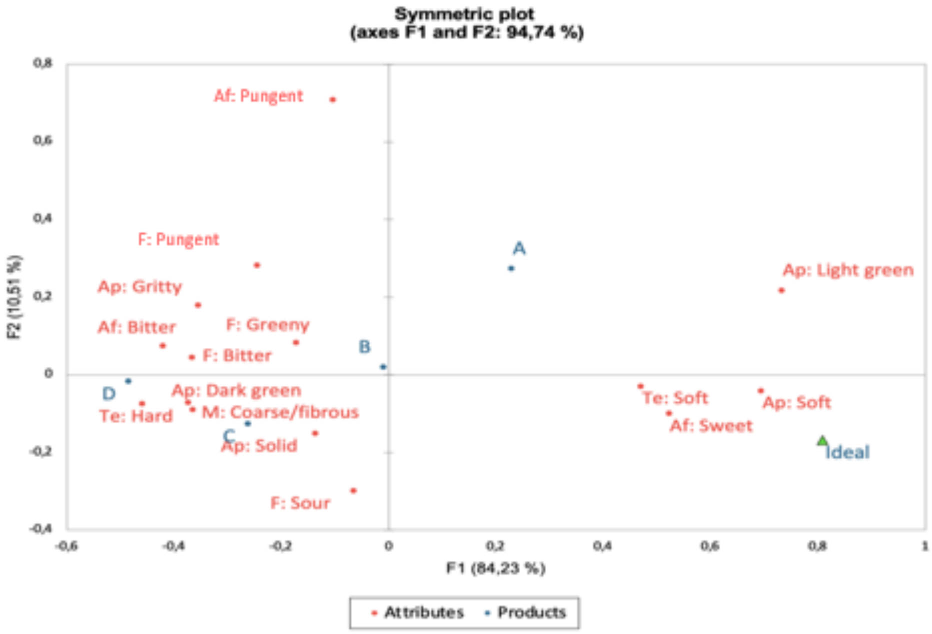

Fig. 3. Symmetric plot of MLF ice cream sensory profile

TABLE 7
SENSORY PROFILE OF MLF ICE CREAM

A	B	C	D
Greeny flavor	Greeny flavor	Greeny flavor	Greeny flavor
Sweet taste	Sweet taste	Sweet taste	Sweet taste
Light green	Pungent taste	Sandy mouthfeel	Sandy mouthfeel
		Bitter aftertaste	Bitter aftertaste
		Bitter taste	Bitter taste
		Solid appearance	Hard texture
		Sour taste	Dark green
		Pungent taste	Pungent taste

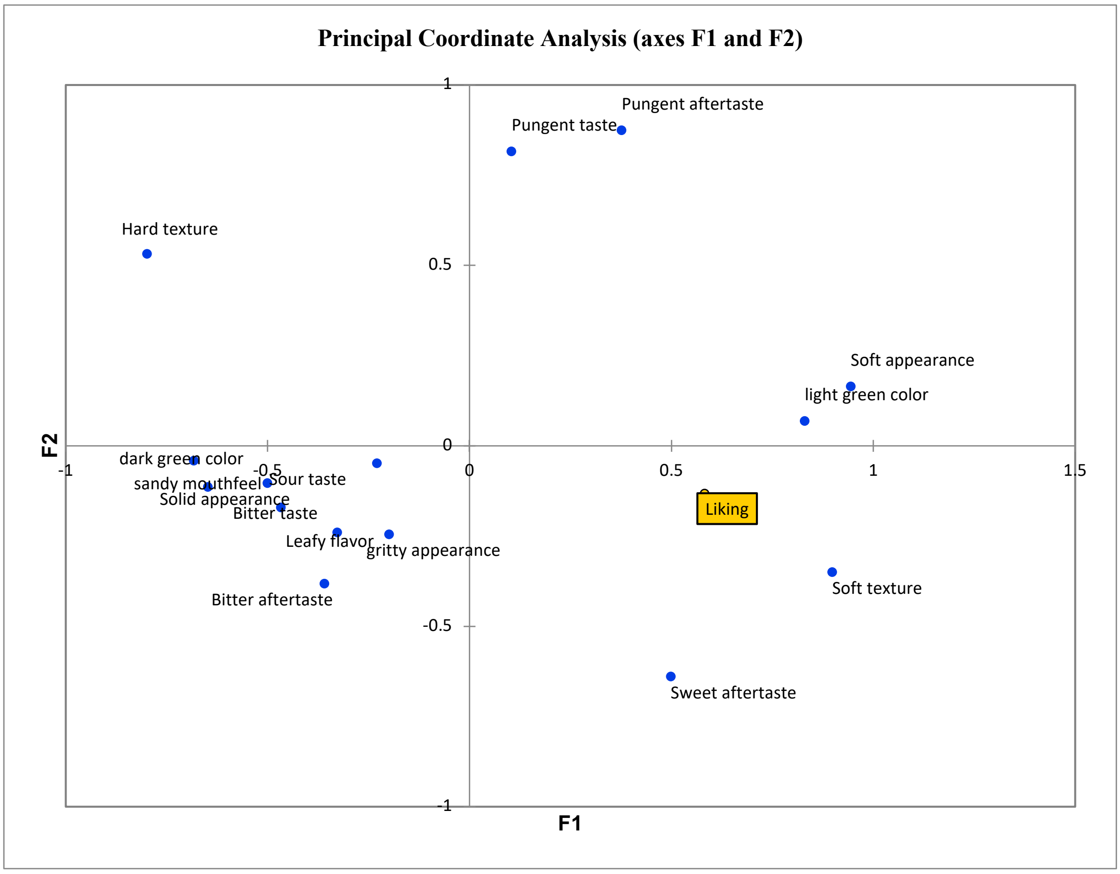

Fig. 4. Correlation of Sensory Characteristics with Preferences

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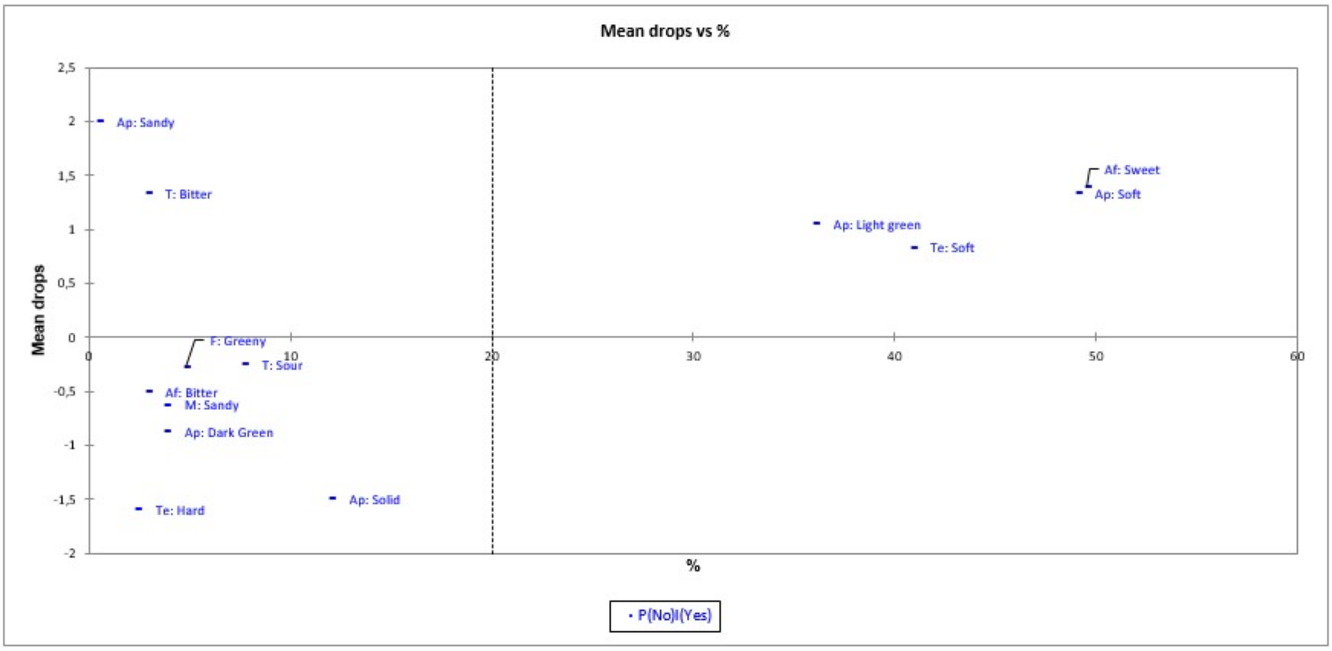

Fig 5. Penalty analysis graph for must-have characteristics

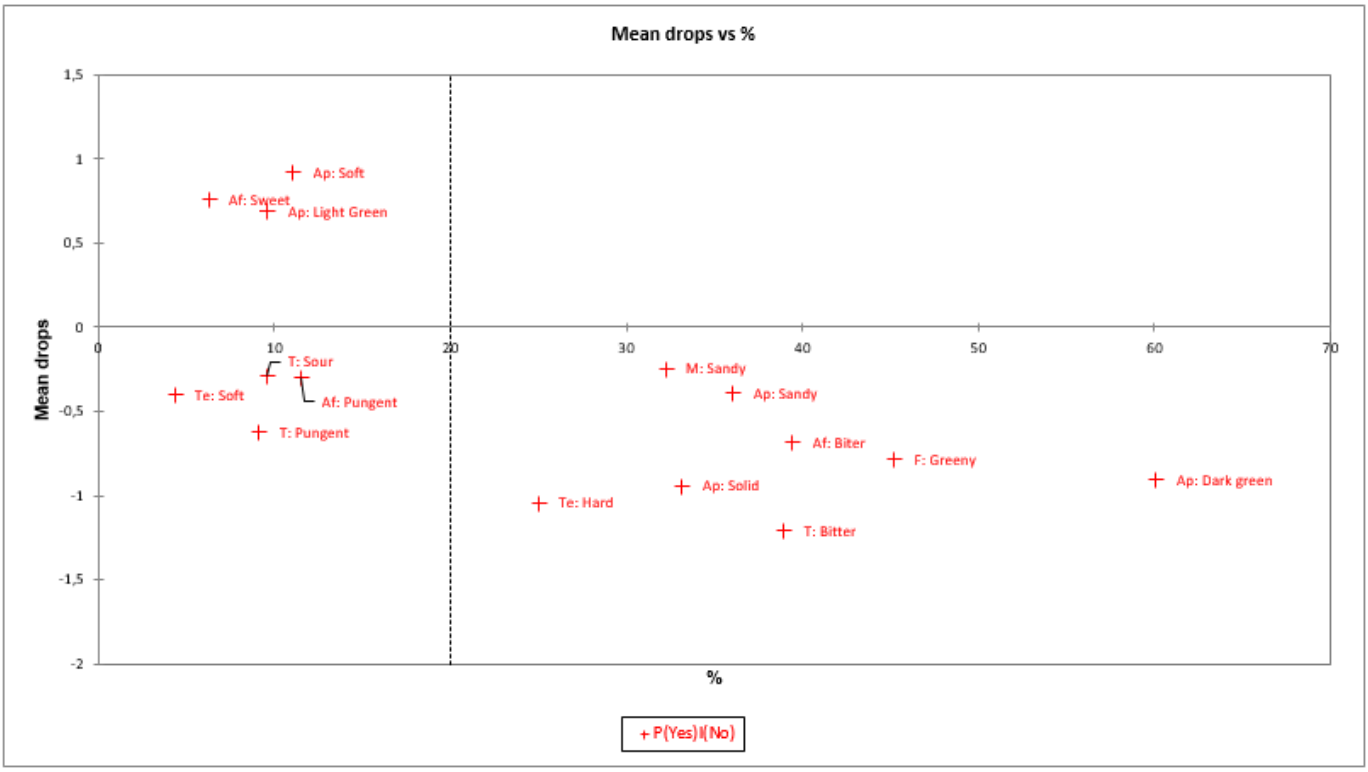

Fig 6. Penalty analysis graph for nice-to-have and must-not-have characteristics

Penalty analysis can be used for both product development and optimization. This is based on consumer response to the product compared to the ideal product for the identification of characteristics influencing the increase or decrease in preference [51]. The results obtained are comprehensively presented in **Table 8.** Penalty analysis conducted on the product helps to generate must-have, does-not-harm, and must-not-have characteristics (**Figure 5 and 6**). The must-have includes characteristics that should be possessed by moringa ice cream. Does-not-harm should be avoided despite not affecting the preference level, while must-not-have characteristics are not needed in ice cream and are capable of reducing the preference level. Must-not-have characteristics are found in many products such as C and D, suggesting that the more moringa powder used, the lower the level of preference for ice cream product. This can be observed from the results of the hedonic test conducted (**Table 9**) where product A had the highest hedonic value. For further development, MLF ice cream should be less bitter, less leafy, and not produce a bitter aftertaste while maintaining a soft texture.

TABLE 8
PENALTY ANALYSIS RESULTS

Must-have	Does-not-harm	Must-not-have
Light green color	Gritty appearance	Dark green color
Soft appearance	Pungent taste	Solid appearance
Soft texture	Sour taste	Bitter taste
Sweet aftertaste	Sandy mouthfeel	Leafy flavor
	Pungent aftertaste	Hard texture
		Bitter aftertaste

TABLE 9
PRODUCT PREFERENCE RESULTS

Sample	Average
A	5.000^{a}
B	4.480^{b}
C	4.140^{bc}
D	3.760^{c}

$^{a-c}$ different superscripts in one column represent significant difference between samples (P≤0.05)

Chemical testing of the best formula

Ice cream with 4% MLF is the best formula preferred by panelists, and the results of the analysis are presented in **Table 10**. The low amount of protein in ice cream is attributed to not incorporating cow's milk as a base ingredient and the inability of minimal MLF (4%) to increase protein content despite the presence of 27.13% (w/w) protein in MLF. Calcium content of ice cream reaches 93.13 mg/100 g because MLF contains 2312.93 mg/100 g. This is higher than calcium content of dairy ice cream in the previous study [52], implying that MLF vegan product can be compared to dairy products.

Ice cream formulated with 4% MLF does not meet Indonesian regulatory standard for nutrient content claims as a source of protein, calcium, and iron. Specifically, calcium and iron contents were 8.46% and 4.63% of the recommended daily intake, respectively, which were both below the minimum requirement of 15%.

TABLE 10
ANALYSIS RESULTS OF THE BEST FORMULA

Parameter	Unit	Average
Water content	%	68.9
Ash	%	0.59
Protein	%	2.38
Fat	%	5.43
Carbohydrate	%	22.70
Calcium	mg/100 g	93.13
Iron	mg/100 g	1.02
Total sugar*)	%	18.45
Saturated fat*)	%	4.87
Cholesterol*)	mg/100 g	0.98
Natrium*)	mg/100 g	24.09
Total Solids	%	24

*) The general requirements analysis to settle a food claim

Protein content was only 3.96% and significantly lesser than the 20% threshold [53]. However, the nutritional profile of ice cream shows an improvement compared to the base ingredient, tempeh milk, which contains only 0.67% protein (w/w), 4.05 mg calcium, and negligible iron content. The observed increase in nutrient levels is attributed to the addition of MLF, known for the richness in protein, calcium, and iron. This formulation still does not fulfill the required levels of protein, milk solids, and total solids as stipulated by Indonesian National Standard (SNI) for ice cream. To address the deficiencies, strategies such as increasing the total solids from tempeh milk or incorporating higher concentrations of MLF may be considered. A major limitation is sensory acceptability because higher levels of MLF are associated with increased bitterness, which negatively impacts consumer preference. Future studies should focus on reducing the bitterness of MLF to enable the incorporation at higher concentrations without compromising sensory quality of the final product.

IV. CONCLUSION

In conclusion, ice cream containing 4% MLF was identified as the most preferred, achieving an average score of 5 out of 7 on the hedonic scale, and showing statistically significant differences compared to other formulas. Increasing MLF concentration led to significant changes in physical properties, including increased hardness, slower melting rate, thicker consistency, and reduced overrun. Sensory evaluation showed that desirable characteristics for MLF-based ice cream included a light green color, soft appearance, smooth texture, and a sweet aftertaste. Undesirable characteristics were associated with a dark green color, solid or dense appearance, bitter taste and aftertaste, greeny flavor, and hard texture. The 4% MLF formula contributed to an increase in calcium content, but did not meet the nutritional requirements for making claims as a source of protein, calcium, or iron according to Indonesian food regulations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

USE OF ARTIFICIAL INTELLIGENCE (AI) TOOLS STATEMENT

We used Grammarly (Grammarly Inc., 2025) to improve the clarity and grammar of the manuscript. The authors reviewed and approved all changes.

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