Analysis of physicochemical and sensory properties of plant-based protein powder drinks

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Abstract. Protein is an essential macronutrient that plays a crucial role in maintaining body structure and supporting various physiological functions in humans. While animal products are traditionally the primary sources of dietary protein, their processing requires precise handling and they contain high levels of saturated fatty acids. As a result, plant-based protein powder drinks are gaining popularity as a healthier alternative, particularly among vegetarians, vegans, and individuals with lactose intolerance. This study aims to evaluate the physicochemical and sensory properties of plant-based protein powder drinks enriched with multigrain. The protein drinks were formulated using three different ratios: 30A70C, 20A80C, and 40A60C, where "A" represents almond powder and "C" represents cocoa powder. The physicochemical properties analyzed included color, solubility, moisture content, crude fiber, and protein content and sensory evaluation conducted using the Check-All-That-Apply (CATA) method to assess the attribute and acceptance test on aroma, taste, texture, aftertaste, and overall acceptability. The results indicated that the best formulation 20A80C had the protein content of 10.01±3.43%, with a solubility of 55.95±1.87%, moisture content of 5.92±0.43%, and crude fiber content of 3.62±0.56%. Sensory analysis using the CATA method revealed that sweet taste, savory taste, chocolate aftertaste, and chocolate aroma positively influenced the overall acceptability of the product. This study provides insights into the physicochemical characteristics and consumer preferences of plant-based protein powder drinks, highlighting their potential as a viable alternative to animal-derived milk-based products.

1 Introduction

Protein is a macronutrient essential for maintaining body structure and supporting various physiological functions in humans [1]. It can be derived from both animal and plant-based food sources. However, animal-derived protein is generally preferred due to its higher protein content. Despite this, animal-based products, such as milk fat, contain high levels of saturated fatty acids, which are associated with an increased risk of heart disease [2-3].

In recent years, there has been an increasing awareness of healthy diets and lifestyles, leading to a greater consumption of plant-based products. Several studies have highlighted the health benefits of almonds, which are a rich source of protein and antioxidants, including α-tocopherols and polyphenols [4], which make almond have the potential to serve as a substitute for animal milk in protein powder drinks. Additionally, multigrain, which consists of a combination of two or more grains, is recognized for its nutritional value and associated health benefits [5]. The inclusion of multigrain in plant-based protein powder drinks may also enhance their flavor profile.

Plant-based protein powder drinks are an emerging category of functional foods and serve as an alternative for individuals who follow vegetarian, vegan, or lactose-intolerant diets. In addition to being a rich protein source, these drinks are high in fiber due to the incorporation of multigrain ingredients. This study formulated plant-based protein powder drinks using a combination of almond powder, isolated soy protein (ISP), palm sugar, date powder, cocoa powder, multigrain powder, and cinnamon. The multigrain powder was composed of brown rice, black rice, red rice, buckwheat, corn grits, barley, rye grain, flaxseed, oat groats, and millet.

While plant-based protein powder drinks have garnered consumer interest, limited research has been conducted to comprehensively analyze their physicochemical and sensory properties. The development of these beverages is expected to provide an alternative protein source for the general population. Therefore, this study aims to evaluate the impact of almond and cocoa powder on physicochemical and sensory properties of multigrain-based protein powder drinks.

2 Methods

2.1 Sample preparation

Plant-based protein powder drinks were produced using the mixing method. The ingredients used in the mixing process consist of pre-powdered base materials. The ingredients used are almond powder, cocoa powder, isolated soy protein (ISP) powder, palm sugar powder, date powder, multigrain powder, and cinnamon powder. Plant-based protein powder drinks are made with 3

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different almond powder and cocoa powder formulations: 20A80C (20% almond and 80% cocoa), 30A70C (30% almond and 70% cocoa), and 40A60C (40% almond and 60% cocoa). The production of plant-based powder is carried out by mixing it to ensure consistency without any lumps.

2.2 Color analysis

Color analysis was performed using the CIELAB method with a Chroma Meter NH310. The device measured and recorded values for L* (lightness), a* (reddish for positive value and greenish for negative value), and b* (yellowish for positive value and blueish for negative value).

2.3 Solubility analysis

The solubility analysis utilized the gravimetric method. The first step began with weighing 3 gr of the sample and dissolving it in 15 ml of warm distilled water at 80°C. Subsequently, filter paper was heated in an oven for 30 minutes at 105°C. The cooled filter paper was weighed in a desiccator. The dissolved sample was filtered using filter paper. Then, the filter paper was dried in the oven for 24 hours at 105°C. The filter paper was cooled in a desiccator and weighed until reaching a constant weight. Each sample was repeated 3 times. Solubility was then calculated using Formula 1 [6], where A = sample weight (g), B = weight of filter paper before filtration (g), and C = weight of filter paper after filtration (g).

$$\%\ Solubility = \frac{A - (C-B)}{A} \times 100\% \quad (1)$$

2.4 Moisture content

Moisture content analysis was carried out using the gravimetric method. Initially, the container was heated in an oven at 105°C for 30 minutes. Next, the container was cooled in a desiccator for 10 minutes and weighed using an analytical balance. Then, 3 g of the sample were added to a cooled container, and the weight was determined. Then, put it in the oven at 105°C for 24 hours. The sample container was cooled in a desiccator for 10 minutes and weighed on an analytical balance. Moisture content was then calculated using Formula 2 [7], where A = weight before drying (g) and B = weight after drying (g).

$$\%\ Moisture\ Content = \frac{A - (C-B)}{A} \times 100\% \quad (2)$$

2.5 Protein content

Analysis of protein used the Kjeldahl method. Initially, 100-250 mg sample was added to the Kjeldahl flask. Then 1.0 ± 0.1 gr K_2SO_4, 40 ± 10 mg HgO, and 2 ± 0.1 ml H_2SO_4 were added, then heated until the liquid becomes clear and cold. Second stage, distilled water was added and homogenized to dissolve the crystals at the bottom of the flask. The flask's contents are transferred into a distillation apparatus, and the flask is rinsed 5-6 times using distilled water. The washing water in the flask is poured into a distillation flask, and 8-10 ml of 60% NaOH-5% $Na_2S_2O_3$ solution is added; a 250 ml Erlenmeyer containing 5 ml of 4% H_3BO_3 solution and 2-4 drops of methylene red-methylene blue indicator are placed under the condenser. Distillation will be carried out to obtain approximately 15 ml of distillate. Third stage is the titration stage with HCl. The titration procedure was carried out for a blank without using a sample, and the standard HCl volume for the blank titration was recorded [8]. The Kjeldahl method indirectly analyzes nitrogen levels. The analysis results are multiplied by a conversion factor of 6.25 to obtain the protein value in food ingredients [9]. The nitrogen content and protein were calculated using Formula 3 and Formula 4.

$$\%\ N = \frac{(ml\ HCl\ sample\text{-}HCl\ blanko) \times N\ HCl \times 14{,}077}{mg\ sample} \times 100\% \quad (3)$$

$$\%\ P = \%\ N \times conversion\ factor \quad (4)$$

2.6 Crude fibre analysis

The analysis of crude fibre was conducted using the gravimetric method. It began with weighing 1 gram of the sample and adding 1.25% H_2SO_4 in a quantity of 50 ml. The solution was boiled for 30 minutes, followed by adding 3.25% NaOH in 50 ml and boiling again for another 30 minutes. The filter paper was weighed. The solution was filtered, and the filter paper was washed with 25 ml of 1.25% H_2SO_4, 50 ml of hot distilled water, and 25 ml of 96% ethanol. Subsequently, the filter paper was transferred to an aluminium cup and dried in an oven at 105°C for 2.5 hours or until a constant weight was achieved. It was then cooled in a desiccator for 15 minutes before being weighed. The crude fibre of the sample can be calculated using Formula 5 [10].

$$\%\ Crude\ Fiber = \frac{g\ sample\ after\ dried - g\ filter\ paper}{g\ sample} \times 100\% \quad (5)$$

2.7 Sensory analysis

The sensory analysis carried out included the aroma, taste, texture, aftertaste, and overall liking attributes of the plant-based protein powder drink. The sensory analysis method used uses the check-all-that-apply or CATA method. The CATA method was carried out with samples presented randomly to 134 panelists and each panelist was provided with 3 brews of plant-based protein powder drinks randomly with a random sample number code and the sample was not given a comparison sample. The CATA method collects sensory product information based on consumer perceptions by providing a checklist if there is the presence of sensory attributes in plant-based protein drinks [11].

2.8 Statistical analysis

All of analysis method was done in triplicate (n=3). The test data were then analysed using one-way Analysis of Variance (ANOVA) with a confidence level of 95% followed by the Duncan test using SPSS software. $P \leq 0.05$ was considered to indicate a statistically significant difference. Physicochemical analyses are reported as mean ± SD. Sensory analysis using Cochran's Q test, graphical plot or symmetric plot, principal coordinate analysis, and mean impact using XLSTAT with CATA Analysis.

3 Results and discussion

3.1 Physical characteristics

The color analysis of powder plant-based protein powder drinks is shown in Table 1. Results showed that different formulations of chocolate powder could affect the color. The results of the research show that different high levels of cocoa powder can influence the color to be less bright in 40A60C due to using lower levels of cocoa powder and high almond powder compared to others. Cocoa powder functions as an ingredient or agent with an important role in forming dark brown color [12]. However, 30A70C that contain lower levels of cocoa powder than 20A80C, resulting in darker color. This can be caused by human error, where the sample powder is stirred unevenly, or caused by the color analysis machine detecting the wrong color.

Table 1. Color of plant-based protein powder drinks (powder).

Sample	L*	a*	b*	C*	h*
20A80C	36.96 ± 0.20^{c}	10.69 ± 0.30^{a}	14.63 ± 0.67^{b}	18.12 ± 0.7^{b}	86.82 ± 0.01^{a}
30A70C	23.08 ± 0.84^{a}	11.83 ± 1.14^{a}	8.66 ± 0.72^{a}	14.67 ± 1.33^{a}	87.84 ± 0.20^{c}
40A60C	25.54 ± 1.20^{b}	11.00 ± 1.3^{a}	9.85 ± 1.22^{a}	14.78 ± 1.70^{a}	87.45 ± 0.24^{b}

$^{a\text{-}c}$Different superscript letters indicate significant differences among formulation at $p<0.05$ using one way ANOVA with Duncan's Post Hoc.

Color analysis of liquid plant-based protein powder drinks is shown in Table 2. The results indicate that variations in cocoa powder formulations influence the color of the final product. The results of the research show that different levels of cocoa powder can influence the color to become lighter (high L*) in 20A80C due to the use of lower cocoa powder compared to others. as well as the result of other powder ingredients which produce a light color, such as ISP which has a cream or yellowish-white color and almond powder which has a light brownish cream color when brewed [13]. 40A60C uses higher levels of cocoa powder than 30A70C, and 30A70C exhibits a darker color. This discrepancy may be due to human error, where the dissolved sample powder was stirred unevenly, or the color analysis machine detected the wrong color.

Table 2. Color of plant-based protein powder drinks (liquid).

Sample	L*	a*	b*	C*	h*
20A80C	27.73 ± 0.68^{b}	7.03 ± 0.33^{a}	5.97 ± 1.07^{a}	9.25 ± 0.86^{a}	86.87 ± 0.16^{a}
30A70C	23.82 ± 1.09^{a}	9.23 ± 1.07^{b}	5.81 ± 1.08^{a}	10.92 ± 1.38^{a}	87.58 ± 0.21^{b}
40A60C	24.18 ± 1.54^{a}	8.25 ± 0.23^{ab}	4.62 ± 1.45^{a}	9.52 ± 0.82^{a}	87.54 ± 0.26^{b}

Different superscript letters indicate significant differences at $p<0.05$ using one way ANOVA with Duncan's Post Hoc.

3.2 Chemical characteristics

Analysis of the chemical characteristics of plant-based protein powder drinks is shown in Table 3. The resulting solubility tests are quite different and proximate tests are not different in the product formulation.

The solubility analysis results in Table 3 indicate that the characteristics of plant-based protein powder drinks exhibit relatively low solubility. The highest solubility is observed in 20A80C, with a result of 55.95% of powder dissolved in water, where this sample does not significantly differ from samples 30A70C and 40A60C. The lowest solubility is found in 30A70C, with a result of 49.65%. The difficulty in dissolving plant-based protein powder drinks is attributed to the relatively high-moisture content in the powder, resulting in larger and non-porous particles that hinder dissolution in water [14]. Furthermore, these drinks contain soy protein, which is primarily composed of globulins that tend to aggregate near their isoelectric point (pH 4.1–4.6), making dissolution more difficult [13]. Globulin is the primary protein type in soybean which are water-insoluble and salt-soluble [15].

Table 3. Chemical characteristics of plant-based powder drinks.

Sample	Solubility (%)	Moisture Content (%)	Protein (%)	Crude Fiber (%)
20A80C	55.95 ± 1.87^{a}	5.92 ± 0.43^{a}	10.01 ± 3.43^{a}	3.62 ± 0.56^{a}
30A70C	49.65 ± 0.77^{b}	5.92 ± 0.18^{a}	8.26 ± 0.76^{a}	3.79 ± 0.75^{a}
40A60C	51.91 ± 3.19^{ab}	5.93 ± 0.13^{a}	6.64 ± 1.35^{a}	3.81 ± 1.03^{a}

Different superscript letters indicate significant differences at $p<0.05$ using one way ANOVA with Duncan's Post Hoc.

The results of the moisture content analysis in Table 3 indicate that the characteristics of plant-based protein powder drinks can generally be considered to meet the Indonesian National Standard (SNI) 01-4320-1996 for Traditional Powdered Drinks. According to SNI 01-4320-1996, the maximum allowable moisture content for traditional instant drinks is 3-5%, and the moisture content results for samples 30A70C, 20A80C, and 40A60C are higher, with no significant difference observed among the samples. This occurrence can be attributed to the manufacturing process of plant-based protein powder drinks, which does not involve re-drying. Instead, the production involves mixing various powdered ingredients, leading to higher moisture content in the powdered drink without reducing the moisture content of the ingredients through re-drying processes [16].

The protein analysis results in Table 3 show that the highest protein value is found in sample 20A80C, likely due to the significant use of almond powder. However, the protein content obtained does not significantly differ between samples 30A70C and 40A60C. The almond powder contains 21.43 grams of protein per 100 grams [17], and chocolate powder contains 17.9 grams per 100 grams, resulting in relatively high protein content in sample 20A80C [18]. According to the Indonesian National Standard (SNI), 01-4270-1996, for cereal milk, the minimum protein content in powdered drinks should be 5%, and the results obtained from the samples sufficiently meet this requirement. Following the nutritional content claims, for a product to be considered a source of protein, it should have 20% of the nutrition label reference per 100 grams (solid form) or 10% per 100 ml (liquid form). Meanwhile, for a high or rich source of protein claim, the product should have 35% of the nutrition label reference per 100 grams (solid form) or 17.5% per 100 ml (liquid form). These regulations are stipulated in the Indonesian Food and Drug Authority Regulation Number 13 of 2016 concerning the Supervision of Claims on Labels and Advertisements for Processed Food. Therefore, plant-based protein powder drinks cannot yet be considered a source or high source of protein.

The results of the crude fibre analysis in Table 3 indicate that the levels of crude fibre are not significantly different between samples 30A70C, 20A80C, and 40A60C. Upon examining Table 3, it is observed that sample 20A80C has a lower value of crude fibre; this difference is not statistically significant when compared to samples 30A70C and 40A60C. The reduction in crude fibre in 20A80C may be attributed to the reduced use of chocolate powder in this sample. The almond powder contains 14.3 grams of fibre per 100 grams [17], while chocolate powder contains 32.1 grams per 100 grams [18]. Crude fibre is a non-starch polysaccharide or fibre component in food that chemical substances cannot hydrolyze through heating with acid and alkali solutions. It represents only 1/5 of dietary fibre [19]. A higher crude fibre yield is associated with higher moisture content, as high crude fibre can enhance the moisture content trapped within the matrix [20].

3.3 Sensory characteristics

Based on the tests carried out, several data results were obtained and processed using XLSTAT with CATA Analysis. The CATA test distinguished between genders, specifically females and males, in evaluating the attributes of the samples. Three samples, labelled 259 (30A70C), 613 (20A80C), and 973 (40A60C), were presented to both genders for assessment of taste, aroma, texture, and aftertaste. The results of Cochran's Q Test for females and males are presented in Table 4.

Table 4. Comparison of Cochran’s Q test on aroma and taste between female and male.

Attributes	p-values	
	Female	Male
Spice Aroma	0.975	0.414
Chocolate Aroma	0.020	0.234
Musty Aroma	0.112	0.513
Rancid Aroma	0.169	0.692
Cereal Aroma	0.459	0.025
Sweet Taste	0.025	0.020
Bitter Taste	0.000	0.000
Bland Taste	0.819	0.135
Savory Taste	0.030	0.378
Salty Taste	0.819	0.662
Sandy Texture	0.519	0.542
Smooth Texture	0.305	0.692
Dense Texture	0.185	0.296
Liquid Texture	0.638	0.522
Thick Texture	0.237	0.016
Light Texture	0.961	0.148
Spice Aftertaste	0.285	0.007
Chocolate Aftertaste	0.191	0.928
Musty Aftertaste	0.075	0.199
Rancid Aftertaste	0.565	0.584
Cereal Aftertaste	0.010	0.008
Sweet Aftertaste	0.128	0.089
Bitter Aftertaste	0.000	0.000
Mouthfeel Oil Aftertaste	0.203	0.291

Based on the results of Cochran's Q test in Table 4, values above the p-value of 0.05 cannot be considered key attributes as they result in insignificant differences concerning the product. The significance level used is 0.05. Attributes with a p-value above 0.05 are considered less statistically significant. Cochran's Q test results for the Female CATA indicate key product attributes: chocolate aroma ($p = 0.020$), sweet taste ($p = 0.025$), bitter taste ($p = 0.000$), savory taste ($p = 0.030$), musty aftertaste ($p = 0.075$), cereal aftertaste ($p = 0.010$), and bitter aftertaste ($p = 0.000$). Cochran's Q test for the Male CATA identifies key attributes: cereal aroma ($p = 0.025$), sweet taste ($p = 0.020$), bitter taste ($p = 0.000$), thick texture ($p = 0.016$), spice aftertaste ($p = 0.007$), cereal aftertaste ($p = 0.008$), and bitter aftertaste ($p = 0.000$).

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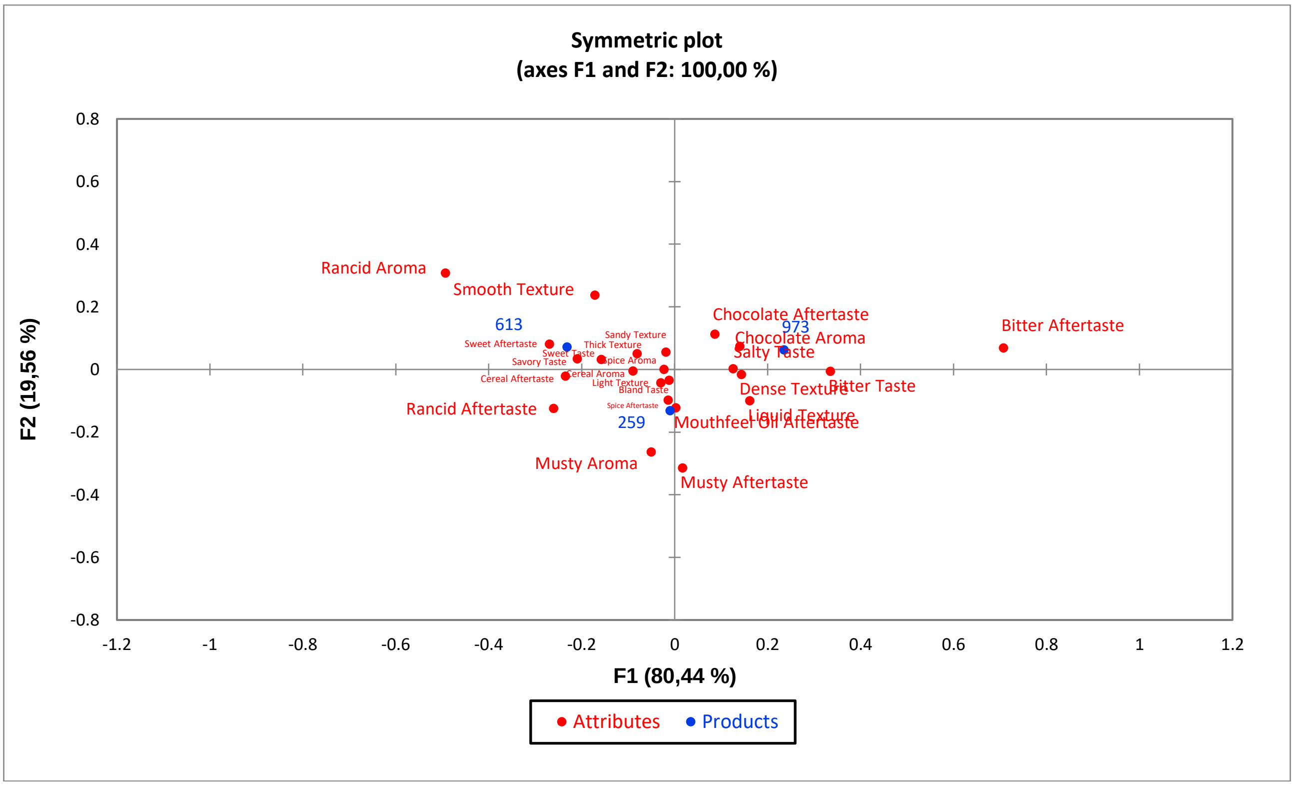


Fig. 1. Symmetric plot for each sample in female CATA. 613 represent 20A80C, 259 represent 30A70C and 973 represent 40A60C

Based on Figures 1 and 2, a symmetrical plot illustrates the distinctive product characteristics attributed to each sample. Each sample is positioned within a specific quadrant, delineating the key attributes that characterize it. Figure 1 reveals that, for sample 259 (30A70C), the female respondents describe characteristics situated in quadrant 3 (bottom-left), encompassing spice aroma, musty aroma, cereal aroma, bland taste, light texture, spice aftertaste, musty aftertaste, cereal aftertaste, and mouthfeel oil aftertaste. The characteristics of sample 613 (20A80C), as articulated by female respondents, reside in quadrant 1 (top-left) and include spice aroma, rancid aroma, cereal aroma, sweet taste, savory taste, sandy texture, smooth texture, thick texture, cereal aftertaste, and sweet aftertaste. Sample 973 (40A60C) exhibits characteristics positioned in quadrant 2 (top-right), described by female respondents as comprising chocolate aroma, bitter taste, salty taste, chocolate aftertaste, and bitter aftertaste.

Based on Figure 2, the characteristics described by males for sample 259 (30A70C) are situated in quadrant 3 (bottom-left), including sweet taste, bland taste, savory taste, musty aroma, thick texture, chocolate aftertaste, sweet aftertaste, and mouthfeel oil aftertaste. The characteristics of sample 613 (20A80C), as articulated by male respondents, reside in quadrant 1 (top-left), encompassing cereal aroma, sweet taste, dense texture, liquid texture, chocolate aftertaste, cereal aftertaste, and sweet aftertaste. Furthermore, sample 973 (40A60C) exhibits characteristics positioned in quadrant 2 (top-right), described by male respondents as comprising bitter taste, salty taste, chocolate aroma, rancid aroma, sandy texture, smooth texture, light texture, and bitter aftertaste.

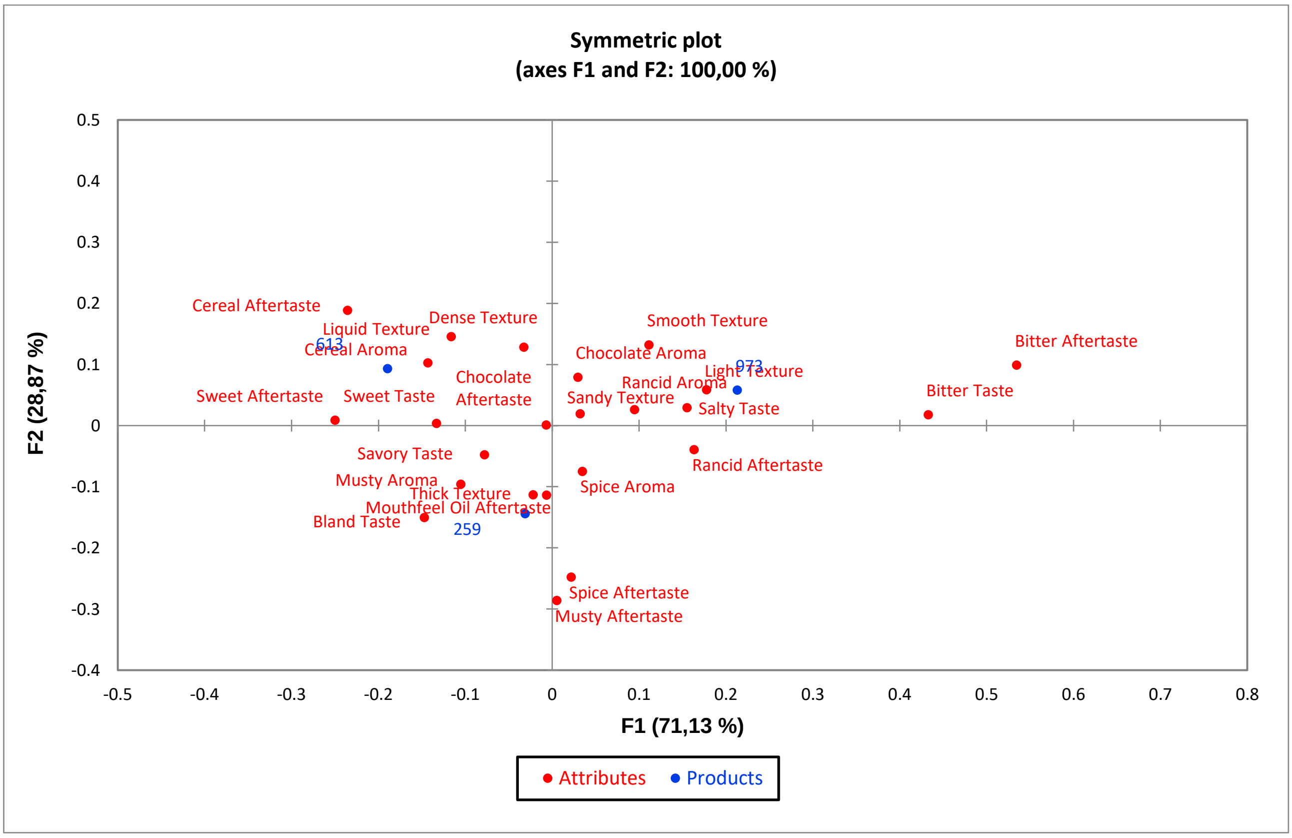
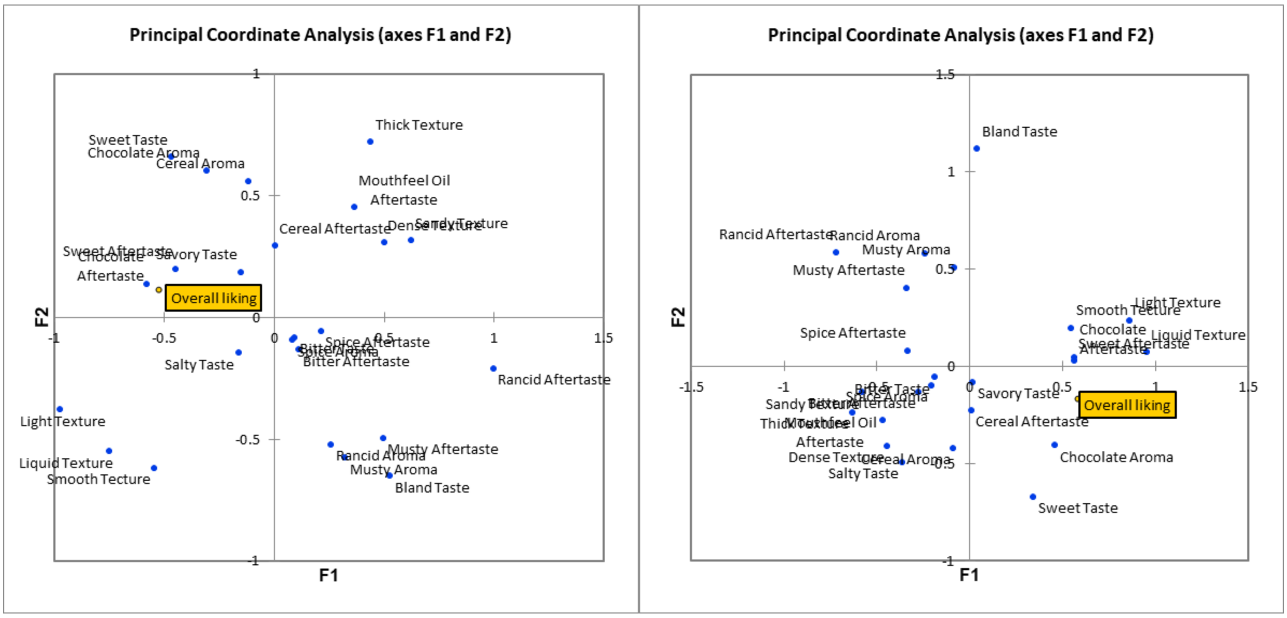


Fig. 2. Symmetric plot for each sample in male CATA. 613 represent 20A80C, 259 represent 30A70C and 973 represent 40A60C

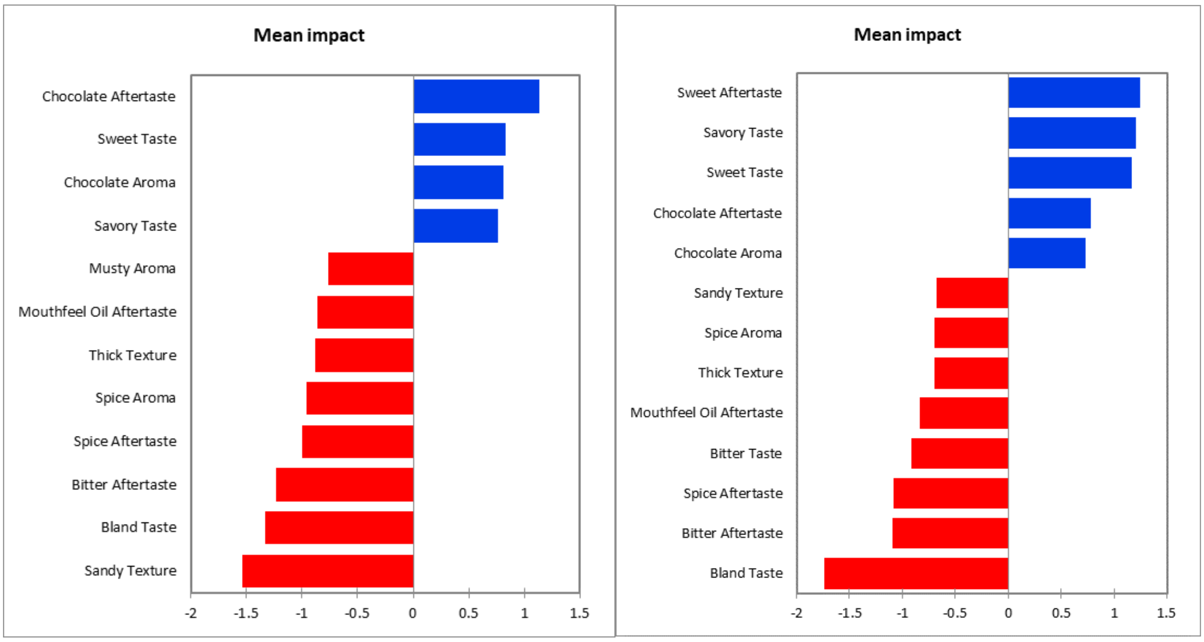
Based on Figure 3, the attributes favored by females include savory taste, sweet aftertaste, and chocolate aftertaste. These three attributes are considered preferences as they are close to the focal point of liking. Attributes opposite to the liking quadrant are considered to be avoided. Some attributes to be avoided include spice aroma, musty aroma, rancid aroma, bitter taste, bland taste, spice aftertaste, bitter aftertaste, musty aftertaste, and rancid aftertaste. Furthermore, the attributes favored by males are chocolate aroma, savory taste, and cereal aftertaste. Avoidable attributes include musty aroma, rancid aroma, spice aftertaste, musty aftertaste, and rancid aftertaste.



(a) (b)

Fig. 3. Principal coordinate analysis for satisfaction for each (a) female and (b) male CATA.

Based on Figure 4, attributes that positively impact according to females are sweet taste, savory taste, chocolate aftertaste, and chocolate aroma. Meanwhile, attributes that have less favorable impact include sandy texture, bland taste, bitter aftertaste, spice aftertaste, spice aroma, thick texture, mouthfeel oil aftertaste, and musty aroma. The attribute with the most significant positive influence is the chocolate aftertaste, and reducing this attribute in the product would have a significant impact, negatively affecting the sensory quality of the product. The attribute with the highest negative impact that should be eliminated or reduced is sandy texture, where reducing this attribute would significantly enhance the sensory attributes of the product. Furthermore, attributes that positively impact according to males are sweet aftertaste, savory taste, sweet taste, chocolate aftertaste, and chocolate aroma. Attributes that negatively impact the product include bland taste, bitter aftertaste, spice aftertaste, bitter taste, mouthfeel oil aftertaste, thick texture, spice aroma, and sandy texture. The attribute with the most significant positive influence is sweet aftertaste, and the attribute with the highest negative impact that should be eliminated or reduced is bland taste.



(a) (b)

Fig. 4. Mean impact of attributes on (a) female and (b) male CATA.

4 Conclusion

Color analysis of plant-based protein powder drinks indicates that varying formulations of chocolate powder significantly impact color attributes. The cocoa powder content affects color brightness, with 20A80C exhibiting less brightness due to lower cocoa powder and higher almond powder content. Solubility analysis reveals relatively low solubility in plant-based protein drinks, with 20A80C showing the highest solubility. Moisture content analysis indicates compliance with the Indonesian National Standard for Traditional Powdered Drinks. However, the protein content does not meet the criteria for being considered a source or a high source of protein, with the highest protein was sample 20A80C in this study. Crude fibre levels among samples do not differ significantly, with 20A80C having slightly lower crude fibre potentially attributed to reduced chocolate powder. Cochran's Q test results highlight key attributes in Female and Male Consumer Affective Testing (CATA), providing insights into preferred and avoided attributes for each gender and depicting characteristics and preferences of each sample. The influence of attributes such as chocolate aroma, sweet taste, and aftertaste on product preferences is also evident. Color did not alter the preferences as the difference between formulation is considered minor. Recommendations for product improvement involve adjusting attributes like sandy texture and chocolate aftertaste to enhance overall sensory quality. Understanding sensory attributes can optimize plant-based protein powder drink formulations to align with consumer preferences.

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