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The Effect of the Addition of Red Kidney Bean Skin Powder (*Phaseolus vulgaris L.*) on the Physical, Chemical, and Organoleptic Characteristics of Red Kidney Bean Tempeh

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A B S T R A C T

Red kidney bean tempeh production is generally made by beans without the epidermis, but in this study, the epidermis was removed from the red beans, dried and powdered, and then added back to the tempeh red beans. This research aims to determine the effect of adding red bean skin powder on the physical, chemical, and organoleptic properties of the tempeh produced. The design used in this research was a completely randomized design (CRD) with five treatments and three replications. The treatment in this research was wet peeled red beans without skin (control) and the addition of red kidney bean skin powder starting from 15%, 30%, 45%, and 60% in red kidney beans before fermentation. Research data was analyzed using ANOVA and Duncan's New Multiple Range Test (DNMRT) at the 5% level. The results of this research showed that the treatments caused significantly different texture values, water content, ash content, protein content, crude fiber content, and iron (Fe) content, but no significant differences in fat content or carbohydrate content. The best treatment based on physical, chemical and organoleptic analysis was treatment C (addition of 30% red bean skin powder) with an average texture value of 2.01 N/cm^2, water content of 61.63%, ash content of 0.72%, protein content of 15.83%, fat content of 1.51%, carbohydrate content of 20.30%, crude fiber content of 6.95%, iron content of 5.00 mg/g coliform content of 0.32 APM/g, and salmonella negative/g. The organoleptic test values were color 6.20 (preferred), texture 6.15 (preferred), taste 4.05 (less preferred), and aroma 4.45 (less preferred).

1. INTRODUCTION

1.1. Research Background

Red kidney beans are one of the legumes that are widely produced more than any other type of beans. According to the Central Statistical Agency (2022), the total production of red kidney beans in Indonesia in 2018 was 67.862 tons. High production levels were often not matched by high utilization as well. Currently, the use of red kidney bean is only consumed as vegetables and baking ingredients. This bean is namely the kidney bean because of its kidney-like shape and its color. Similar to other types of beans, kidney bean contains several anti-nutritional compounds such as phytic acid, hemagglutinin, and anti-trypsin that can inhibit protein digestibility and mineral absorption [1]. Several processes for removing antinutritional compounds include soaking, boiling, acid soaking, and fermentation [2]. Kidney beans contain high level of protein, fiber, and carbohydrate, namely 22.1 g, 4 g, and 28 g in 56.2 gram of kidney beans. Kidney beans also contain calcium, phosphorus, iron, vitamin A, vitamin B1, and bioactive components (flavonoids and phytosterols) [3].

The study published by Ref. [2] had developed tempeh from red beans, and Ref. [4] had also produced tempeh from non-soy beans (red beans, Bogor beans, green beans, and peanuts). The process of making tempeh from non-soy beans is, in principle, the same as the process of making tempeh from soybeans [4]. Generally, in the process of making tempeh, the epidermis that has been separated from the seeds will be discarded, so that it can cause solid waste. Removing the epidermis on red beans during tempeh production reduced the fiber content in tempeh [5]. Furthermore, based on [6], the skin epidermis of soybeans added to tempeh must be powdered so that it can be penetrated by *Rhizopus oryzae* molds. The addition of soybean skin powder to tempeh with ratio of 0%, 20%, 40%, and 60%, obtained increasing in the crude fiber content of the resulting tempeh [6].

1.2. Literature Review

Red kidney beans (*Phaseolus vulgaris*) are the genus *Phaseolus* in Fabaceae family. *Phaseolus vulgaris* is an edible bean, so it usually cultivated and consumed by humans and animals. Dry beans contain nutrition such as complex carbohydrate up to 60%, protein 20-27%, fiber 28%, vitamin and mineral, and low fat. Red kidney beans have a low glycemic index (GI) and they are great sources of nutrients like calcium, magnesium, copper, manganese, iron, potassium, vitamin E, and vitamin K1. Red kidney beans have the potential to treat a wide range of chronic illnesses that affect people globally [7]. *Phaseolus vulgaris* beans are better source of polyphenolic chemicals, which have a variety of health-promoting qualities. Dry beans contain high polyphenols as anti-oxidant, anti-diabetic, anti-inflammatory, anti-mutagenic, and anti-carcinogenic [8]. Moreover, these beans have a very promising future as functional foods and nutraceuticals.

Red kidney beans can be processed into foods with good nutritional value. One of the food products that can be developed is tempeh. Tempeh is fermented food originally from Indonesia that usually made from soybeans and other legumes. Tempe is produced through fermentation process by several Rhizopus sp. The steps making tempeh are peeling the skin of beans, boiling, starter inoculation, and then incubate at room temperature for 24 to 36 hours using plastic or other packaging [9]. Tempeh has been recommended as superfood because of its high vitamin B12, protein, ergosterol, and some mineral contents [10]. Compare to the unfermented soybeans, tempeh has better physicochemical and nutritive properties. Several studies have proved that tempeh has many beneficial effects, such as lowering the heart rate in hypertension rats, decreasing the synthesis of ROS in BV2 cells treated with lipopolysaccharide (LPS) [11], and protects against memory deficits. Tempeh has possible advantages as therapeutic agent in oxidative stress related to neurodegenerative illness [12].

Several studies related to red kidney bean tempeh had been done. [13] reported that overall processing process in tempeh production cause increasing in protein content, a decrease in carbohydrate content, a change in amino acid composition, a decrease in total isoflavone level, and concentration of antitrypsin inhibitory activity and oligosaccharide. Generally, in the process of making tempeh, the epidermis skin that has been separated from the seeds will be discarded. This can cause solid waste from tempeh production process. The red bean epidermis skin has fiber that can increase fiber content in red bean tempeh. Peeling the epidermis of kidney beans during making tempeh may reduce the fiber content of tempeh [5]. The epidermis skin of soybean made into powder can be penetrated by Rhizopus. The parts of tempeh containing intact seed coat do not have a strong/compact structure. It happens because the hyphae of mycelium in the mold are not able to penetrate the soybean skin. Therefore, in this study added red kidney bean epidermis powder to increase fiber content and cohesiveness in the resulting tempeh and evaluate the physical, chemical, and organoleptic characteristics of red bean tempeh produced, and the best concentration of red bean skin powder addition in red kidney bean tempeh production.

1.3. Research Objective

The purpose of this study was to determine the effect of the addition of red kidney bean skin powder on the physical, chemical, and organoleptic characteristics of red kidney bean tempeh, and to determine the best concentration of red kidney beans skin powder. This study used a completely randomized design (CRD) with 5 treatments and 3 replications.

2. MATERIALS AND METHODS

This study was conducted in Andalas University laboratories. The equipment used in this study was equipment for making tempeh and several tools for analysis. While the materials were red kidney beans and tempeh yeast. The chemicals used in this research were selenium catalyst mixture, H_2SO4, NaOH 30%, H_3BO_4, HCl, n-hexane, ethanol 96%, physiological saline solution, Lactosa Broth Media, and Salmonella Shigella Agar Media.

2.1. Preparation of sample

250 gram red kidney beans were obtained from the traditional market were soaked for 7 hours in 1 liter of water. After that, the beans were boiled in 1 liter water for 5 minutes. The water was removed and the red kidney beans were cooled until the temperature at 30^0 C. The skin of the red kidney bean was separated from the bean. The beans were washed in 3 times, and the skin was dried using food dehydrator for 10 hours at 70^0 C. Next, the bean skin was ground using chopper and sieved with a 20-mesh sieve.

2.2. Red kidney beans tempeh production

250-gram skinless red kidney beans were boiled with 1 liter of water for 10 minutes until the water boiled. Red kidney beans were incubated in closed container for 24 hours. After that, the soaking water was removed and the beans were rinsed until there was no mucus on the beans. Next step was boiling again with 1 liter of water and 2.5 ml of vinegar, boiled until boiling and left for 10 minutes. After that, the cooking water was discarded, the red kidney beans were cooled. Red kidney beans skin powder was added according to the treatments along with 0.6 gram of yeast, then mixed until evenly distributed. Furthermore, it was packed in plastic that had been perforated, then placed in a dark place for fermentation process for 48 hours.

2.3. Analytical methods

All parameters in this study were conducted in triplicates and followed: moisture content, ash, protein, fat, carbohydrate, and crude fiber content for skin red kidney beans powder. The observations in red kidney bean tempeh were texture value, moisture, ash, protein, fat, carbohydrate, crude fiber, iron content, coliform and salmonella contamination, multiple comparation organoleptic test. Moisture and protein content were measured according to The Indonesian National Standard (SNI) 3144:2015). Ash and fat level content were measured using method adopted from [14]. Crude fiber level was evaluated based on SNI 01-2894-1992.

Iron content was calculated according to atomic absorption spectrophotometry by wet destruction. Coliform contamination using presumptive and confirmatory tests. While salmonella contamination was evaluated by salmonella shigella agar. Descriptive test on tempeh based on SNI 3144:2015. Multiple comparation organoleptic test by comparing red kidney beans tempeh with tempeh sold in market.

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3. RESULT AND DISCUSSION

3.1. Composition of red kidney beans skin

Table 1 shows the chemical component analysis results of red kidney beans epidermis skin. This analysis was conducted to determine the effect of the addition of epidermis skin powder on tempeh produced. Red kidney beans skin powder contains low water content because it had been dried before powdering. The low water content causing other chemical components to be high. The fiber content was 28.10 % of the total carbohydrate of kidney bean epidermis. Generally, kidney bean skin is composed of cellulose, hemicellulose, and lignin. In kidney bean skin, there is more 50% cellulose content.

The epidermis of red bean contains polyphenol compounds in the form of procyanidins around 7-9%. Ash content in red bean tempeh without skin was obtained at 1.100%, while when compared to red bean tempeh with skin, it was obtained at 1.210% [15]. In the process of peeling the kidney bean epidermis can reduce the iron content by 98.7%-99.9% [16].

Table 1. chemical composition of red kidney bean skin powder

Component	Content (%)
Moisture	8.48 ± 0.24
Ash	4.23 ± 0.14
Proteins	9.66 ± 0.11
Fats	2.96 ± 0.11
Carbohydrates	74.67 ± 0.44
Fiber	28.10 ± 0.26

3.2. Red kidney beans tempeh

3.2.1. Texture

Texture observation was performed to determine the physical properties of materials related to the durability or strength of a material against pressure using Texture Analyzer Tool. Table 2 shows the average value of hardness in red kidney bean tempeh.

Table 2 indicates that the results of addition red kidney beans skin powder to red kidney beans tempeh which was significantly different at the 5% level on texture value of red kidney beans tempeh.

Table 2. Characteristics of Red Kidney Bean Tempeh

Characteristics	Addition of red bean skin powder				
	P1 (Control)	P2 (15%)	P3 (30%)	P4 (45%)	P5 (60%)
Texture (N/Cm^2)	1.45 ± 0.01a	1.79 ± 0.07b	2.01 ± 0.05c	2.45 ± 0.08d	2.96 ± 0.09e
Moisture (%)	63.05 ± 0.30d	62.85 ± 0.24d	61.63 ± 0.19c	60.27 ± 0.21b	59.03 ± 0.23a
Protein (%)	13.54 ± 0.28a	14.21 ± 0.15b	15.83 ± 0.13c	16.49 ± 0.26d	17.63 ± 0.29e
Fat (%)	1.63 ± 0.21	1.57 ± 0.49	1.51 ± 0.19	1.20 ± 0.20	1.14 ± 0.11
Carbohydrate (%)	21.35 ± 0.66	20.72 ± 0.75	20.30 ± 0.24	21.10 ± 0.33	20.80 ± 0.60
Raw fiber (%)	4.85 ± 0.32 a	5.87 ± 0.21 b	5.87 ± 0.21 b	7.92 ± 0.12d	8.71 ± 0.34 e
Ash (%)	0.41 ± 0.01 a	0.63 ± 0.03 b	0.72 ± 0.02 c	0.92 ± 0.02 d	1.39 ± 0.06 e
Iron	3.47 ± 0.50	4.39 ± 0.39	5.00 ± 0.11	5.73 ± 0.28	6.50 ± 0.06
Coliform contamination	0.36	0.34	0.32	0.29	0.28
Salmonella contamination	Negative	Negative	Negative	Negative	Negative

The level of texture obtained in red kidney bean tempeh showed that the more addition of red kidney skin powder, the higher the texture value of red kidney bean tempeh. The added red kidney bean skin powder fills the empty cavity between the red kidney beans tempeh, so that texture produced in tempeh will be denser. Increased texture value is also related to the water content of the resulting red kidney bean tempeh. When the moisture content of the product is low, it means the texture is compact. The texture produced by each treatment can be seen in Figure 1.

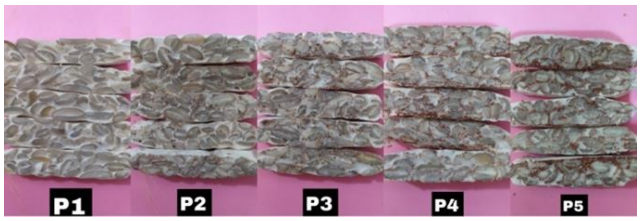

Figure 1. The slices of red kidney beans tempeh in each treatment.

Figure 1 shows that the more addition of red kidney skin powder, the cavity between red beans in tempeh will be filled by red bean skin powder. The study performed by [17], koro beans tempeh with black soybean substitution has a texture value of 1.53-3.09 N/Cm^2, the value of 1.53 N/Cm^2 is categorized with soft texture. So that, the texture value obtained in P1 treatment (control) was not much different.

3.2.2. Moisture content

Moisture content is the amount of water contained in the material stated in percent. The higher the moisture content of a food ingredient, the more likely it is to spoil [18]. The result of analysis variance showed that the addition of red kidney bean skin powder was significantly different at the 5% level on the water content value of red bean tempeh. Based on raw material analysis, red bean skin powder contains 8.48% water content. The water content in red beans that have been boiled and soaked was 56.83% [19]. The reduction of water level occurs because the water content of red bean skin powder. It has lower water concentration than red beans. The more addition of red bean skin powder would reduce the water content of tempeh (Table 2).

The study conducted by Ref. [4] on non-soybean tempeh obtained the water content value of red bean tempeh of 61,79%. Besides that, the research done by [20] showed that water level of red bean tempeh of 65,79%. The value of water content in red bean tempeh in treatment P1 (without adding red bean skin powder) of 63,05% was not much different from the results of previous studies. The value of water level of red bean tempeh in this study was in accordance with SNI 3144-2015 which states that the maximum water content of tempeh is 65%.

3.2.3. Protein content

Protein is an organic substance that is important for the body because this substance functions as a building and regulating substance in the body. Based on Table 2, the results of analysis variance showed that the addition of red kidney bean skin powder was significantly different at 5% level on the value of red bean tempeh protein content. According to [21], the protein content in red beans that have been boiled and soaked is 15,79% and red bean skin powder contains 9,66% protein. The redder the bean skin powder, the higher the protein content of red bean tempeh produced. During the fermentation process, there is a change in the amount of amino acid level due to the breakdown of complex protein compounds into simpler compounds by protease enzymes produced from *Rhyzopus oligosporus* and the overall number of amino acids increases after the fermentation process [22]. In the Ref [6], the protein content in soybean tempeh increased with the addition of soybean skin powder, which was 12,14 – 16,25%.

According to study by Ref. [21], the protein content contained in red bean tempeh was 13,71%. Following that, [20] showed that the protein content of red bean tempeh was 13,45%. Based on SNI 3144:2015, the protein content in tempeh is at least 15%. In this study, the control treatment and the addition of 15% did not reach the minimum level of tempeh protein.

3.2.4. Fat content

The result of variance analysis showed that the addition of red bean skin powder in tempeh production was not significantly different at the 5% level on the value of fat content of red bean tempeh. Based on the analysis of the raw materials, red bean skin powder contains 2,96% fat. The fat content in red beans that have been boiled was 3,87% [21]. The low-fat content in red kidney bean skin powder compared to kidney beans resulted decreasing in the fat content of kidney bean tempeh. During the fermentation process, mold hydrolyzes fat into fatty acids. The fatty acids produced are linoleic, linolenic, and oleic fatty acids. *Rhyzopus oligosporus* uses thses fatty acids as energy source during fermentation, so that fat content can decrease [21]. In the analysis of fat content contained in red bean tempeh without addition of red bean skin powder was 1,63%, almost the same as the results of research done by [23].

3.2.5. Carbohydrate level

The addition of red bean skin powder in red bean tempeh process was not significantly different at 5% level on the value of tempeh carbohydrate content based on the result of analysis variance. Carbohydrate level decreased due to the addition of red bean skin powder [24].

3.2.6. Crude fiber level

Crude fiber is a food component that cannot be hydrolyzed by chemicals or strong acids and strong bases such as sulfuric acid and sodium hydroxide. While food fiber is a complex carbohydrate found in plant cell walls, which consists of lignin, cellulose, hemicellulose, which cannot be digested by digestive enzymes [25]. Table 2 shows the value of crude fiber content of tempeh from each treatment. The result of analysis variance showed that the addition of red kidney bean skin powder to the crude fiber content of red bean tempeh was significantly different at the 5% level. Red kidney bean skin powder contains 28,10% crude fiber. The crude fiber content in boiled kidney beans was 2,82% [26]. Based on this, it caused increasing the crude fiber content of tempeh in each treatment. The crude fiber content in soybean tempeh with the addition of soybean skin flour increased which was 4,22 – 6,56 % [6]. This study showed that the crude fiber content in red bean tempeh without adding red bean skin powder (control) was 4,85%.

3.2.7. Ash content

Ash is the residual combustion of unburned organic components. Ash content consist of mineral present in food ingredients [27]. The results of analysis variance showed that the addition of red bean skin powder was significantly different at the 5% level on the ash content of red bean tempeh. The analysis resulted an increase in the ash content of red bean tempeh due to the addition of red bean skin powder. The ash content of red bean skin powder was 4,23%. Meanwhile, the ash content of red beans that had been boiled and soaked was 1,34% [19]. The ash content of control treatment was 0,41%.

3.2.8. Iron content

Iron (Fe) is micronutrient that is essential for human body in maintaining body functions, both at the level of cells, tissues, and organs [28]. Iron levels produced by tempeh from each treatment can be seen in Table 2. The result of analysis variance indicated that the addition of red bean skin powder was significantly different at the 5% level on the value of iron content of the red bean tempeh. Iron level increased due to the addition of red bean skin powder which was related to ash content. The higher the ash content, the higher the content of mineral elements in food products [20]. The fermentation process can also increase the availability of iron because of the hydrolysis of phytic acid in fermentation process [25]. During fermentation, phytic acid levels are getting lower due to the activity of the phytase enzyme produced by the tempeh mold. Phytic acid present in food can bind to minerals and proteins, causing a decrease in the solubility of the compounds it binds [29].

3.2.9. Coliform contamination

Coliform is a bacterium that has a normal habitat in the intestines of humans and animals and becomes an indicator of the presence of pathogenic bacteria. These bacteria are commonly used as sanitation indicators in water and food [30]. Table 2 shows that the addition of red bean skin powder decreased the amount of coliform in red bean tempeh. Coliform can ferment lactose by producing acid and gas when incubated at 35-37^0 C [31].

Red beans do not contain lactose [32] and red beans skin contain more than 50% cellulose [8]. It can be concluded that the redder bean skin powder added, the less likely coliform was to develop.

3.2.10. Salmonella contamination

Salmonella is one of the gram-negative bacteria that is pathogenic and an agent that often causes foodborne disease. Table 2 shows the analysis of Salmonella contamination in red bean tempeh with the addition of red bean skin powder in each treatment. All of treatments resulted negative result which was suitable with SNI 3144:2015. This can be interpreted that the red bean tempeh products from this study were safe for consumption.

Salmonella can be found in food due to contamination that can come from water that is exposed to wastewater pollution

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containing Salmonella or can occur indirectly through human hands or tools used [33].

3.3. Multiple comparison organoleptic test

This organoleptic test aims to determine the level of difference and the level of preference of red bean tempeh for each treatment compared to the Azaki brand tempeh on the market. The parameters observed in red bean tempeh were color, texture, taste, and aroma. The soring is as follows: 1 = very different, less preferred; 2 = different a lot, less preferred; 3 = different a bit, less preferred; 4 = slightly less preferred; 5 = not different and preferred; 6 = slightly different, more preferred; 7 = different a bit more, preferred; 8 = different a lot, more preferred; 9 = different a lot, more preferred. Observations on the plural comparison test of red bean tempeh for each treatment can be viewed in Table 3.

Table 3. Organoleptic test results of plural comparison of red beans tempeh

The addition of red bean skin powder (%)	Plural comparison organoleptic parameters			
	Color	**Texture**	**Flavor**	**Aroma**
P1 = 0	5.15 + 0.74b	5.35 + 0.74a	4.35 + 1.26b	5.05 + 0.68c
P2 = 15	4.45 + 1.63ab	6.10 + 1.65b	4.30 + 1.21b	4.60 + 1.42bc
P3 = 30	6.20 + 1.73c	6.15 + 1.26b	4.05 + 1.05ab	4.45 + 1.09bc
P4 = 45	4.10 + 1.11a	4.95 + 1.09a	3.40 + 1.27a	3.85 + 1.13ab
P5 = 60	4.00 + 1.29a	4.85 + 0.74a	3.30 + 1.21a	3.45 + 1.23a

Note: Numbers in the same column followed by different lowercase letters are significantly different according to DNMRT at the 5% level.

The plural color comparison test value (Table 3) showed no difference in color between P1 and P2 treatment tempeh, which were white. While the color in the P3 treatment, the addition of red bean skin powder produced a reddish white tempeh, the dominant panelist assessment preferred it. Furthermore, in the multiple comparison test of the texture of the tempeh produced, it appeared that it was getting denser with the addition of red bean skin powder. This is because red bean skin powder contains high crude fiber. Fiber is a polysaccharide in food ingredients as a texture booster. The higher the fiber content, the product with a firmer texture will be produced and the product becomes harder [34]. However, the more (more than 30%) the addition of red bean skin powder, the less panelists liked the texture of the tempeh.

The result of the multiple comparison test (Table 3) on the taste of tempeh in the P1 treatment produced tempeh has a distinctive taste of tempeh, however, the addition of red bean skin powder which is increasingly in each treatment will reduce the distinctive taste of tempeh. The reduction in the distinctive taste of tempeh is due to the powdered red bean skin and leaves a flat taste. Furthermore, the results of the multiple comparison test on the aroma of tempeh with the addition of more red bean skin powder in each treatment made the panelists less like the aroma of the tempeh produced. This is due to the languorous aroma of red bean skin powder. Red beans contain the enzyme lipoxygenase which causes a languid aroma [35]. The results of the multiple comparison test of all red bean tempeh treatments can be seen in Figure 2.

Based on the bar chart analysis of the multiple comparison test of red kidney bean tempeh, it can be concluded that the panelists preferred the P3 treatment (30% addition of red kidney bean skin powder). The panelists preferred the P3 treatment for the texture and color of red bean tempeh with a score for texture of 6.15 (slightly different and preferred) and color 6.2 (slightly different and preferred).

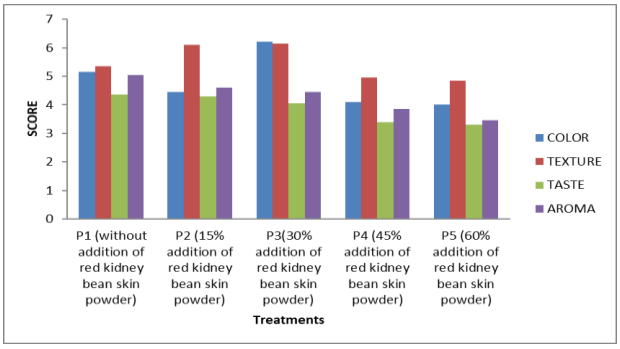

Figure 2. Bar Chart of Red Kidney Bean Tempeh Multiple Comparison Test

4. CONCLUSION

Based on the existing body of research, the following conclusions can be drawn. The incorporation of red bean skin powder into red bean tempeh resulted in notable variations in texture, moisture content, ash content, protein content, crude fiber content, and iron content. The results of the multiple comparison tests conducted on several attributes, including color, texture, taste, and scent, did not yield statistically significant differences when compared to the levels of fat and carbohydrate content.The most effective intervention involved the incorporation of red bean skin powder at a concentration of 30% (referred to as the P3 treatment). The tempeh exhibited textural properties with a value of 2.01 N/cm2. It had a moisture content of 61.63%, ash content of 0.72%, protein content of 15.83%, fat content of 1.51%, carbohydrate content of 20.30%, crude fiber content of 6.95%, iron content of 5.00 mg/gr, coliform content of 0.32 APM/g, and tested negative for Salmonella/g. The results of the plural comparison test indicate that color received a rating of 6.20, indicating a higher preference compared to the comparator. Similarly, texture received a rating of 6.15, indicating a higher preference compared to the comparator. In terms of taste, the rating was 4.05, suggesting a minor difference from the comparator. Lastly, the aroma received a rating of 4.45, again indicating a slight difference from the comparator.

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