



Analysis of the Fat, Tannin, and Iron Content of *Snack bars* Utilizing Banana Flour, Sorghum Flour, and Added Tamarind Leaf Flour

Devi Kumala Ningrum^{1*} | Rizki Nurmalya Kardina²

^{1,2}Department of Nutrition, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Indonesia

***Corresponding Author:**

Rizki Nurmalya Kardina, Department of Nutrition, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Indonesia.

Email: rizki_kardina@unusa.ac.id

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ABSTRACT

Hypercholesterolemia is a non-communicable disease caused by an increase in total body fat content. Additionally, it can be caused by the habit of consuming foods high in saturated fat. Therefore, one approach is to modify food products by using banana flour, sorghum flour, and tamarind leaf flour in the production of snack bars. This research aimed to analyze the differences in fat, tannin, and iron levels in snack bars made from banana flour, sorghum flour, and the addition of tamarind leaf flour. The methodology of this study was based on a experimental design, specifically implementing a Completely Randomized Design (CRD) to structure the experiment. This experiment used snack bar samples consisting of three formulation ratios of Banana Flour: Sorghum Flour : Tamarind Leaf Flour, namely F1 (80%:10%:10%), F2 (75%:10%:15%), and F3 (70%:10%:20%). Fat content was tested using the Soxhlet method, tannin was measured using the UV-Vis spectrophotometry method, and iron content was determined using the atomic absorption spectrophotometry method. One Way ANOVA was applied to assess fat and tannin levels, while the Kruskal Wallis procedure was employed for analyzing iron concentration. The analysis indicated that F1 had the most favorable low-fat value of 13.72%, while F3 achieved the highest concentrations of tannin and iron, recorded at 4.2 mg, and 0.057 mg, respectively. The study concludes that there are significant differences in tannin content with a *p*-value 0,001 and iron content with a *p*-value 0,026, while fat content do not show a significant difference with a *p*-value 0,056.

Keywords: Hypercholesterolemia, Snack Bars, Sorghum, Banana, Tamarind Leaves, Iron, Tannins

INTRODUCTION

A common global health issue is non-communicable diseases (NCDs), such as hypercholesterolemia. Hypercholesterolemia is a condition that has been continuously increasing worldwide, including in Indonesia, which significantly contributes to the occurrence of morbidity and mortality realated to cardiovascular diseases. Hypercholesterolemia frequently affects adults and the elderly. According to several studies, the risk of increased cholesterol levels rises with age. In adulthood, men tend to have higher cholesterol levels than women because cholesterol serves as a precursor for testosterone production. Among elderly individuals, women frequently

present with greater cholesterol concentrations than men, primarily as a result of declining estrogen levels (Saputri & Novitasari, 2021).

According to the World Health Organization (WHO), the global prevalence of hypercholesterolemia is quite high, reaching between 16 to 33 million cases, and Indonesia is among the affected countries. In Indonesia, hypercholesterolemia is also significantly prevalent. Based on data from the 2018 Basic Health Research (Riskesdas), 34,820 individuals aged over 15 years were found to have high cholesterol levels, and this number is likely to increase over time. Furthermore, based on a 2022 report from the Indonesian Health Ministry (Kemenkes) hypercholesterolemia affects around 28% of the country's citizens. In East Java Province, data from the Integrated Development Post (Posbindu) and Primary Healthcare Facilities (FKTP) in 2016 reported that out of 8,225 individuals, 36.1% were identified as having high cholesterol levels.

Hypercholesterolemia are associated with an increased risk of cardiovascular illnesses, including coronary artery conditions and atherosclerosis, which involve the narrowing or blockage of blood vessels (Fitrianti *et al.*, 2019). The causes of hypercholesterolemia are not only genetic or due to other diseases but can also result from an unhealthy lifestyle that triggers an increase in cholesterol levels (Waznah, 2022). Poor lifestyle choices and unhealthy eating patterns such as the regular consumption of foods high in saturated fats, including coconut milk, butter, cream, fast food, and fried foods like fritters, which are commonly consumed as snacks can lead to hypercholesterolemia because these foods are high in fat (Khairunnisa *et al.*, 2023).

Based on the explanation of dietary causes of hypercholesterolemia such as the consumption of fried foods or junk food a possible solution is to consume functional food products that are rich in tannins and low in fat (Indriyani *et al.*, 2023). Additionally, food products with iron content also offer a solution, as iron has a relationship with lipids, including changes in cholesterol metabolism in response to altered iron levels. For example, higher iron levels are associated with a reduced risk of hypercholesterolemia. (Rockfield *et al.*, 2018). Cholesterol reduction can be achieved by selecting products that contain antioxidants such as tannins, which have the ability to inhibit the absorption of glucose and fat in the intestines, thereby lowering total cholesterol levels (Sakaganta, 2021). One of the healthy low-fat snack products is a snack bar, innovated with the addition of tamarind leaf flour.

In addition to lowering hypercholesterolemia levels, this snack bar also adds value to tamarind leaves, which are typically used only as traditional herbal medicine. As reported by the World Health Organization (WHO), the consumption of herbal medicine has been reintroduced as an alternative treatment, especially during the Covid-19 pandemic. Herbal consumption increased during the pandemic, as it was believed to be effective in boosting the immune system (Kusumo *et al.*, 2020). However, after the Covid-19 pandemic, herbal consumption declined from 79% to 56.59% (Saima Perdani *et al.*, 2021).

Tamarind leaves are a part of the tamarind tree that have been developed as herbal or alternative medicine. Their high antioxidant content such as tannins, saponins, and flavonoid provides medicinal benefits due to their antibacterial activity, particularly in young tamarind leaves (Husain *et al.*, 2022). A study conducted by Assagaf *et al.*, (2015) tamarind leaf extract was found to reduce cholesterol levels in male Wistar rats. Additionally, research by Sakaganta & Sukohar (2021) reported that the flavonoid and salicylate compounds present in bay leaves can naturally reduce blood cholesterol levels.

A snack bar is a convenient type of food product that is easy to consume, has a long shelf life, and is made from a variety of food ingredients (Aminah, 2019). There are various types of ingredients used to make snack bars, each with its own benefits. In this study, the snack bar was formulated using three main ingredients: banana flour, sorghum flour, and tamarind leaf flour. Banana flour can serve as a substitute for wheat flour, being rich in vitamins and gluten-free (Permana & Efendi, 2024). In addition, banana flour is high in fiber, which helps reduce the absorption of bad cholesterol in the digestive tract (Pratama, 2022). Sorghum flour, on the other hand, is high in fiber and minerals, has water-soluble properties, and contains bioactive compounds that are beneficial for their anti-inflammatory effects and ability to lower cholesterol (Hermení *et al.*, 2023). The third key ingredient is tamarind leaf flour, which contains tannins that can help reduce cholesterol levels (Risfianty & Indrawati, 2020). Tamarind leaf flour is added due to its relatively high tannin content of 0.1894 mg, compared to banana flour (0.13 mg) and sorghum (0.062 mg). Furthermore, the use of tamarind leaves in snack bars helps reduce waste, unlike traditional herbal drinks like *jamu sinom*, which typically only utilize the leaf extract. Considering the prior context, this experiment aims to evaluate the fat, tannin, and iron composition of the developed snack bar formulation.

MATERIAL AND METHODS

The material used in this research are banana flour obtained from the marketplace under the brand “Moringa”, sorghum flour from the marketplace under the brand “Tambiyaku”, and tamarind leaves which are either young or old, collected directly from the tamarind tree. The leaves are then soaked to minimize their bitter taste, making them the base ingredient for the snack bar. The sugar used is power form, then it is melted, and the raw sugar is obtained from the marketplace with the brand name ‘ Ie is Organic Foods’. The sample size for the cnack bar made from sorghum flour with the addition of tamarind flour, using 3 different formulas and repeated 3 times.

This study was conducted in two different locations: the Nutrition Laboratory, Chemistry Laboratory, and Biomolecular Laboratory at Universitas Nahdlatul Ulama Surabaya (UNUSA), as well as the Nutrition Laboratory at Universitas Airlangga (UNAIR). The Nutrition Laboratory at UNUSA was used for the production process of the snack bar, while the Chemistry and Biomolecular Laboratories at UNUSA were used to analyze fat content. The Nutrition Laboratory at UNAIR was used to analyze tannin and iron content. An experimental method was employed in this study, utilizing a Completely Randomized Design (CRD) with three types of treatment and triplicate trials. When data exhibited a normal distribution, statistical analysis was carried out using One Way ANOVA with Duncan’s post-hoc procedure, in contrast for non- normally distributed data, the Kruskal Wallis test was applied, followed by a Pairwise comparison. The experimental design table is presented below :

Table.1 Experimental Design

Treatment	Replication 1	Replication 2	Replication 3
F1	F1.1	F1.2	F1.3
F2	F2.1	F2.2	F2.3
F3	F3.1	F3.2	F3.3

The formulation ratio using the composition of Banana Flour : Sorghum Flour : Tamarind Leaf Flour consists of three treatments as follows :

1. F1 = 80% : 10% : 10%
2. F2 = 75% : 10% : 15%
3. F3 = 70% : 10% : 20%

Production of Tamarind Leaf Flour

The production of tamarind leaf flour begins with sourcing tamarind leaves, followed by separating the leaves from the stems, and then proceeding to the soaking process. The equipment needed during the tamarind leaf flour processing includes a large bowl, tray, microwave/oven, blender, sieve, spoon, baking tray, and spatula. The soaking is done using water mixed with salt, vinegar, and baking soda for 12 hours. The soaked tamarind leaves are then drained and dried initially under sunlight, and then dried in a microwave or oven at 35°C for 5 hours. The dried tamarind leaves are then ground using a blender, and the resulting powder is sifted to obtain tamarind leaf flour.

Production of *Snack bar*

The production of the snack bar begins with the procurement of ingredients, including banana flour, sorghum flour, tamarind leaf flour, and complementary ingredients such as eggs, palm sugar, cashew nuts, salt, and sesame seeds. The equipment required for making the snack bar includes a knife, cutting board, large bowl, digital scale, spoon, whisk, spatula, baking tray, and oven. After collecting all necessary components, the next step is to weigh each ingredient according to the specific formulation listed in the table below:

Table.2 Composition of the *Snack bar* Product

Flour Formulation (%) Ingredients	F1	F2	F3
	(80%:10%:10%)	(75%:10%:15%)	(70%:10%:20%)
Banana Flour	80 gr	75 gr	70 gr
Sorghum Flour	10 gr	10 gr	10 gr
Tamarind Leaf Flour	10 gr	15 gr	20 gr
Egg	60 gr	60 gr	60 gr
Palm Sugar	15 gr	15 gr	15 gr
Cashew Nuts	20 gr	20 gr	20 gr
Salt	1 gr	1 gr	1 gr
Sesame Seeds	1 gr	1 gr	1 gr
Water	20 ml	20 ml	20 ml

After weighing each ingredient, the next step is to crush the cashew nuts into small pieces and roast them at 120°C for 15 minutes. Then, melt the palm sugar with a little water, and mix all the prepared ingredients according to each formula. The well-mixed dough is then shaped into rectangular bars, weighed, sprinkled with sesame seeds, and baked at 130°C for 40 minutes.

Fat Content Testing

The fat content was analyzed using the *Soxhlet* method with an n-hexane solution and snack bar samples. The required equipment includes a *Soxhlet* apparatus, analytical digital balance, fat flask, stirring rod, beaker glass, filter paper, cotton, desiccator, oven, condenser, distillation flask, mortar and pestle, and petroleum jelly. The first step is equipment preparation, including drying the fat flask in an oven at a temperature of 100°C–105°C, followed by cooling it in a desiccator and weighing it. Next is sample preparation, where the sample is ground using a mortar and pestle,

weighed, and wrapped in filter paper. The sample is then extracted using a Soxhlet apparatus with n-hexane solution for 5 hours. The extract is then separated for 30 minutes, and the extracted fat is heated in the oven at 150°C for 30 minutes. After cooling, fat measurement is carried out, followed by the calculation of its content using a designated formula:

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$$\% \text{ fat} = \frac{W_2 - W_1}{W} \times 100\%$$

Information :

W : Sample weight (g)

W1 : Empty fat flask weight (g)

W2 : Flat flask + extracted fat weight (g)

Tannin Content Testing

The tannin content test is carried out using the *UV-Vis Spectrophotometry* method with standard solutions of BSA, acetate buffer, FeCl₃, HCl, tannic acid, methanol, and SDS-TEA. The tools required for the tannin analysis include test tubes, filter paper, mortar and pestle, vortex mixer, centrifuge, and *spectrophotometer*. The first step is sample preparation, where the sample is ground using a mortar and pestle, weighed, and placed into a test tube. Next, the sample is extracted using a vortex mixer, followed by separation of the extract via centrifugation and cooling with the BSA standard solution. The extract is centrifuged again, and the supernatant is processed using a combination of acetate buffer, SDS-TEA, FeCl₃ solution, and HCl, then mixed using a vortex. The absorbance is measured using a spectrophotometer. After that, a standard solution is prepared by dissolving tannic acid in methanol to form a stock solution, which is tested and calculated using the following formula:

$$\text{Total tannins} = \frac{C \times V \times \text{Diluting factor}}{m \text{ (g)}}$$

Information:

C : Total tannin concentration from the tannic acid standard curve (ppm atau mg/L)

V : Sample

Volume (L) Df : Dilution

Factor (Ml) M : Sample

weight (g)

Iron Content Testing

Determination of iron content was conducted using the *Atomic Absorption Spectrophotometry* (AAS) method with concentrated nitric acid (HNO₃), iron standard solution, and hydrochloric acid (HCl). The equipment required for iron content testing included an atomic absorption spectrophotometer, an Fe hollow cathode lamp, an analytical balance, beaker glasses,

an oven, volumetric flasks, funnels, porcelain crucibles, an electric furnace, filter paper, and a desiccator. The first step was the preparation and weighing of the sample, followed by a dry destruction process in an electric furnace for 2 hours. After cooling to room temperature, the filtrate was transferred and measured using the *atomic absorption spectrophotometer* (AAS) equipped with an iron cathode lamp. A 100 mg/L stock Fe standard solution was prepared by diluting the iron standard with 1N HNO₃ and distilled water was added until the liquid reached the specified line on the volumetric flask. Absorbance was measured at the appropriate wavelength, and the iron content in the sample was determined based on the regression equation derived from the calibration curve and obtained using the equation shown below:

$$\text{Metal level (mg Fe/g)} = \frac{C \times V \times Df}{BS}$$

Keterangan:

C : Concentration ($\mu\text{g/mL}$)

V : Sample Solution Volume

(ml) Df : Dilution Factor

BS: Sample Weight (g)

RESULTS AND DISCUSSION

Fat Content

The normality test results for fat content showed a p-value of 0.114 (>0.05), indicating a normal distribution. Therefore, the data can be analyzed using a One-Way ANOVA test. The following is the fat content analysis using One-Way ANOVA:

Table.3 Differences in Fat Content of *Snack bar* Products

o	de	Co	N	Mean $\pm$ SD (%)	ρ – value
		F1	3	13,72 $\pm$ 1,25	0,056
		F2	3	16,79 $\pm$ 1,34	
		F3	3	14,66 $\pm$ 1,10	

As shown in table 3, variations can be identified in the average fat levels among snack bars formulated with banana flour, sorghum flour, and various amounts of tamarind leaf flour, with the highest fat content found in F2 at 16.79% (75%:10%:15%), followed by F3 at 14.66% (70%:10%:20%), while the lowest fat content is found in F1 at 13.72% (80%:10%:10%). Based on the One Way ANOVA results, the ρ value obtained for fat content was 0,056, which is higher than the α threshold (0.05), suggesting that the differences in mean fat content are not statistically significant. Therefore, the data did not permit continuation with the DMRT (Duncan's Multiple Range Test) as a post hoc analysis.

The fat content of this snack bar product is relatively high, primarily derived from sorghum flour at 3.3 grams, followed by tamarind leaf flour at 1.52 grams, and lastly banana flour at 0.4 grams. Tamarind leaf flour contributes to the increase in fat content (Harsitha *et al.*, 2021). The fat content analysis in this study showed no significant differences, which may be due to the soaking process in acidic and alkaline conditions using vinegar and baking soda during the preparation of tamarind leaf flour. The soaking was carried out for 12 hours using salt, baking soda, and vinegar, where such treatment can affect the fat content.

The use of vinegar in an acidic environment can accelerate the hydrolysis reaction of fats, which is the breakdown of fats into smaller components such as free fatty acids and glycerol. As a result, fats may not be fully detected during analysis, leading to lower observed fat content. Similarly, sodium bicarbonate or baking soda, which is alkaline in nature, can also reduce the fat content in a product (Litaay *et al.*, 2021). A similar outcome was observed in the research conducted by Febrianto *et al.*, (2014) nothing that treatment of corn kernels with baking soda at specific concentrations of 1%, 3%, and 5% for several hours resulted in lower fat content as the concentration increased. Furthermore, a study by Herpandi *et al.*, (2019) showed that soaking putak fish bones in sodium bicarbonate at concentrations of 0%, 0.5%, 1%, 1.5%, 2%, and 2.5% for 3 hours also led to a decrease in fat content with higher concentrations. Similarly, the study by Suryaningrum *et al.*, (2022) indicated that soaking in vinegar and sodium bicarbonate solutions can accelerate fat hydrolysis, thereby reducing fat content.

The fat content obtained from F1 to F3 relatively did not show significant differences; however, these levels have met the quality standards set by SNI 01-4216-1996 concerning the Quality Requirements for Snack Bar Formula Foods, which specify a minimum fat content of 3% and a maximum of 30%. The snack bars in this study are considered safe for consumption because the fat content derived from banana flour and tamarind leaf flour is relatively low, aligning with the Ministry of Health's recommendation of a daily fat intake limit of approximately 67 grams or 20%–25% of total energy. Therefore, these snack bars can be consumed by individuals with hypercholesterolemia. This is supported by the study conducted by Muryati, (2020) which stated as a macronutrient, fat plays a crucial part in preserving the body's overall health and function. If fat intake is well-regulated and not excessive, the body remains healthy; however, excessive fat consumption can lead to health issues such as hypercholesterolemia.

Tannin Content

The normality test results for tannin levels showed a p-value of 0.637 (>0.05), indicating that the data are normally distributed. Therefore, the analysis can proceed using a One-Way ANOVA test. The following is the tannin analysis using the One-Way ANOVA test:

Table.4 Differences in Tannin Content of *Snack bar* Products

o	de	Co	N	Mean $\pm$ SD (mg)	ρ – value
		F1	3	3,7 $\pm$ 0,02 ^a	0,001
		F2	3	3,9 $\pm$ 0,03 ^b	
		F3	3	4,2 $\pm$ 0,03 ^c	

As shown in table 4, the tannin content varies across snack bars made with banana flour, sorghum flour, and tamarind leaf flour vary, with the highest tannin content found in F3 at 4.3 mg (70%:10%:20%), followed by F2 at 3.9 mg (75%:10%:15%), and the lowest in F1 at 3.7 mg (80%:10%:10%). The tannin content analysis using the One-Way ANOVA procedure, resulted in a ρ -value of 0.001, which falls within the threshold of significance ($\alpha = 0.05$), indicating a significant difference in the average tannin levels. Since there is a significant difference, a follow up analysis was carried out using Duncan's Multiple Range Test (DMRT) technique. Findings from the DMRT showed that the tannin content in the snack bars differs significantly across all formulations. The tannin levels in the snack bars made with banana flour, sorghum flour, and tamarind leaf flour differed significantly between F1 and F2, F2 and F3, and F1 and F3

The findings of this study indicate that increasing the proportion of tamarind leaf flour correlates with a higher tannin concentration in snack bar. In other words, as the percentage of

tamarind leaf flour increases, the tannin content in each formulation also increases. The tannin content in the snack bar is primarily derived from tamarind leaf flour, which contains 0.1894 mg of tannin (Deepak *et al.*, 2016). These results are in agreement with the research conducted by Fu'adah *et al.*, (2021) which showed an increase in tannin content with higher concentrations of moringa leaf flour substitution. Similarly, Adhamatika *et al.*, (2021) although using different ingredients, reported the same trend—namely, that a higher percentage of dried tea leaves results in increased tannin content.

Tannins are natural compounds that interact with proteins. Their role in reducing hypercholesterolemia involves forming complexes with proteins, which then coat the intestinal walls and ultimately inhibit cholesterol absorption or regulate the activity of lipoprotein hydrolysis (Mutia *et al.*, 2018; Nugroho *et al.*, 2022). Furthermore, research conducted by Wang *et al.*, (2023) revealed a notable reduction in triglyceride and blood cholesterol levels following an 8-week intervention with 0.5% tannin supplements in a group of sheep.

Iron Content

The outcome of the normality assessment for iron levels yielded a for iron content showed a p-value of 0.01, which is below 0.05, suggesting that the data deviate from a normal distribution. Therefore, the data were further examined through the Kruskal-Wallis method. Below is the analysis of iron concentration carried out using the Kruskal-Wallis method:

Table.5 Differences in Iron Content of *Snack bar* Products

o	de	Co	N	Mean $\pm$ SD (mg)	ρ – value
		F1	3	0,053 $\pm$ 0,01 ^a	0,026
		F2	3	0,055 $\pm$ 0,05 ^b	
		F3	3	0,057 $\pm$ 0,02 ^c	

Based on Table 5, it can be seen that the average iron content of the snack bar made with banana flour, sorghum flour, and tamarind leaf flour varies, with the highest iron content found in F3 at 0.057 mg (70%:10%:20%), followed by F2 at 0.055 mg (75%:10%:15%), and the lowest in F1 at 0.053 mg (80%:10%:10%). The analysis of iron content using the Kruskal-Wallis test showed a p-value of 0.026, which is $\leq \alpha$ (0.05), demonstrating a statistically meaningful disparity in iron levels across the samples. Since a significant difference was found, the analysis was continued with a Pairwise comparison test. The Pairwise test results indicated a significant difference in iron content between F1 and F3, while other formulas such as F2 and F3 did not show a significant difference.

Based from this investigation revealed a notable variation attributed to the incorporation of tamarind leaf flour, which influenced the iron content. This can be seen from the increasing iron levels in F1 (0.053 mg), F2 (0.055 mg), and F3 (0.057 mg), indicating that as the percentage of tamarind leaf flour increases, the iron content also increases. In addition, banana flour and sorghum flour also contributed to the iron content in the snack bar. This finding is consistent with the study by which stated that increasing proportion of moringa leaf flour in the formulation contributes to elevated iron levels. Similarly Zaddana *et al.*, (2022) reported that a greater amount of moringa leaf flour result in higher iron content. In this study, the baking process of the snack bars using an iron baking tray for 40 minutes may have contributed to the increase in iron concentration, this observation aligns with the findings of Kulkarni *et al.*, (2013) which found a

significant 16.2% increase in iron levels in snacks cooked in iron pots, Additionally, Sharma *et al.*, (2021) observed a substantial increase in total iron content in foods cooked using iron trays or ingots. Although there were significant differences in iron levels from F1 to F3, none of the formulations met the SNI 01-4216-1996 quality standard for snack bar formulas, which requires a minimum iron content of 16 mg. This means that the iron content in each formula did not meet the established quality standard. One way to meet the SNI 01-4216-1996 iron content requirement for snack bar formula is by incorporating ingredients with a high iron content.

CONCLUSION AND SUGGESTION

There was no significant difference in fat content was observed among the snack bar formulations composed of banana flour, sorghum based ingredients, and varying proportions of tamarind leaf flour. However, there was a significant difference in tannin content among the formulations, with the highest being in F3 (70%:10%:20%) at 0.42% and the lowest in F1 (80%:10%:10%) at 0.37%. A significant difference in iron content was also observed between formulations F1 and F3, with F3 (70%:10%:20%) having the highest iron content at 0.057 mg and F1 (80%:10%:10%) having the lowest at 0.053 mg in snack bars made from banana flour, sorghum flour, and tamarind leaf flour.

Suggestions for future researchers, it is recommended that future studies include additional variables related to the nutritional content of the snack bar product, such as vitamin B, vitamin E, phosphorus, calcium, temperature, and processing time, for a more extensive exploration in future studies. It is also advised that future research maintain consistency in ingredient processing, such as using precise soaking durations. Suggestions for the public, the recommended formulation as an alternative functional food product for individuals with hypercholesterolemia is Formula F3.

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