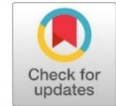




Differences in fatty acids, zinc, and copper profiles among processed *Kedawung* (*Parkia roxburghii* G. Don) products and their relevance to cardiovascular health

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ABSTRACT

Background: *Kedawung* is an underutilized legume which are known for its nutrient-rich qualities that can improve through various processing methods, including fermentation and flouring. Nutrients such as fatty acids, zinc, and copper play important roles in lipid metabolism and antioxidant defense and there are therefore relevant to cardiovascular health.

Objective: This study aimed to examine differences in fatty acid, zinc, and copper profiles among three processed *Kedawung* products boiled *Kedawung* seeds (BK), *Kedawung* tempeh (TK), and *Kedawung* tempeh flour (TPK) and to evaluate their potential relevance to cardiovascular health.

Materials and Methods: The study employed gas chromatography to analyze fatty acids and atomic absorption spectrophotometry to analyze zinc and copper in BK, TK, TPK samples. A normality test was conducted using Shapiro-Wilk, followed by one-way ANOVA or Kruskal–Wallis tests for normally and non-normally distributed data, respectively.

Results: The highest total fatty acid content was observed in TK (97.965 ± 12.002 %w/w), while TPK showed the lowest value (38.144 ± 29.677 %w/w). BK contained seven types of fatty acids, TK contained six, and TPK contained three. Stearic acid, linoleic acid, and lignoceric acid were present in all three samples. Zinc and copper contents were highest in TPK (62.529 ± 8.0205 ppm and 17.987 ± 4.097 ppm) and lowest in TK (12.854 ± 2.065 ppm and 2.639 ± 0.080 ppm).

Conclusion: Different processing methods significantly influence the fatty acid, zinc, and copper profiles of *Kedawung*. Processed *Kedawung* products present nutritional characteristics with potential relevance to cardiovascular health, although further studies are required to confirm their functional effects.

Keywords: Copper; fatty acid; food processing; kedawung; zinc

BACKGROUND

Legumes are an important source of nutrients, including protein, carbohydrates, water-soluble vitamins, and minerals, and play a significant role in human nutrition.¹ In Indonesia, legume-based foods are widely consumed and are considered one of the main food commodities after rice and corn.² Among legumes, soybean is one of the most widely utilized, and demand for its consumption increases every year. In 2020, soybean demand reached approximately 3.28 million tons, while domestic production was only 0.63 million tons, resulting in about 81% of total soybean needs being met through imports.³ Furthermore, soybean imports showed an increasing trend during 2016–2020, with an average annual growth rate of 4.13%.⁴ Because domestic soybean production remains inadequate and reliance on imports continues to grow, this situation raises concerns regarding food security and sustainability.^{3,4} These overall conditions highlight the need to explore locally available legumes with comparable nutritional potential.

Kedawung is an underutilized legume-type plant that grows in many Asian countries, including Indonesia. It is a medium-sized tree with grayish-brown bark and a height of 15–25 m. Seed production begins at approximately six years of age, with pods containing flattened, egg-shaped seeds that have a slightly bitter taste and a pungent smell.^{5,6} *Kedawung* is popularly used in traditional medicine to treat various conditions, including high blood pressure, piles, diabetes, dysentery, and urinary tract infections.⁵ From a nutritional perspective, *Kedawung* seeds contain substantial amounts of fat (35.5%), protein (28.8%), carbohydrates (22.0%), and ash (5.7%).⁵ Importantly, previous studies have reported that these nutrients remain relatively stable during basic processing methods such as soaking and heating and may even increase during the legume fermentation process, suggesting that *Kedawung* has strong potential for further food product development.^{7,8}

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Processing techniques are known to influence the nutritional quality and functionality of legumes. Fermentation, commonly applied in legume-based foods, has been shown to improve nutrient bioavailability and increase the content of bioactive compounds.⁷ A previous study by Sathya and Siddhuradju found that fermentation had a positive effect on *Kedawung* seeds by increasing their protein, fat, essential fatty acid, and mineral content.⁸ This finding is corroborated by a study by Sahara et al., which reported that unsaturated fatty acid levels in rice bran were significantly increased after fermentation.⁹ Additionally, a study by Atere et al. showed that fermentation significantly increased the amino acid and fatty acid profiles of locust bean (*Parkia biglobosa*).¹⁰ These improvements are largely attributed to enzymatic activity during fermentation, which modifies macronutrient and micronutrient composition.¹¹ In Indonesia, fermentation is commonly applied in the form of tempeh, a traditional fermented legume product that functions both as a food preparation method and as a functional food.¹² Within this framework, tempeh-style fermentation provides a relevant model for evaluating the nutritional modification of *Kedawung*, where fermentation represents one possible processing strategy to enhance its nutritional value.

Despite its nutritional benefits, fermented legume products generally have a limited shelf life due to ongoing enzymatic activity. To improve product stability and extend shelf life, additional processing methods such as flouring are often applied.¹¹ Flouring is a technique that involves drying and grinding food materials to reduce particle size.¹³ The application of heat during this process may result in an increase in the ash content of food products.¹⁴ As a result, flouring may indirectly increase mineral content that has already been enhanced during the fermentation of *Kedawung* seeds. Sathya and Siddhuradju reported that zinc and copper contents increased from 2.7 mg/100 g to 3.1 mg/100 g and 0.8 mg/100 g to 1.0 mg/100 g, respectively, following fermentation.⁸ However, a recent study by Famuwagun and Taiwo reported that the drying process applied to locust beans reduced ash content and increased fat content, although the changes were not statistically significant.¹⁵ Meanwhile, Olalusi et al. reported that the ash and fat contents of the same legume showed fluctuating results depending on the type of sun-drying method used.¹⁶ These findings indicate that different processing conditions may lead to varying nutritional outcomes. Therefore, it is important to evaluate how flouring, when applied after fermentation, affects the nutritional profile of *Kedawung*.

Beyond changes in general nutrient composition, processing methods may influence specific nutrients with functional roles in human health. Fatty acids, zinc, and copper are nutrients of particular interest due to their roles in lipid metabolism, antioxidant defense, and cardiovascular health.¹⁷ Cardiovascular health is closely related to the prevention of cardiovascular disease, which is defined as a group of disorders affecting the heart and blood vessels, including coronary heart disease, stroke, and other related conditions.¹⁸ In Indonesia, heart disease particularly stroke and coronary conditions remains a leading cause of death, especially in urban areas, with a diagnosed prevalence of 1.5% in 2018.¹⁹ Key risk factors contributing to cardiovascular disease include oxidative stress, dyslipidemia, and the consumption of foods high in fat and cholesterol.²⁰ Certain dietary fatty acids have been reported to influence lipid profiles associated with these risk factors. Oleic, linoleic, and linolenic fatty acids found in fermented legume-based foods, particularly soybean tempeh, play an important role in lowering low-density lipoprotein (LDL) cholesterol levels and increasing high-density lipoprotein (HDL) cholesterol levels.^{12,21,22} Other nutrients, such as isoflavones, zinc, and copper, have also been reported to function as antioxidants and help prevent oxidative stress through their role in superoxide dismutase activity.²³ However, limited information is available regarding how different processing methods influence the levels of these nutrients in *Kedawung*-based products.

Therefore, this study aims to examine differences in fatty acid, zinc, and copper profiles among three forms of processed *Kedawung*: boiled *Kedawung* seeds (BK), *Kedawung* tempeh (TK), and *Kedawung* tempeh flour (TPK). By focusing on *Kedawung* as the primary raw material and evaluating the effects of different processing methods, this research seeks to provide evidence supporting the utilization of *Kedawung* as a locally sourced legume with potential relevance to cardiovascular health.

MATERIALS AND METHODS

The research design was a 1-factor completely randomized design to determine the content of fatty acids, zinc, and copper in 3 variations of *Kedawung* preparations, including boiled *Kedawung* seeds (BK), *Kedawung* tempeh (TK), and *Kedawung* tempeh flour (TPK). Each sample was repeated 3 times and tested in duplo. This research was conducted from August to December 2021 and divided into 2 stages, preliminary research and main research. Preliminary research aims to test varieties and prepare samples while the main research is to test the content of fatty acids, zinc, and copper in the three samples.

Preliminary Research

Kedawung seeds were sourced from a local farmer in Yogyakarta and verified for species uniformity through a variety test at the *Laboratorium Ekologi dan Biosistematik, Departemen Biologi, Universitas Diponegoro*. The yeast brand *Raprima* was commercially produced by PT Aneka Fermentasi Industri in Bandung. The sample preparation was a continuous process. Boiled *Kedawung* seeds (BK) and *Kedawung* tempeh (TK) were processed based on tempeh production method number seven by Saono et al. and were conducted at the researcher's house.²⁴ Meanwhile, the *Kedawung* tempeh flour (TPK) was prepared following the flouring method by Astawan et al. and was conducted at the *Laboratorium Rekayasa, Pusat Studi Pangan dan Gizi, Universitas Gadjah Mada*.²⁵

The steps for the preparation of boiled *Kedawung* seeds sample began with washing the seeds with clean and running water. The seeds were then soaked in clean water for 2 hours and boiled in boiling water (100°C) for 2 hours. After boiling, the seeds were drained, cooled at room temperature, and shell peeled. Next, the peeled seeds were soaked again with room temperature boiled water for 8 hours, boiled again in boiling water (100°C) for 30 minutes, then drained and cooled at room temperature. Finally, the seeds were divided into 2 portions, one for boiled *Kedawung* seeds sample (BK) and the other for the making of *Kedawung* tempeh (TK).

To make *Kedawung* tempeh, *Rhizopus oligosporus* yeast was added to the other half of BK sample. The ratio of yeast to seed was 2 grams of yeast per 1 kg of sample. The yeast was then mixed evenly with the sample, which was packed into perforated polyethylene bags. The inoculated seeds were then incubated for 48 hours in a room protected from direct sunlight, with a room temperature and dry conditions, until it became compact. Finally, the samples were again divided into two portions, one for *Kedawung* tempeh (TK) and the other for *Kedawung* Tempeh Flour (TPK) samples.

The process of preparing *Kedawung* Tempeh Flour (TPK) began with slicing the other half of TK sample which is then steamed for 2 minutes. The steamed tempeh was then cooled at room temperature, levelled on a rack, and dried using a cabinet dryer for 8 hours at 50°C. Once dry, the tempeh samples were ground using a grinder and then passed through a 50-mesh sieve to achieve a fine powder.

Main Research

Fatty acid test was carried out at the *Laboratorium Kimia Organik, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Gadjah Mada* by *CV Chem-Mix Pratama* following AOCS Official Method Ce 1-62 Fatty Acids Compositions by Gas Chromatography with AOCS Official Method Ce 2-66 Preparation of Methyl Ester of Fatty Acids.²⁶ The sample preparation step begins with drying 150 grams of wet weight of boiled *Kedawung* seeds (BK) and *Kedawung* tempeh (TK) samples using an oven with a temperature of 105°C for 5 – 6 hours to get a dry weight of 100 grams, then as much as 100 grams of dry weight of *Kedawung* seeds (BK), *Kedawung* tempeh (TK), and *Kedawung* tempeh flour (TPK) was refined again with a blender, dissolved with n-hexane petroleum benzene solution, extracted by the Soxhlet method, and the oil form of each sample was separated. Then methylation (methyl ester formation) was carried out by adding 150 mL of 0.1 M KOH-methanol, heated in a water bath for 20 minutes at 80°C, the sample was cooled, 5 mL of BF₃ was added, and heated again at the same temperature. The sample was then cooled again, mixed with 2 mL NaCl, added 5 mL n-hexane petroleum benzene. As a final step, the top layer was pipetted and put into a vial filled with anhydrous Na₂SO₄.

The fatty acid composition was analyzed using a Shimadzu GCMS-QP2010S chromatographer (Shimadzu Scientific Instrument, Massachusetts, USA) equipped with an Rtx 5 column (Restek Corporation, Pennsylvania, USA) and helium as the carrier gas. The injector temperature and detector temperature were set at 300°C and 305°C, the carrier gas was ensured to flow smoothly with a pressure of 13.7 kPa, then 1 µL of each sample was injected with a syringe. The column temperature used was a temperature gradient with an initial column temperature of 70°C for 5 minutes, then raised to 300°C with an increase rate of 5°C per minute, then held for 19 minutes. The chromatogram results were then matched with the WILEY229.LIB and NIST12.LIB databases to identify the types of fatty acids detected, and the contents were calculated.

Zinc and copper content tests were conducted at *CV Chem-Mix Pratama, Bantul, Yogyakarta* based on AOAC 999.11 Determination of Lead, Cadmium, Copper, Iron, and Zinc in Foods.²⁷ Each sample (5 grams) was placed in a porcelain cup and ashed in a furnace at 300°C – 550°C for 4 hours until white ash was obtained. The samples were then cooled and dissolved with 10 mL of 6 N HCl while being heated on an electric bath for 2 – 3 minutes. Then the sample was added with 0.1 M HNO₃ diluted with distilled water, shaken, and finally filtered with Whatman filter paper number 41. The next step is to prepare a standard solution of Zinc and

Copper by dissolving 0.4397 grams of $ZnSO_4 \cdot 7H_2O$ and as much as 0.4124 grams of $CuSO_4 \cdot 5H_2O$ in a 100 mL volumetric flask. Then diluted with HNO_3 0.1 M to the limit mark.

The calibration curve was made by measuring the absorbance of each standard solution on SSA (Atomic Absorption Spectrophotometry). Zn^{2+} standard solution with concentration variations of 0 ppm; 1.0 ppm; 2.0 ppm; 4.0 ppm; and 6.0 ppm measured absorbance with SSA at λ_{max} (maximum wavelength) 213.88 nm while Cu^{2+} measured with variations of 0 ppm; 0.2 ppm; 0.4 ppm; 0.8 ppm; 1.4 ppm; and 1.8 ppm and λ_{max} 324.72 nm. Furthermore, method validation was carried out using the linearity test and continued with testing the concentration of Zinc and Copper whose absorbance was measured at λ_{max} 213.88 nm for Zn and 324.72 nm for Cu. The concentration of each mineral in each sample was determined from the regression equation.

The results of fatty acid (%w/w dry weight), Zinc, and Copper (ppm/parts per million) content in each sample were compiled using Microsoft Excel. Fatty acid, Zinc, and Copper contents that were not detected in all samples were described descriptively while data on fatty acid, Zinc, and Copper contents that were detected in all samples were further analyzed with statistical processing software. A normality test was first conducted using Shapiro-Wilk because the number of data was <30. To determine the differences in fatty acid, zinc, and copper content in normally distributed samples, the One-Way ANOVA test was conducted with a further test in the form of the Tukey HSD test to determine which variables had significant differences. While in samples that were not normally distributed, the Kruskal-Wallis Test was carried out with a further test in the form of the Mann-Whitney Test. Results were considered significant if the p-value ≤ 0.05 .

RESULTS

Based on Table 1, it is known that there are 7 types of fatty acids in BK, 6 types in TK, and 3 types in TPK, which are dominated by saturated fatty acids. The highest fatty acid content was found in TK (97.965 ± 12.002 %w/w) and the lowest in TPK (38.144 ± 29.677 %w/w). The three samples contained stearic acid, lignoceric acid, and linoleic acid. There was a significant difference in the average lignoceric acid content between the three samples based on the Tukey test. However, there is no difference in the average content of stearic acid and linoleic acid between the three samples. Based on the table below it can also be seen that the zinc and copper contents tend to decrease in TK but increase in TPK. Zinc and copper contents were highest in TPK (62.529 ± 8.0205 ppm and 17.987 ± 4.097 ppm). There were differences in the average zinc content in the three samples, but significant differences only between TPK and BK also TPK and TK, while there were no significant average differences between BK and TK. Copper content between the three samples also had a significant average difference based on the Mann-Whitney test.

Table 1. Fatty Acids, Zinc, and Copper Profiles on Each Samples

Nutrients	Samples			p-value
	BK	TK	TPK	
Saturated Fatty Acids or SFA (%w/w)				
Capric acid (C10:0)	5.675±0.401	4.402±0.850	N/A	N/A
Myristic acid (C14:0)	13.880±1.914	N/A	N/A	N/A
Stearic acid (C18:0)	16.327±5.803 ^a	8.453±2.861 ^a	6.873±0.403 ^a	0.148**
Lignoceric acid (C24:0)	6.315±0.130 ^a	3.985±0.253 ^b	1.950±0.748 ^c	0.000*
Monounsaturated Fatty Acids or MUFA (%w/w)				
Palmitoleic acid (C16:1)	9.960±1.174	19.720±1.814	N/A	N/A
Polyunsaturated Fatty Acids or PUFA (%w/w)				
Linoleic acid (C18:2n6c)	0.732±0.866 ^a	42.005±5.489 ^a	29.321±28.526 ^a	0.058*
Unsaturated Trans Fatty Acid (%w/w)				
Elaidic acid (C18:1 trans9)	7.130±6.903	19.400±0.735	N/A	N/A
Total Fatty Acids (%w/w)	60,019±17.191	97.965±12.002	38.144±29.677	N/A
Zinc (ppm)	13.570±8.736 ^a	12.854±2.065 ^a	62.529±8.0205 ^b	0.000*
Copper (ppm)	4.009±0.719 ^a	2.639±0.080 ^b	17.987±4.097 ^c	0.027**

The use of distinct superscript letters indicates significant average difference among samples. (*) refers to ANOVA (Analysis of Variance) test, while (**) indicates the Kruskal-Wallis test. The abbreviation N/A signifies that certain fatty acids were not detectable during the testing process. The initials BK, TK, and TPK represent boiled *Kedawung* seeds, *Kedawung* tempeh, and *Kedawung* tempeh flour, respectively.

DISCUSSION

The requirement for a compound to be analyzed using the gas chromatography method is that the compound must be extracted and methylated into its oil form with a minimum amount of 1 μ L.²⁸ Previous research by Afifah et al. stated that the minimum amount of *Tempe Gembus* needed to produce oil extracts for fatty acid analysis was 100 grams of dry weight.²⁹ The same opinion was also stated by Truzzi, et al. who used 100 gram of Antarctic fish (*Trematomus bernacchii*) muscle to observed its fatty acid content using gas chromatography.³⁰ So that in this study the sample weight used was 100 grams of dry weight of BK, TK, and TPK. The dry weight of BK and TK was obtained from the oven process of 150 grams of wet samples of BK and TK.

The similarity between the three samples is that the dominant fatty acid content is saturated fatty acid (SFA). This is in line with previous research by Hidayati, et al. which states that fatty acids in *Kedawung* seeds in their raw form are dominated by saturated fatty acids.³¹ In addition, Atere, et al. also reported that *Kedawung* seeds fermented into *Iru* (Nigerian specialty food) with various types of starters have saturated fatty acids that are more dominant than other types of fatty acids.¹⁰ Ijarotimi, et al. in their research also stated that the dominant fatty acid content in *Kedawung* seed flour, fermented *Kedawung* seed flour, and germinated *Kedawung* seed flour is saturated fatty acid.³²

The dominant saturated fatty acid content in BK is stearic acid. This is because stearic acid is a type of saturated fatty acid that has a high concentration in tropical plant seeds.³³ However, steric acid and the other three types of fatty acids decreased and even disappeared in both TK and TPK. This is likely due to the fermentation process itself. The fermentation process by mold can decompose most of the fat in fermented food ingredients.²¹ In the process of making *Senegalia macrostachya* tempeh, the same results were obtained; it was found that the fat content decreased by 30% in 48 hours of fermentation time.³⁴ *Rhizopus sp.* plays important role in turning fat into free fatty acids, which are metabolized as an energy source with the help of lipase enzymes.²¹ The decrease in total fat content may affects the fatty acid content.

The process of washing, soaking, and heating before fermentation can also reduce fatty acid content, especially in short-chain (C4 – C6) and medium-chain (C8 – C12) fatty acids.³⁵⁻³⁷ Fatty acids (C4 – C10) are soluble in both hot and cold water. In addition, these fatty acids and fatty acids (C12 – C14) are volatile.³⁸ This could explain the decrease and even loss of capric acid and myristic acid content in TK and TPK. Previous research by Yulianti et al. also revealed that the decrease in fat content due to the soaking process occurred in soybeans, mung beans, and peanut, though they did not specifically mention reduction of several types of fatty acids.³⁹ However, it can be assumed that are also some reductions in fatty acid composition since that fats are made up of triglycerides and fatty acid chains. Meanwhile, the effect of boiling showed fluctuating fatty acids in cowpea, chickpea, and kidney bean.⁴⁰ Although classified as long-chain fatty acids (C12 – C24), the content of steric acid and lignoceric acid also decreased in TK and TPK. Continuous application of heat for a long period of time will increase the temperature to the melting point of long-chain fatty acids and can reduce the levels of these fatty acids.⁴¹ Therefore, there is a significant average difference in lignoceric acid between the three samples.

In addition to saturated fatty acids, BK is also known to contain monounsaturated fatty acid (MUFA), palmitoleic acid and polyunsaturated fatty acid (PUFA), linoleic acid. Both types of fatty acids increased in TK. These results are in line with previous research by Sathya and Siddhuraju, which revealed that the fermentation process can increase the levels of linoleic acid contained in *Kedawung* seeds.⁸ Salehodin, et al. in their research also stated that %MUFA and %PUFA increased until the fourth and fifth days of fermentation at 22 and 25°C, which is classified as room temperature.⁴² Similarly, Kanghae, et al. in previous research, reported that soybean fermentation prepared with *Rhizopus oligosporus* resulted in a higher oleic and linoleic acids compared to its unfermented soybean form.⁴³ This phenomenon is a result from *Rhizopus oligosporus* lipolytic activity, which leads to an increased amount of free fatty acids by 25-26%.²¹

However, both types of unsaturated fatty acids (UFAs) decrease in TPK. The heating process in making TPK converts fatty acid components into volatile compounds such as aldehydes, ketones, and hydrocarbons.⁴³ All of these compounds are lost during long-term heating.⁴⁴ Similar results were revealed by Ali, et al., who stated that %PUFA (one of which is linoleic acid) in unroasted groundnut seeds decreased by 10,47% 9 hours of heating.⁴⁵ Mohamed, et al. on the other hand also stated that the extraction process with the Soxhlet method, which involves high heat, reduced the fatty acid content more significantly when compared to cold extraction.⁴⁶ The cis bonds in unsaturated fatty acids, which reduce intermolecular attraction and thus lower the melting point, are bent and less strong. Therefore, the greater the number of cis bonds, the more the melting point of a fatty acid decreases, causing the fatty acid to evaporate faster due to heat.⁴⁷

The formation of elaidic acid is also a form of compensation of heating during sample preparation in both preliminary and main research. Elaidic acid is classified as a type of trans unsaturated fatty acid. Trans fatty acids are formed through partial hydrogenation reactions caused by heating, either at high temperatures or for long periods of time. Heating also induces thermal polymerization and oxidation reactions that lead to the formation of trans fatty acids.⁴⁸ Hydrolysis reactions further contribute to the formation of trans fatty acids.⁴⁹ This reaction occurs more easily in foods with long-chain saturated fatty acids that have the same number of C atoms, in this case stearic acid, which also has 18 C atoms.⁵⁰ The loss of elaidic acid in TPK is likely due to its melting point of approximately 45°C.⁵¹

Apart from fatty acids, the three samples also contained zinc and copper, which were statistically significant. The content of these two minerals decreased in TK but increased dramatically in TPK. This result is different from the study by Sathya and Siddhuraju which revealed that the content of these two minerals increased in fermented *Kedawung* seeds compared to the raw form.⁸ The presence of phytase enzyme activity can decompose phytic acid (a mineral-binding compound), thereby increasing mineral bioavailability.⁵² The difference in results may be due to variations in raw materials, microorganisms, environmental conditions, and fermentation duration.⁵³

However, Damanik, et al. also Sine and Sutarto revealed similar results, where a decrease in ash content occurred during the fermentation process in the production of *Tempe Gembus* and *Tempe Gude*.^{44,52} The determination of ash content amount is an important step in proximate analysis for evaluating the mineral content of foods.⁵⁴ The process of making tempeh involves soaking and boiling, which can trigger a reduction in mineral content due to the water-soluble nature of minerals. For example, soaking legumes like black beans (*Phaseolus vulgaris* L.) results in the loss of minerals such as iron, zinc, and copper.⁵⁵ In addition, fermentation is also associated with an increase in tannin levels due to the hydrolysis of condensed tannins such as proanthocyanidins into phenols. Tannins binds to various types of minerals, but their levels decrease during fermentation process due to the activity of phenyl oxidase bacteria.⁵⁵ However, phenols, which are the result of tannin hydrolysis during the fermentation process, also contributes to binding various types of minerals, thereby inhibiting the bioavailability of these minerals.⁵⁵

Although it decreased in TK, the zinc and copper content of TPK increased drastically. This increase is attributed to the drying process involved in making TPK. This result aligns with the research by Lisa, et al., which states that the ash content of white oyster mushroom flour is directly proportional to the increase in temperature and duration of drying. Ash content reflects the total minerals contained in food.⁵⁶ It is suggested that the increase in temperature and prolonged exposure to heat reduces the water content and increase the levels of other ingredients left behind, one of which is minerals.⁵⁶ This explanation supports the dramatic increase in zinc and copper content in TPK, which was the sample with the longest heat exposure time.

The Indonesian Heart Association (*Perhimpunan Dokter Spesialis Kardiovaskular Indonesia* /PERKI) recommends eating a diet rich in unsaturated fatty acids while minimizing saturated fatty acids and trans fatty acids to prevent cardiovascular disease.⁵⁷ Total fat consumption per day should be <35% of total energy, with <7% of total fat per day for saturated fatty acids, <1% trans fatty acids, and the rest is met by consumption of unsaturated fatty acids.⁵⁸ Saturated fatty acids, especially palmitoleic acid and linoleic acid, can reduce LDL and increase HDL.⁵⁹ Reducing saturated fat intake while increasing the intake of polyunsaturated and monounsaturated fat consumption is linked to a reduced risk of cardiovascular disease, other leading causes of death, and overall mortality.⁵⁸

These recommendations are relevant to this research, therefore, the consumption of BK, TK, and TPK has a potential nutritional benefit for cardiovascular health. The total fatty acid content of TK (97.965 ± 12.002 %w/w) was higher than the total fatty acid of *Tempe Gembus* (79.44 ± 3.44 %w/w).⁴⁴ The content of essential fatty acids, one of which is linoleic acid (ω -6) in TK (42.005 ± 5.489 %w/w) is also higher when compared to Soybean Tempeh (37.56 %w/w) and *Tempe Gembus* (26.83 ± 1.49 %w/w).^{44,60} Research by Denardi-Souza, et al. mentioned that, in addition to the increase in fatty acids, the presence of *Rhizopus oligosporus* during fermentation triggers protease enzyme activity, which significantly increases essential amino acids.²² Essential amino acids, such as aromatic amino acids like include phenylalanine, tyrosine, and tryptophan, are also known to decrease serum LDL cholesterol and increase HDL cholesterol, contributing to better cholesterol management.⁶¹

Consumption of TK and TPK is more advisable because they contain less saturated fatty acids than BK. The zinc and copper content in TK ($12,854 \pm 2,065$ ppm and $2,639 \pm 0,080$ ppm) and TPK ($62,529 \pm 8,0205$ ppm and $17,987 \pm 4,097$ ppm) are also much higher than the average zinc and copper content of Soybean Tempeh which is only around 1.74 ppm for zinc and 1.69 ppm for copper.⁵⁹ The loss of fatty acids, especially

unsaturated fatty acids in TPK due to processing can be compensated by consuming twice as much TPK to match the total fatty acids content of BK or three times more to match that of TK.

However, the increase in the content of elaidic acid (a trans unsaturated fatty acid), which is associated with cardiovascular risks, in TK, as well as a decrease in the content of zinc and copper, which in fact can act as antioxidants, requires careful consideration.^{57,59} On the other hand, in TPK, elaidic acid is not detected, nor is palmitoleic acid, but the content of zinc and copper increased dramatically. Based on these findings, further *in vivo* studies are needed for both samples to determine their effectiveness in preventing cardiovascular disease.

CONCLUSIONS

This study shows that different processing methods can meaningfully influence the fatty acid, zinc, and copper profiles of *Kedawung* (*Parkia roxburghii* G. Don). Significant differences were observed in lignoceric acid, zinc, and copper levels among boiled *Kedawung* seeds, *Kedawung* tempeh, and *Kedawung* tempeh flour, whereas stearic and linoleic acid contents remained relatively unchanged. These findings indicate that fermentation and flouring modify the nutritional composition of *Kedawung* in distinct ways. Overall, processed *Kedawung* products exhibit nutritional characteristics that may be relevant to cardiovascular health. Nevertheless, further studies are needed to assess nutrient bioavailability and to clarify their functional health effects through *in vivo* investigations.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

DECLARATION USE OF AI

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