

Effects of Germination and Fermentation on Fatty Acid Profiles of Legume-Based Tempeh: A Systematic Review with Perspective on Cardiovascular Health

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Abstract

Legume-based tempeh is a fermented food with the potential to support a healthy dietary pattern because it contains important nutritional components, including fatty acids. Germination and fermentation are known to influence lipid composition and fatty acid profiles in legumes, thereby potentially improving their nutritional value from a cardiovascular health perspective. This systematic review aimed to evaluate the effects of germination, fermentation, and their combination on the fatty acid profiles of legume-based tempeh. The PRISMA method was used to select and review 14 articles retrieved from the ScienceDirect and Scopus databases. The findings indicate that germination and fermentation can modify the fatty acid profile of tempeh, particularly through changes in saturated and unsaturated fatty acid contents. Several studies reported a decrease in saturated fatty acids and an increase in unsaturated fatty acids, including oleic acid, linoleic acid, and linolenic acid, although the magnitude of these changes varied depending on the type of legume, germination time, fermentation duration, microorganism, and analytical method used. Among the tempeh varieties reviewed, cowpea tempeh subjected to 24 h germination followed by 24 h fermentation showed a relatively favorable fatty acid profile, characterized by increased unsaturated fatty acids and an estimated linoleic acid to linolenic acid ratio of approximately 2.3:1.

Keywords: fatty acid; fermentation; germination; legume; tempeh

1 Introduction

Coronary heart disease (CHD) is a critical public health issue worldwide, affecting both developed and developing countries. Although CHD can occur at any age, it predominantly affects adults and the elderly. According to the World Health Organization, cardiovascular diseases account for approximately 17.9 million deaths annually [1] and are projected to reach 23.3 million deaths by 2030 [2]. In the United States, CHD causes approximately 478,000 deaths per year, while in Europe, an estimated 20,000–40,000 individuals per one million population are affected [3]. In Indonesia, the prevalence of heart disease diagnosed by physicians across all age groups is reported to be 0.85% [4], placing the country among those with the highest CHD prevalence in Asia [5]. These statistics highlight

the urgent need for effective CHD prevention strategies.

Dietary intervention is one of the most effective approaches for reducing CHD risk. Legumes are highly recommended due to their favorable lipid composition. Lipids in legumes primarily consist of glycerol and fatty acids [6] including monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are associated with reduced CHD risk [7]. Both fatty acids are omega-3. Besides omega-3, fatty acids that are beneficial for health are oleic fatty acid (omega-9) and linoleic fatty acid (omega-6), which also been reported to lower cardiovascular risk and improve insulin production [8]. However, legumes also contain saturated fatty acids (SFA), which can elevate low-density lipoprotein (LDL)

cholesterol and reduce high-density lipoprotein (HDL) cholesterol, thereby increasing CHD risk [3]. Consequently, reducing SFA and enhancing unsaturated fatty acid content in legume-based foods is essential.

Previous research show that the fatty acid composition in legumes can be modified by processing, one of which is fermentation. Tempeh is a traditional fermented legume-based food widely consumed in Indonesia and increasingly recognized as a functional food. While soybean (*Glycine max*) is the most common raw material, various other legumes, including mung beans (*Vigna radiata*), red beans (*Phaseolus vulgaris*), peanuts (*Arachis hypogaea*), lentils (*Lens culinaris*), lupins (*Lupinus albus*), pigeon peas (*Cajanus cajan*), velvet bean (*Mucuna pruriens*), jack bean (*Canavalia ensiformis*), and cowpeas (*Vigna unguiculata*) have been reported as alternative substrates [9]. Although different legumes yield similar fatty acid profiles, their composition and concentrations vary depending on processing conditions [6]. In general, the tempeh fermentation process involves microorganisms (yeast). Various types of yeast can be used to make tempeh, such as *Rhizopus oligosporus*, *Rhizopus stolonifera*, *Rhizopus arrichus*, *Rhizopus orizae*, *Mucor javanicus*, and *Mucor rouxii* [10]. The addition of yeast is crucial in the tempeh fermentation process because it affects the quality of the tempeh. If yeast is not added to the tempeh fermentation process, the tempeh will spoil [11].

In addition to the type of raw material, fermentation also impacts the nutritional content of legumes. According to previous research, fermentation can increase MUFA levels and decrease SFA and PUFA levels in sacha inchi legumes due to the activity of the lipase enzyme, which hydrolyzes triglycerides into fatty acids and glycerol [12]. The duration of fermentation time also influences the fatty acid composition by reducing the presence of SFA fatty acids and increasing the presence of MUFA and PUFA fatty acids [13]. The maximum activity of the lipase enzyme which hydrolyzes triglycerides into free fatty acids occurs during fermentation for 36 hours [14]. Meanwhile, other studies state that myristic, palmitic, stearic, oleic, and lignoceric fatty acids increase with increasing fermentation time, but lauric, linoleic, linolenic, arachidic, eicosanoic, and docosanoic fatty acids do not change during 21-24 hours of fermentation in long beans because the microorganisms used in the fermentation

process do not use lipids as the main energy and carbon during their growth [15].

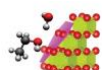
Another technique that can change the fatty acid composition of legumes or tempeh is the germination process. This process often occurs in grains, where the seeds grow to form new embryos in the form of roots, stems, or shoots [16]. Germination has a significant impact on the nutritional content of legumes. According to previous research, germination can improve the omega-3 fatty acid profile in soybeans because during germination there is increased activity of the lipase enzyme to mobilize fat reserves as an energy source for the embryo [17].

The combination of the two processes above, germination and fermentation, has been reported to further modify the fatty acid profile of the resulting tempeh products. The combined germination-fermentation treatment can reduce the SFA fatty acid ratio and increase the MUFA and PUFA fatty acids in jack bean tempeh and jack bean sprout tempeh [13]. Other research states that the combination of germination-fermentation can increase the MUFA content and form gamma-linolenic acid in velvet bean tempeh and velvet bean sprout tempeh [18]. According to previous study, gamma-linolenic fatty acid is good for skin health because it can improve parameters and reduce dehydration of the skin [19].

Despite growing interest, systematic evaluations of the combined effects of germination and fermentation on fatty acid profiles of various tempeh types remain limited. In research [20] discussed the effect of germination on the relationship between protein digestibility and proteolysis in several types of legumes. Furthermore, research [21] discussed the effect of a combination of germination and fermentation on antinutrients in various types of legumes. Therefore, this review article discusses the effect of germination and fermentation on the fatty acid profile of tempeh from various types of legumes. This systematic review aims to: (1) evaluate the effects of germination, fermentation, and their combination on fatty acid profiles of legume-based tempeh, and (2) recommend tempeh products with optimal fatty acid profiles for CHD prevention.

2 Method

This systematic review was conducted to evaluate the effects of germination and fermentation on the fatty acid profiles of tempeh derived from various legumes and to identify the most suitable tempeh types for cardiovascular



health. The review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [22]. Search articles from various types of databases, such as ScienceDirect and Scopus.

2.1 Search Strategy

Scientific articles were retrieved from ScienceDirect and Scopus databases. The search focused on peer-reviewed research articles investigating germination, fermentation, tempeh, legumes, and fatty acids. The criteria for selected articles are limited to English and Bahasa Indonesia only. Keywords and Boolean operators were applied as combinations of: “germination OR germinate OR sprout OR sprouts”, “fermentation OR fermentation process OR fermentation technique”, “tempe OR tempeh”, “legume OR legumes OR Leguminosae OR fabaceae”, and “fatty acid OR fatty acids OR volatile compound”. The specific keywords used can be seen in **Table 1**.

Table 1. Keywords Search Strategy from Various Databases

Code	Germination Keyword
1	Germination
2	Germinate
3	Sprout
4	Sprouts
5	1 OR 2 OR 3 OR 4
Code	Fermentation Keyword
6	Fermentation
7	Fermentation process
8	Fermentation technique
9	6 OR 7 OR 8
Code	Tempeh Keyword
10	Tempe
11	Tempeh
12	10 OR 11
Code	Legume Keyword
13	Legume
14	Legumes
15	Leguminosae
16	Fabaceae
17	13 OR 14 OR 15 OR 16
Code	Fatty Acid Keyword
18	Fatty acid
19	Fatty acids
20	Volatile compound
21	18 OR 19 OR 20
Code	Combined Keyword
22	5 AND 9 AND 12 AND 17 AND 21

2.2 Selected Criteria

Two simple criteria were established for article selection:

2.2.1 Inclusion

Original research articles evaluating the effects of germination and/or fermentation that included data of changes in the fatty acid profiles of tempeh produced from legumes were included, whether fermented legumes, fermented legume sprouts, or the tempe product itself. Studies addressing nutritional changes were included if fatty acid data were reported.

2.2.2 Exclusion

Review articles, book chapters, duplicate publications, and studies unrelated to fatty acid profiles of legume-based tempeh. Articles with raw legumes as the main sample (not in the form of tempeh) even if germinated are not included in the criteria. Duplicates articles are also not included in the criteria.

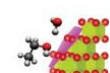
2.3 Articles Selection

A total of 352 articles were identified from a systematic search of the Scopus databases (171 articles) and ScienceDirect (181 articles). Duplicate articles were removed using Excel, resulting in 297 articles. Initial screening was performed based on inclusion and exclusion criteria, resulting in 108 articles. These 108 articles were then screened based on eligibility criteria. Of these 108 articles, 94 articles met the eligibility criteria, such as articles not related to germination and fermentation, legumes, tempeh, and irrelevant outcomes (other than fatty acid and nutrient profiles) and were not selected. Finally, 14 articles were selected for the review article.

Figure 1 shows a flowchart illustrating the article selection process and **Table 2** shows a list of articles that used as data.

2.4 Data Extraction

Data extraction was carried out based on the category of the influence of germination and fermentation on the fatty acid profile and nutritional content of tempeh from various types of legumes.



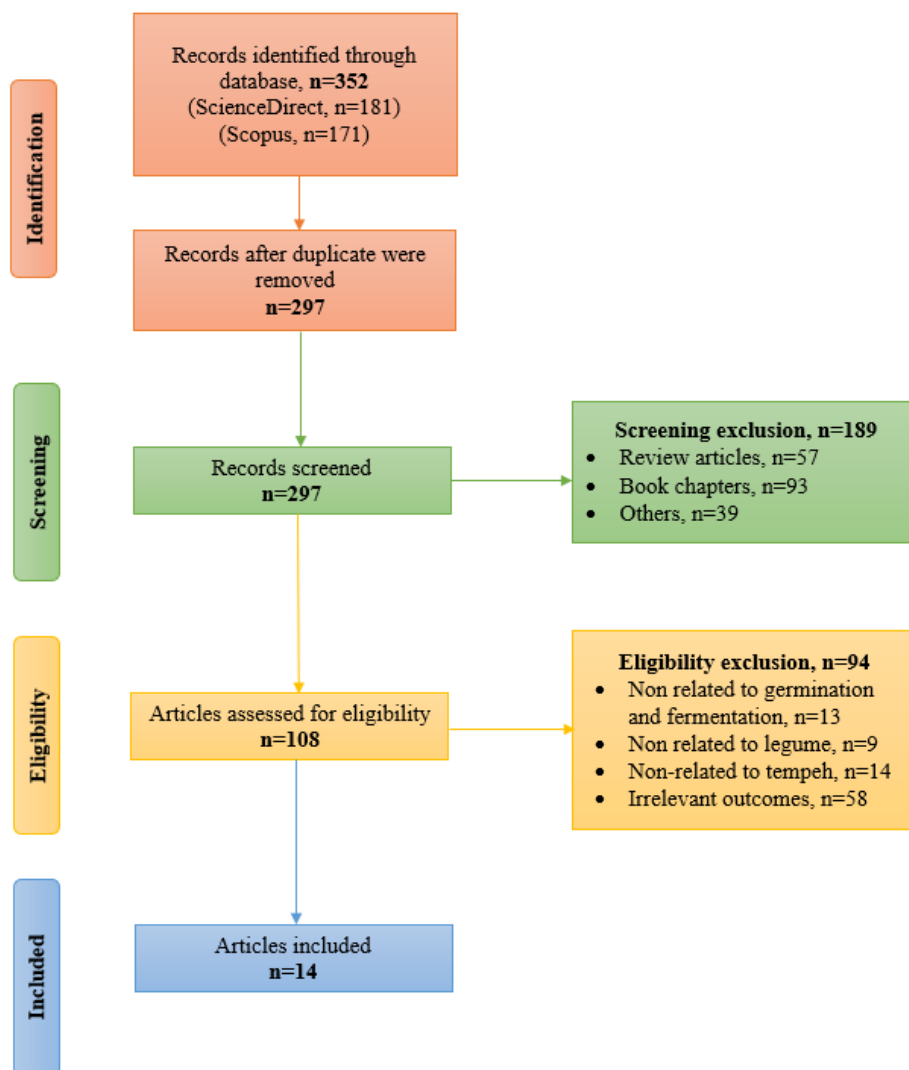
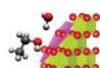


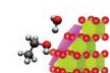
Figure 1. PRISMA Flowchart Illustration of Article Selection and Extraction Process

Table 2. Summary of Selected Articles Used as Data Sources in the Systematic Review

No	Author/Year	Legume Type	Treatment Condition	Fermentation Microorganism	Analytical Method for Fatty Acid Determination	Main Fatty Acid Result	References
1.	Prinyawiwatkul et al., 1996	Cowpea	24 h germination	<i>Rhizopus microspores var. oligosporus</i>	Gas Chromatography (GC)	Palmitic, Stearic, Oleic, Linoleic, Linolenic	[15]
			24 h germination and 24 h fermentation			Palmitic, Stearic, Oleic, Linoleic, Linolenic	
2.	Feng et al., 2007	Soybean	16 h germination and 51 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Mass Spectrometry (GC-MS)	Propanoic, 2-methylbutanoic	[23]
		Barley	16 h germination			Propanoic, 3-methylbutanoic	



				and 28 h fermentation				
3.	Ijarotimi Kenshinro, 2012	& African Locust	168 h germination	Not detected	Gas-Liquid Chromatography (GLC)	Stearic, Palmitic, Oleic	[24]	
			168 h germination and 24 h fermentation			Stearic, Palmitic, Oleic, Linoleic		
4.	Kadar 2020	et al., Soybean	24 h germination and 48 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography- Mass Spectrometry (GC-MS)	Palmitic, Stearic	[25]	
5.	Riley 2022	et al., Common vetch <i>Vicia sativa</i> Amelia	192 h germination	<i>Rhizopus oligosporus</i>	Gas Chromatography- Mass Spectrometry (GC-MS)	Caproic	[26]	
			24 h germination and 72 h fermentation			Myristic		
		<i>Vicia sativa</i> Jose	192 h germination			Caproic		
			24 h germination and 72 h fermentation			Capric, Myristic		
		<i>Vicia villosa</i> Latigo)	192 h germination			Caproic		
			24 h germination and 72 h fermentation			Capric		
6.	Riyanto 2024	et al., Soybean	24 h germination and 48 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography- Mass Spectrometry (GC-MS)	Linoleic, butanoic, palmitic, stearic	[27]	
7.	Astawan 2025	et al., Soybean	48 h fermentation	<i>Rhizopus spp.</i>	Gas Chromatography- Mass Spectrometry (GC-MS)	Not detected	[28]	
			28 h germination and 48 h fermentation			Not detected		
			120 h fermentation			Not detected		



			28 h germination and 120 h fermentation			Not detected	
			168 h fermentation			Not detected	
			28 h germination and 168 h fermentation			Oleic, Linoleic	
8.	Herring et al., 1991	Soybean	70 h fermentation	<i>Rhizopus</i>	Gas Chromatography (GC)	Palmitic, Stearic, Oleic, Linoleic, γ -linolenic, α -linolenic	[29]
9.	De Reu et al., 1994	Soya	28 h fermentation	<i>Rhizopus microspores var. oligosporus</i>	Gas Chromatography (GC)	Linoleic	[30]
10.	Milinda et al., 2021	Cashew	48 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Flame Ionization Detector (GC-FID)	Oleic	[14]
		Soybean	48 h fermentation			Linoleic	
11.	Suryanti et al., 2021	Lead tree	96 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Mass Spectrometry (GC-MS)	Nonanoic, 9,12-octadecanoic	[31]
12.	Nurhaeni et al., 2021	Moringa	48 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Mass Spectrometry (GC-MS)	Omega-3, omega-6	[32]
13.	Afifah et al., 2025	Sacha inchi	72 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Flame Ionization Detector (GC-FID)	Linoleic, linolenic	[12]
14.	Pamekas et al., 2025	Soybean	24 h fermentation	<i>Rhizopus oligosporus</i>	Gas Chromatography-Mass Spectrometry (GC-MS)	Butyric	[33]
			240 h fermentation			Not detected	

3 Result and Discussion

Fourteen studies were included in this review, with seven reporting significant effects of combined germination and fermentation on fatty acid profiles. A total of 7 articles showed that the combination of germination and fermentation had an effect on the fatty acid profile of various types of tempeh, particularly on unsaturated fatty acids.

3.1 Effect of Germination on the Types of Saturated and Unsaturated Fatty Acids in Tempeh

To discuss the effect of germination step before fermentation on the fatty acids in tempeh, a standardized coding system was established to improve clarity and consistency throughout the manuscript. In this article, NGF represents non-

germinated and non-fermented samples; Gx represents samples germinated for x hours; Fy represents samples fermented for y hours; and GxFy represents samples germinated for x hours followed by fermentation for y hours. The complete coding system is summarized in **Table 3**.

The results show that germination and fermentation treatments affected the occurrence of fatty acids in different types of tempeh (**Table 4**). In soybean tempeh and barley tempeh, the non-germinated and non-fermented samples did not show the detection of short-chain saturated, including propanoic acid (3:0) and 2-methylbutanoic acid (5:0). However, after the combined germination and fermentation treatment, both fatty acids were detected. This indicates that the combination of germination and fermentation may contribute to the formation or release of short-chain saturated fatty acids in these tempeh products.

Table 3. Coding System for Tempeh Treatment Conditions

No.	Code	Treatment	Description
1	NGF	Non-germinated and non-fermented	Sample without germination and fermentation treatment
2	Gx	Germinated only	Sample germinated for x hours without fermentation
3	Fy	Fermented only	Sample fermented for y hours without prior germination
4	GxFy	Germinated and fermented	Sample germinated for x hours followed by fermentation for y hours

Notes: G = germination; F = fermentation; x and y indicate the duration of germination and fermentation, respectively, expressed in hours.

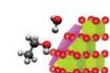
A different pattern was observed in common vetch tempeh. In *Vicia sativa*, several short saturated fatty acids, including butanoic acid (4:0), caproic acid (6:0), enanthic acid (7:0), caprylic acid (8:0), and nonylic acid (9:0), were detected after germination treatment. Interestingly, additional medium-chain and long-chain saturated fatty acids such as lauric acid (12:0), myristic acid (14:0), pentadecylic acid (15:0), and stearic acid (18:0) were detected in combined germination and fermentation treatment. Similar trends were also

found in other cultivar, although the detected fatty acid types varied among them. These findings suggest that the response of fatty acid profiles to germination and fermentation is cultivar-dependent, even within the same legume species. The combined treatment appears to broaden the diversity of saturated fatty acids detected, especially medium- and long-chain saturated fatty acids.

In soybean tempeh treated with G24F48, saturated fatty acids such as butanoic acid (4:0), palmitic acid (16:0), and stearic acid (18:0) were detected, whereas these fatty acids were not detected in the untreated sample. In addition, linoleic acid (18:2), an unsaturated fatty acid, was also detected after the same treatment. This result indicates that germination followed by fermentation may not only affect saturated fatty acids but also contribute to the detection of unsaturated fatty acids. The presence of linoleic acid is nutritionally relevant because it is an essential polyunsaturated fatty acid.

During germination, lipid metabolism occurs due to increased enzyme activity originating from the seeds to produce energy for embryo growth. This enzyme is a lipase enzyme that will break down triglycerides into fatty acids and glycerol, where this phenomenon will change the fatty acid composition of seeds that have not undergone germination [34].

Another interesting aspect of soybean tempeh is the effect of fermentation time on fatty acids. Several studies have reported that the optimal fermentation time for tempeh production is approximately to 48 hours. For example, bosok tempeh is produces through extended fermentation time up to 168 hours shows the change of oleic and linoleic acid. In contrast, fresh tempeh and semangit tempeh did not show any fatty acids. Based on the data for fresh and semangit tempeh, although both underwent germination similarly to bosok tempeh, oleic and linoleic acids were not detected. These findings suggest that fermentation time may influence the fatty acid profile. According to previous research, longer fermentation time can improve the fatty acid profile because the use of *Rhizopus* fungi can produce lipase enzymes, which break down fat into fatty acids and glycerol [35]. The longer the fermentation time, the greater the fungal or



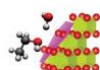
microorganism population and the production of lipase enzymes.

The combination of germination and fermentation also affected the fatty acid composition. For example, soybean tempeh treated G24F48 showed the presence of new fatty acids that were previously undetectable in soybean, namely butanoic, palmitic, stearic, and

linoleic acids. It was suggested that lipase enzyme activity produced by fungi during fermentation. Microbial activity can influence fatty acid degradation or resynthesis due to the presence of other enzymes produced by the microbes themselves, depending on temperature, pH, fermentation time, and substrate [12].

Table 4. The Effect of Germination on the Types of Saturated and Unsaturated Fatty Acids in Tempeh

No	Type of Tempeh	Fatty Acid	Treatment (in hours)			References
			NGF	G/F	GF	
1.	Soybean tempeh (<i>Glycine max</i>)	Saturated fatty acid	G0F0		G16F51	(23)
		Propanoic (3:0)	-		√	
		2-metyhl-butanoic (5:0)	-		√	
	Barley tempeh (<i>Hordeum vulgare</i>)	Saturated fatty acid	G0F0		G16F51	
		Propanoic (3:0)	-		√	
		2-metyhl-butanoic (5:0)	-		√	
2.	Common vetch tempeh					[26]
	<i>Vicia sativa</i> Amelia	Saturated fatty acid		G192	G24F48	
		Butanoic (4:0)		√	√	
		Caproic (6:0)		√	-	
		Enanthic (7:0)		√	-	
		Caprylic (8:0)		√	√	
		Nonylic (9:0)		√	√	
		Lauric (12:0)		√	√	
		Myristic (14:0)		-	√	
		Pentadecylic (15:0)		-	√	
	Stearic (18:0)		-	√		
	<i>Vicia sativa</i> Jose	Saturated fatty acid		G192	G24F48	
		Propionic (3:0)		√	√	
		Butanoic (4:0)		√	√	
		Valeric (5:0)		√	-	
		Caproic (6:0)		√	-	
		Enanthic (7:0)		√	-	
		Caprylic (8:0)		√	√	
		Nonylic (9:0)		√	-	
		Capric (10:0)		√	√	
		Lauric (12:0)		-	√	
		Myristic (14:0)		-	√	
		Pentadecylic (15:0)		-	√	
	Stearic (18:0)		-	√		
	<i>Vicia sativa</i> Latigo	Saturated fatty acid		G192	G24F48	
		Propionic (3:0)		√	-	
Butanoic (4:0)			√	√		
Valeric (5:0)			√	-		
Caproic (6:0)			√	-		



	Enanthic (7:0)		√	-	
	Caprylic (8:0)		√	√	
	Nonylic (9:0)		√	-	
	Capric (10:0)		-	√	
	Lauric (12:0)		-	√	
	Myristic (14:0)		-	√	
	Pentadecylic (15:0)		-	√	
3. Soybean tempeh (<i>Glycine max</i>)	Saturated fatty acid	G0F0	G24F48		[27]
	Butanoic (4:0)	-		√	
	Palmitic (16:0)	-		√	
	Stearic (18:0)	-		√	
	Unsaturated fatty acid	G0F0	G24F48		
	Linoleic (18:2)	-		√	
4. Soybean tempeh (<i>Glycine max</i>)	Saturated fatty acid		G24F48		[25]
	Palmitic (16:0)			√	
	Stearic (18:0)			√	
5. Soybean tempeh (<i>Glycine max</i>)					[28]
Fresh tempeh	Saturated & Unsaturated fatty acid		F48	G28F48	
	-		-	-	
Semangit tempeh	Saturated & Unsaturated fatty acid		F120	G28F120	
	-		-	-	
Bosok tempeh	Unsaturated fatty acid		F168	G28F168	
	Oleic (18:1)		-	√	
	Linoleic (18:2)		-	√	

Notes: The meaning of “-” is not detected, while “√” is detected. Furthermore, the grey-shaded columns indicate variables that were not determined. The code of treatment refers to Table 3.

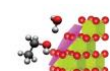
3.2 The Effect of Germination on the Quantity of Saturated and Unsaturated Fatty Acids in Tempeh

Germination not only affects the presence or absence of fatty acids in tempeh, as discussed previously. However, germination also influences the quality of saturated and unsaturated fatty acids in tempeh. This fatty acid quality can be determined by the presence, quantity, or specific essential fatty acids contained in tempeh. **Table 5** shows the effect of germination on the quantity of saturated and unsaturated fatty acids in various types of tempeh.

All types of tempeh contain an average of saturated and unsaturated fatty acids. **Table 5** shows an interesting finding occurred when tempeh was subjected to germination and fermentation. The average saturated fatty acid content decreased when subjected to germination and a combination of germination and

fermentation, while unsaturated fatty acids increased. For example, in cowpea tempeh, saturated fatty acids, such as lauric, myristic, and palmitic acids, decreased after 24 hours of germination compared to those without germination. Another interesting finding occurred when cowpea tempeh was subjected to a combination of germination and fermentation for 24 hours, indicating an increase in unsaturated fatty acids, such as oleic, linoleic, linolenic, and eicosanoic acids. These unsaturated fatty acids, such as omega-3 and omega-6, are good for the body. This proves that fermentation has a positive effect on tempeh.

On the other hand, a more in-depth analysis revealed that cowpea tempeh contained both medium- and long-chain saturated fatty acids, as well as unsaturated fatty acids consisting of monounsaturated and polyunsaturated fatty acids



(see **Table 6**) in non-germinated and non-fermented, germinated only, and combined germination and fermentation treatments. **Table 6** shows the distribution of saturated and unsaturated fatty acids in cowpea and African locust tempeh. The results demonstrated that germination reduced the levels of medium-chain and long-chain saturated fatty acids. This phenomenon is presumably associated with the activation of the β -oxidation pathway in glyoxysomes during seed germination. Endogenous lipase enzymes hydrolyze stored triacylglycerols, while medium-chain fatty acids, owing to their higher solubility and accessibility compared to long-chain saturated fatty acids, are mobilized more rapidly to support sprout development [36]. However, long-chain fatty acids in cowpea increased after GF treatment. This increase was presumably associated with the synthesis of structural membrane lipids by microorganisms, as well as a concentration effect resulting from the reduction of other carbon-containing components during the fermentation process [37]. Nevertheless, the consumption of long-chain saturated fatty acids has been associated with an increased risk of cardiovascular disease. In contrast, short-chain and medium-chain saturated fatty acids generally exhibit neutral physiological effects [38]. It was presumably attributed to the rapid oxidation pathway of medium-chain saturated fatty acid, which prevents the accumulation of triglycerides that may adversely affect vascular health [39].

A similar trend was observed for the unsaturated fatty acid profile of cowpea tempeh, particularly mono-unsaturated and poly-unsaturated fatty acids. Germination treatment reduced the levels of both mono- and poly-unsaturated fatty acids. However, cowpea was subjected to the combined germination and fermentation shows an increase in both types of unsaturated fatty acids was observed.

Nevertheless, both types of unsaturated fatty acids exert beneficial effects on cardiovascular health through distinct protective mechanism. Poly-unsaturated fatty acids are widely associated with a reduced risk of coronary heart disease by lowering low-density lipoprotein (LDL) cholesterol levels in the bloodstream, whereas mono-unsaturated fatty acids tend to maintain high-density lipoprotein (HDL) cholesterol at favorable levels [40]. The average fermentation

treatment provides a decreased in the quantity of saturated fatty acids and an increase in unsaturated fatty acids. For example, African locust tempeh showed an increase in unsaturated fatty acids such as palmitoleic, linoleic, and linolenic acids, after being germinated for 168 hours and fermented for 24 hours. Palmitoleic and linoleic fatty acids increased by approximately 20-fold following the combined germination and fermentation treatment compared with the germination only treatment in African locust tempeh. This increase indicates successful tempeh fermentation, as microorganism promoted the hydrolysis of lipids into fatty acids through activation of the lipase enzyme [12]. In addition, the presence of unsaturated fatty acids is likely associated with the biosynthesis of new compounds by microorganisms [41]. This biosynthesis process occurs through dehydrogenation or desaturation reaction that lead to the formation of unsaturated fatty acids. The desaturation reaction occurs through the activity of the desaturase enzyme, which converts saturated fatty acids into unsaturated fatty acids [42]. In this case, microorganisms are used during the fermentation process. Therefore, the formation of unsaturated fatty acids occurs more frequently during the fermentation process. For example, stearic acid (18:0) which has a single bond undergoes dehydrogenation into a double bond and forms oleic acid (18:1), linoleic (18:2), or linolenic (18:3). Thus, the quantity of stearic acid will decrease, while oleic, linoleic, or linolenic acids will increase. Therefore, more unsaturated fatty acids occur in the fermentation process. Meanwhile, an example of the desaturase reaction mechanism in stearic acid is shown in **Figure 2**.

Fatty acid quality is not only determined by the quantity of saturated fatty acids, decreasing their content and increasing their content of unsaturated fatty acids. The type of fatty acid also influences its quality. Oleic, linoleic, and linolenic acids are essential fats found in legumes [43]. Both cowpea and African locust tempeh contain these types of fatty acids (see **Table 5**). The body needs these three fatty acids because they are essential for health, acting as omega-3 and omega-6 fatty acids. However, the body cannot produce these three fatty acids on its own. Therefore, they can be obtained from foods such as tempeh [44].

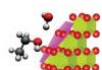
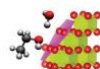


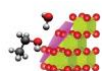
Table 5. The Effect of Germination on the Quantity of Saturated and Unsaturated Fatty Acids in Various Types of Tempeh

No	Type of Tempeh	Treatment	Fatty Acid Profile	Other Nutrients	References
1.	Cowpea tempeh (<i>Vigna unguiculata</i>)	G0F0	Saturated fatty acid (mg/100g) - Lauric (12:0) = 74.6 - Myristic (14:0) = 2.9 - Palmitic (16:0) = 596.0 - Stearic (18:0) = 130.2 - Arachidic (20:0) = 29.0 - Behenic (22:0) = 64.8 - Lignoceric (24:0) = 38.2 Unsaturated fatty acid (mg/100g) - Oleic (18:1) = 160.4 - Linoleic (18:2) = 765.0 - Linolenic (18:3) = 370.3 - Eicosenoic (20:1) = 6.3	Simple sugar and oligosaccharide: xylose, fructose, glucose/galactose, sucrose, maltose, raffinose, stachyose.	[13]
		G24F0	Saturated fatty acid (mg/100g) - Lauric (12:0) = 21.9 - Myristic (14:0) = 2.8 - Palmitic (16:0) = 567.5 - Stearic (18:0) = 131.3 - Arachidic (20:0) = 29.3 - Behenic (22:0) = 65.1		



		• Lignoceric (24:0) = 44.1 Unsaturated fatty acid (mg/100g) • Oleic (18:1) = 158.0 • Linoleic (18:2) = 746.0 • Linolenic (18:3) = 353.7 • Eicosenoic (20:1) = 6.0	
	G24F24	Saturated fatty acid (mg/100g) • Lauric (12:0) = 7.0 • Myristic (14:0) = 9.9 • Palmitic (16:0) = 754.5 • Stearic (18:0) = 182.7 • Arachidic (20:0) = 36.4 • Behenic (22:0) = 76.9 • Lignoceric (24:0) = 50.7 Unsaturated fatty acid (mg/100g) • Oleic (18:1) = 232.5 • Linoleic (18:2) = 1010.7 • Linolenic (18:3) = 439.7 • Eicosenoic (20:1) = 8.9	
2.	African locust tempeh (<i>Parkia biglobosa</i>)	G0F0 Saturated fatty acid (mg/100g) • Lauric (12:0) = 0.0003	Proximate: protein, fat, ash, fiber, carbohydrate. Minerals: P, K, Na, Ca, Mg, Fe, Zn, P/Ca, Na/K, Cu, Mn.

[33]

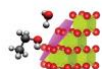


-
- Myristic (14:0) = 0.0003
 - Palmitic (16:0) = 17.256
 - Stearic (18:0) = 18.086
 - Arachidic (20:0) = 3.815
 - Behenic (22:0) = 7.742
 - Lignoceric (24:0) = 0.734
- Unsaturated fatty acid (mg/100g)**
- Palmitoleic (16:1) = 0.014
 - Oleic (18:1) = 18.594
 - Linoleic (18:2) = 33.687
 - Linolenic (18:3) = 0.0002
-

- G168F0 **Saturated fatty acid (mg/100g)**
- Lauric (12:0) = 0.001
 - Myristic (14:0) = 0.001
 - Palmitic (16:0) = 15.479
 - Stearic (18:0) = 18.073
 - Arachidic (20:0) = 3.306
 - Behenic (22:0) = 8.444
 - Lignoceric (24:0) = 2.022
-



		Unsaturated fatty acid (mg/100g) - Palmitoleic (16:1) = 0.006 - Oleic (18:1) = 20.833 - Linoleic (18:2) = 0.001 - Linolenic (18:3) = 0.001		
		G168F24 Saturated fatty acid (mg/100g) - Lauric (12:0) = 0.001 - Myristic (14:0) = 0.001 - Palmitic (16:0) = 12.879 - Stearic (18:0) = 19.721 - Arachidic (20:0) = 5.165 - Behenic (22:0) = 11.678 - Lignoceric (24:0) = 0.008 Unsaturated fatty acid (mg/100g) - Palmitoleic (16:1) = 0.142 - Oleic (18:1) = 20.154 - Linoleic (18:2) = 28.700 - Linolenic (18:3) = 0.403		
3.	Soybean tempeh <i>(Glycine max)</i>	G24F48 Saturated fatty acid - Palmitic (16:0) - Stearic (18:0)	Carbohydrate: lyxose. Amino acids: tyrosine, threonine. Nitrogenous base: uracil.	[34]



4. Soybean tempeh (<i>Glycine max</i>)	G0F0	-	Sugars: trehalose, raffinose, fructose, glucose. Others: glycerol, adenosine, uracil, genistein	[29]
	G24F48	Saturated fatty acid • Butanoic (4:0) • Palmitic (16:0) • Stearic (18:0) Unsaturated fatty acid: linoleic (18:2)	Amino acids: glycine, tryptophan, cystathionine, 2-aminobutyric acid. Sugars: trehalose, raffinose, fructose, glucose. Others: glycerol, adenosine, uracil, genistein	

Notes: some included studies did not report untreated control data and only presented data for treated samples (article 3). Furthermore “-” indicates that is not reported/detected. The code of treatment refers to Table 3.

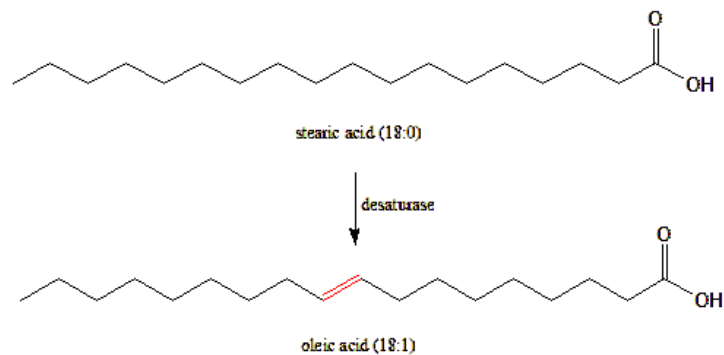


Figure 2. Desaturation Reaction of Stearic Acid (18:0) into Oleic Acid (18:1)

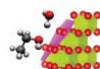


Table 6. Distribution of Saturated and Unsaturated Fatty Acids in Cowpea and African Locust Tempeh

Types of Tempeh		Treatment	Fatty Acid (mg/100g)				
			Saturated Fatty Acid			Unsaturated Fatty Acid	
			Short-chain	Medium-chain	Long-chain	Mono-	Poly-
Cowpea Tempeh (<i>Vigna unguiculata</i>)		G0F0	-	74.6	861.1	166.7	1135.3
		G24F0	-	21.9	839.9	164	1099.7
		G24F24	-	7	1111.1	241.4	1450.4
African Locust Tempeh (<i>Parkia biblobosa</i>)		G0F0	-	0.0003	47.633	18.608	33.687
		G24F0	-	0.001	47.325	20.839	0.002
		G24F24	-	0.001	49.452	20.296	29.103

Notes: The meaning of “-” is not detected. The code of treatment refers to Table 3.

3.3 Effect of Germination on the Ratio of Saturated and Unsaturated Fatty Acids in Tempeh

The ratio of saturated and unsaturated fatty acids in food products, especially tempeh, is important because it has an impact on health. According to [45], the maximum daily total fat that is safe to consume is around 30% of total energy, consisting of 10% saturated fatty acids, 10% monounsaturated fatty acids, and 10% polyunsaturated fatty acids. Saturated fatty acids have a negative impact on health by increasing total cholesterol and LDL levels, while monounsaturated fatty acids can reduce LDL levels and increase HDL [46]. Lowering LDL and increasing HDL has a positive impact on health, especially the heart. The ideal ratio of saturated to unsaturated fatty acids is small, meaning saturated fatty acid content decreases and unsaturated fatty acid content increases.

Based on **Table 5**, the average tempeh treated with germination experienced a decrease in saturated fatty acids and an increase in unsaturated fatty acids. This means the ratio between saturated and unsaturated fatty acids is small. This is because during the germination process, germinating seeds require energy through the mobilization of fat into fatty acids and glycerol with the help of the lipase enzyme. These fatty acids can be used as an energy source for the beans to germinate, thus reducing [47]. In addition, free fatty acids in the form of saturated fatty acids are broken down through the β -oxidation process to form acetyl-CoA [48]. These saturated fatty acids are more easily broken down during β -oxidation because they have straight chains and are more

stable than unsaturated fatty acids, which have double bonds. Therefore, saturated fatty acids are reduced by their use in this process.

The increase in unsaturated fatty acids likely occurs due to the presence of desaturase enzymes, which are active during germination. These enzymes convert single bonds into double bonds by removing hydrogen atoms from saturated fatty acids to unsaturated fatty acids. This reaction is called desaturation or dehydrogenation. During the imbibition process, enzymes already present in the seed are activated [49].

In addition to germination, fermentation also affects the fatty acid profile of tempeh, particularly unsaturated fatty acids. For example, cowpea tempeh that germinated and fermented for 24 hours (see **Table 5**) exhibited a low omega-6 to omega-3 ratio (n6:n3) both before and after fermentation. Before fermentation, the n6:n3 ratio was 746.0:353.7, which slightly changed to 1010.7:439.7 after fermentation. The n6:n3 ratio refers to the proportion of omega-6 fatty acids relative to omega-3 fatty acids present in food. A high n6:n3 ratio is certainly not ideal for health because it triggers a number of health problems, such as increased inflammation and chronic diseases [50]. The n6:n3 ratio that is good for health is around 1:1 to 5:1 [51]. The n6:n3 ratio in the study was around 2.1:1 to 2.3:1, meaning it is good for health and safe for heart disease.

Several data presented in **Table 5** were further processed to obtain the ratios of total saturated and unsaturated fatty acids. **Figure 3** illustrates the ratios of saturated and unsaturated fatty acids in cowpea tempeh and African locust tempeh. In cowpea tempeh, germination as well as the combined germination and fermentation treatments reduced saturated fatty acids while

increasing unsaturated fatty acids. This phenomenon was presumably associated with the reduction of saturated fatty acids during the early germination phase due to the high rate of lipid degradation through the β -oxidation pathway within glyoxysomes, which supplies energy for sprout growth [52]. However, when the process was continued with microbial fermentation, a reversal of the trend was observed, in which unsaturated fatty acids increased significantly.

This phenomenon was presumably associated with the activity of fungal desaturase enzymes capable of introducing double bonds into saturated hydrocarbon chains, further enhanced by the release of unsaturated fatty acids from bound lipid matrices through extracellular lipase-mediated enzymatic hydrolysis [53]. Nevertheless, cowpea tempeh across all treatments was consistently dominated by unsaturated fatty acids (>58%).

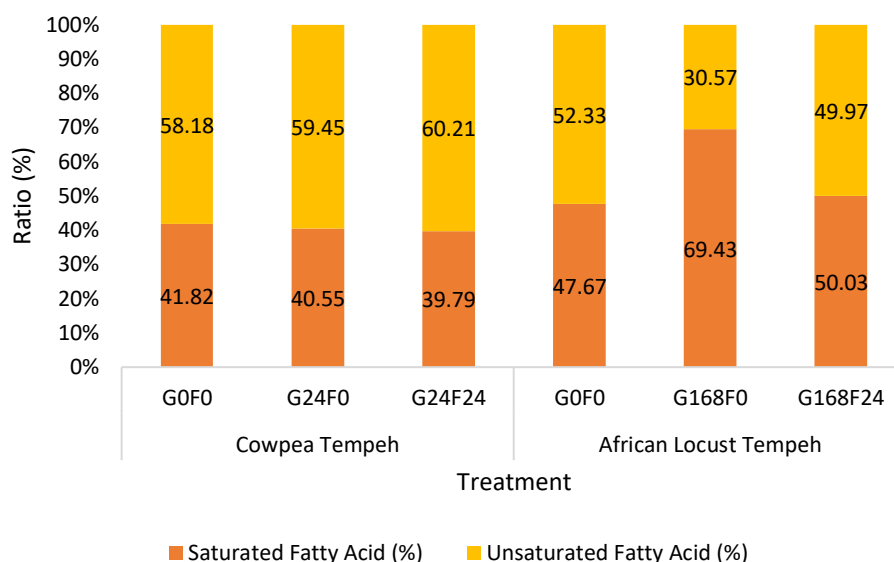


Figure 3. Ratio of Saturated & Unsaturated Fatty Acids in Cowpea and African Locust Tempeh. The code of treatment refers to Table 3.

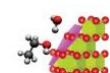
In contrast, African locust tempeh exhibited highly dynamic at each treatment. Under the initial condition (G0F0), the ratio of saturated and unsaturated fatty acids was relatively balanced. However, when the seeds underwent germination for 168 h, a sharp increase in the ratio of saturated fatty acids was observed, while unsaturated fatty acids decreased drastically. This phenomenon indicates the extensive utilization of saturated fatty acids by *Parkia biglobosa* seed cells, presumably triggered by the activity of plant lipoxygenase enzymes, which degrade the double bonds of unsaturated fatty acids to support sprout growth pathways. Following the combined germination and fermentation treatment, the trend shifted significantly, as the proportion of unsaturated fatty acids increased again. This phenomenon was presumably associated with the ability of fermentative microorganisms to restore unsaturated fatty acids through enzymatic activities during fermentation. From the perspective of the product's functional value, the combination of germination and fermentation

represents a promising processing approach for producing healthier African locust tempeh.

If the process is terminated at the germination only, the resulting product may be less suitable for cardiovascular disease, as its fatty acid profile becomes predominantly composed of saturated fatty acids (69.43%), which may contribute to elevated low-density lipoprotein (LDL) levels. In contrast, the 24 h fermentation intervention was able to effectively remodel the lipid composition by restoring the proportion of unsaturated fatty acids to a nearly balanced level of approximately 50%, thereby producing a product that is considerably safer and more favorable for cardiovascular health.

3.4 Tempeh Types with Potentially Favorable Fatty Acid for Cardiovascular Health

As previously known, saturated fatty acids can contribute to heart disease. Conversely, unsaturated fatty acids can reduce the risk of heart disease. Therefore, to determine the best type of legumes in terms of fatty acid profile, one can look at the balance between saturated fatty acids, which



should be limited, and unsaturated fatty acids. Furthermore, the presence of essential fatty acids, such as oleic (18:1), linoleic (18:2), and linolenic (18:3), should also be considered [44].

Based on the explanation above, germination, fermentation, and a combination of germination and fermentation processes affect the fatty acid profile of various types of tempeh. The health benefits of tempeh are determined by its nutritional content, including fatty acids, amino acids, antioxidants, and other factors. For example, the fatty acid content of a food, such as tempeh, can impact heart health. Fatty acids that are good for preventing and reducing heart disease are unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid. From all the types of tempeh described, it can be seen which types are good for heart health. **Table 7** shows tempeh types with potentially favorable fatty acid profiles for cardiovascular health.

Based on the available compositional data, cowpea tempeh (G24F24) showed the most favorable fatty acid-related indicators among the reviewed tempeh types. However, this interpretation is based on fatty acid composition only and should not be considered direct evidence for coronary heart disease prevention. Besides, soybean tempeh (G24F24) may have improved nutritional characteristics after germination and fermentation. However, because quantitative fatty acid data were limited, its fatty acid profile cannot be directly compared with cowpea tempeh. However, bosok tempeh (G28F168) showed the presence of unsaturated fatty acids after germination and prolonged fermentation. However, due to the absence of quantitative data, its cardiovascular relevance should be interpreted cautiously.

Table 7. Tempeh Types with Potentially Favorable Fatty Acid for Cardiovascular Health

No.	Type of Tempeh	Treatment	Quantitative/Qualitative Criteria Used
1.	Cowpea tempeh	G24F24	Quantitative data available. This tempeh showed relatively high unsaturated fatty acids, particularly oleic acid, linoleic acid, and linolenic acid. Linoleic acid was reported at 1010.7 mg/100 g and linolenic acid at 439.7 mg/100 g, resulting in an estimated linoleic acid to linolenic acid ratio of approximately 2.3:1.
2.	Soybean tempeh	G24F24	Mainly qualitative data available. Linoleic acid was detected after treatment, whereas it was not reported before treatment. Other nutritional compounds, including amino acids such as glycine, tryptophan, cystathionine, and 2-aminobutyric acid, were also reported after treatment.
3.	Bosok tempeh	G28F168	Mainly qualitative data available. Unsaturated fatty acids, particularly oleic acid and linoleic acid, were detected after treatment but were not reported before treatment.

Notes: The code of treatment refers to Table 3.

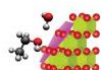
4 Conclusion

Based on this systematic review, germination and fermentation may influence the fatty acid profile of tempeh produced from various legumes. In several reviewed studies, these processes were associated with changes in saturated fatty acids and unsaturated fatty acids, including oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). However, the magnitude and direction of these changes varied depending on the type of legume, germination time, fermentation duration, and microorganism used. Among the reviewed tempeh types, germinated and fermented cowpea tempeh showed a relatively favorable fatty acid profile based on the available compositional data. This was indicated by its relatively high

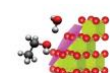
unsaturated fatty acid content and an estimated linoleic acid to linolenic acid ratio of approximately 2.3:1. Germinated and fermented cowpea tempeh may be considered a promising candidate for further nutritional evaluation in coronary heart disease prevention. Further studies including comprehensive nutritional analysis, and clinical outcomes are required to clarify its potential relevance to cardiovascular health.

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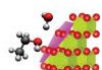
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