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Nutritional Changes in the Process of Rendang Tempeh's Production (Gulai, Kalio, and Rendang)

Fachrur Rozi[1], Wellyalina[1], Meisya Az-Zahra Rachmayanti[1], Rini[1], Rahadian Zainul[2] and Daimon Syukri[1,*]

[1]Department of Food and Agricultural Product Technology, Universitas Andalas, Limau Manis Campus, Padang, Indonesia;
[2]Department of Chemistry, Faculty of Mathematic and Natural Science, Padang State University, Padang, Indonesia
[*]Corresponding author's e-mail: dsyukri@ae.unand.ac.id

Food processing, such as cooking, can affect the bioactive components of the ingredients. In making rendang tempeh, several stages must be gone through, such as curry (gulai) and kalio formation; therefore, the production of rendang tempeh will take time and might be impacted by the changes in nutrients of the tempeh. This research was conducted to observe changes in chemical components, such as proximate components, antioxidants, fatty acids, and isoflavones, during the process of making tempeh rendang. This study processed tempeh into gulai, kalio, and rendang. Chemical analyses such as proximate analysis (water, fat, protein), antioxidant, fatty acid, and isoflavone analysis were carried out at each stage of making the rendang tempeh. Based on the study's results, it was known that the proximate profile increases during the rendang-making process. Linoleic acid, as the main fatty acid content in tempeh, was decreased, as was the isoflavone content. In this study, From the research, it was concluded that the cooking process changes the profile of the bioactive compounds in tempeh, which might be affected by the decrement of phytochemicals. In this study, the degradation pattern of phytochemical compounds, especially isoflavones, remains unknown, as well as whether the compounds are degraded or changed into aglycone forms with higher bioactivity. Therefore, the observation on molecular perspective on nutritional compounds of rendang tempeh during processing from tempe as raw material to rendang tempeh needs to be further conducted to obtain comprehensive information regarding the benefit of transformation of tempe to become a processed food "rendang tempeh."
Keywords: Fermented, gulai, kalio, rendang, heat process, functionality, proximate, soybean.

INTRODUCTION

Tempeh is a traditional food originating from Indonesia. Tempeh is a source of plant-based protein that is usually served in daily diet. Tempeh is made from the soybean fermentation process by *Rhizopus sp.* This fermentation process produces several hyphae that stick the soybeans together. Tempeh contains substances that are beneficial for the body, such as protein, fat, isoflavones, and minerals (Ramulo and Surya, 2021). Protein is a macronutrient that forms body tissue, regulates body metabolism, and produces 4 kcal of energy. Twenty types of amino acids form protein; some are classified as essential and non-essential amino acids. Non-essential amino acids are amino acids that the human body can synthesize. In contrast, essential amino acids are a group of amino acids that the human body cannot synthesize, so they can be obtained by consuming certain foods from both animal and vegetable sources. Essential amino acids include isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine (Church *et al.*, 2020). Isoflavones are secondary metabolite compounds in the flavonoid group (Syukri *et al.*, 2018). This compound is commonly found in legume products and their derivatives, such as tempeh. The fermentation process in tempeh increases the content of tocopherol, protein, folic acid, and isoflavones and forms vitamin B_{12} (Tamam *et al.*, 2019). In tempeh, isoflavones are usually found in aglycones (non-sugar) form due to the heating stage of soybeans in making tempeh, which causes the release of glucose from glycosides with the help of enzyme β-glucosidase so that the body more easily absorbs it (Huang *et al.*, 2018; Syukri *et al.*, 2019). The isoflavones commonly found in tempeh are genistein, daidzein, and glycitein, which can act as antioxidants. Genistein and daidzein can replace the role of estrogen hormone when estrogen hormone levels in the human body decrease so that menopausal symptoms can be reduced (Fawwaz *et al.*, 2017).

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The isoflavones contained in tempeh can also increase the ability of calcium absorption in the bones so that it can reduce the risk of developing osteoporosis (Harahap and Suliburska, 2022). Isoflavones can prevent the oxidation of Low-Density Lipoprotein (LDL), thereby reducing the risk of arteriosclerosis and rectal, prostate, breast, and colon cancer (Kim, 2021). Tempeh also contains fatty acids in form of palmitic, stearic, oleic, linoleic and linolenic (Damanik *et al*., 2018). Tempeh is becoming popular in various countries as a food for people with a calorie deficit or who live as vegetarians because tempeh can be used as a meat analog. One of the ways to process tempeh is to make it into rendang. Rendang is a typical Minangkabau food that is famous for its deliciousness but tends to be less suitable if consumed in large quantities because coconut milk is high in saturated fat and contains beef, 100 grams of which contains 70 mg of cholesterol (Syukri *et al*., 2024). Substituting meat with tempeh can help reduce cholesterol consumption. Rendang is a type of gulai that is cooked for quite a long time, usually for 5 to 6 hours, until the color turns dark brown. Rendang is made of beef and coconut milk cooked together and added spices in the form of red chilies, galangal, lime leaves, lemongrass, bay leaves, turmeric leaves, turmeric, shallots, garlic, and other spices. Rendang is rich in protein and fat, considering that the essential ingredients are beef and coconut milk The spices used in rendang also act as antioxidants. Chilies contain vitamins C, A, thiamin, niacin, riboflavin, and E, suitable for improving blood circulation. Ginger contains gingerol, shogaol, and gingeron, have higher antioxidant activity than vitamin E, Tumeric contains curcumins (Rini *et al*., 2016; Ivanović *et al*., 2021; Salem *et al*., 2022). Understanding the impact of various food processing methods and durations on the nutritional composition of food products is crucial. While food processing can reduce anti-nutrient components in raw food ingredients, it also has the potential to diminish nutrients sensitive to pH, temperature, and oxygen. For instance, the heating process can alter the components of amino acids, fatty acids, and isoflavones. Therefore, it is essential to research the antioxidant activity, protein, fat, fatty acids, and daidzein levels in dishes like curry (gulai), kalio, and tempe rendang to ensure their nutritional value is preserved. When making tempe rendang, which uses tempeh as raw material with soft characteristics because it is made from the fermentation process of soybeans, the possibility of changes in nutrition is substantial. The process of making rendang, which goes through several processes, such as forming curry (gulai) and kalio until the rendang is finally formed, will undergo a heating process for an extended period. There is no report on the effect of rendang tempe processing on its nutritional changes. Thus, the finding of this study will be a reference to the practice of developing functional rending tempeh, even if the process will take a long time to process.

MATERIALS AND METHODS

All materials were purchased from the local market in Padang City West Sumatra Indonesia. All chemicals used were pro analysis grade purchased from several brands that are available in Indonesia. The study was conducted from July 2023 to July 2024. The experiments were done at laboratory of Department of Food and Agricultural Product Universitas Andalas. s

***Tempeh Gulai Making Process*:** Gulai is the local name for the form of curry in Indonesia. 300 g of tempeh are cut into 3 cm x 3 cm x 3 cm cubes. Tempeh pieces are fried until half cooked (7 minutes), then drain. Puree 59 g of shallots, 40 g of bird's eye chili, 27 g of garlic, 50 g of roasted candlenuts, 2 g of ginger, 3 g of turmeric, and 0.5 g of coriander. The ground spices are then cooked with coconut milk, eight bay leaves, five lime leaves, three turmeric leaves, and one lemongrass stalk added. All ingredients are heated at a temperature of 90-97 ºC for 30 minutes. After that, the tempeh is added, and the temperature is lowered to 82-88 ºC for 30 minutes (Refdi *et al*., 2022).

***Tempeh Kalio Making Process*:** Kalio is the viscous form of curry. 300 g of tempeh are cut into 3 cm x 3 cm x 3 cm cubes. Tempeh pieces are fried until half cooked (7 minutes), then drain. 59 g shallots, 40 g red chilies, 27 g garlic, 24 g ginger, 6 g cinnamon, 0.5 g coriander, 35 g galangal, 0.3 g cloves, and table salt are blended. The grounded spices are cooked with coconut milk, eight bay leaves, five lime leaves, three turmeric leaves, and two lemongrass stalks. The coconut milk and the spices are cooked at 100-150 ºC for 45 minutes. After that, add the tempeh and lower the temperature to 85-90 ºC for 30 minutes, then lower the temperature again to 83-88 ºC for 30 minutes (Refdi *et al*., 2022).

***Tempeh Rendang Making Process*:** 300 g of tempeh are cut into 3 cm x 3 cm x 3 cm cubes. Tempeh pieces are fried until half cooked (7 minutes), then drain. 59 g shallots, 40 g red chilies, 27 g garlic, 24 g ginger, 6 g cinnamon, 0.5 g coriander, 35 g galangal, 0.3 cloves, and table salt are blended. The grounded spices are cooked with coconut milk, eight bay leaves, five lime leaves, three turmeric leaves, and two lemongrass stalks. All ingredients are heated at 90-97 ºC for 45 minutes. After that, add the tempeh and lower the temperature to 82-88 ºC for 30 minutes, then lower the temperature again to 79-85 ºC for 60 minutes. The drying of the coconut milk is a benchmark for completing the rendang cooking process (Refdi *et al*., 2022).

***Sample Fat Extraction*:** Fat extraction was carried out using the Bligh and Dyer Method (Zainal *et al*., 2022). This fat extraction method uses a mixture of chloroform, distilled water, and methanol. The first step in this method is adding 5 g of sample, 650 mg of distilled water, and 10 ml of methanol into a test tube. The sample was then homogenized using a vortex for 2 minutes at low speed to avoid emulsion. Then, 5 ml of chloroform and 5 ml of distilled water were added and homogenized again using a vortex for 30 seconds. Next, the sample was centrifuged for 20 minutes until three layers were formed: the top layer containing polar compounds, the middle layer containing unextracted residue (interphase), and the bottom layer containing lipids. The top and middle layers are then separated using a dropper pipette, while the bottom layer is filtered into a glass bottle. The fat extract was then aired for 1 hour, covered, and stored at 4 ºC for further analysis using GC-MS. GCMS analysis was performed according to Rini *et al*. (2021). The fat was methylated according to Rini *et al*. (2022) before GCMS analysis.

***Isoflavone Sample Preparation*:** A 1 g sample was macerated for 24 hours in a 25 ml volumetric flask using 10 ml of 70% ethanol. After 24 hours, the sample was placed in an ultrasonic bath for 15 minutes. Next, the sample was centrifuged for 30 minutes and filtered using cellulose nitrate membrane filters into a vial tube for subsequent injection into HPLC (Syukri *et al*., 2018).

***Statistical Analysis*:** Duncan's multiple range test was used to analyze the statistics at the 5% probability level. R program was utilized to do One-way ANOVA. The mean observations from three replicates of each of the four distinct treatments make up the data.

RESULTS AND DISCUSSION

***Raw Material Analysis*:** Raw material analysis was carried out on the main research ingredient, tempeh, which had yet to be processed. This analysis is carried out to determine the components of the raw materials. The analysis included proximate analysis in the form of water content, fat content, protein content, antioxidant activity, fatty acid content, and identification of daidzein compounds. The results of the analysis of water content, fat content, protein content, and antioxidant activity can be seen in Table 1.

Table 1. Raw material analysis result.

Analysis Component	Result ± Standard deviation
Water Content (%)	62.84 ± 0.00
Fat Content (%)	4.26 ± 0.06
Protein Content (%)	20.30 ± 0.30
Antioxidant Activity(%)	49.65 ± 2.22
Daidzein Level (ppm)	54.67

The results of the analysis of the water content of the tempeh raw material were 62.84%. This analysis proves that the raw materials' water content is according to the National Standardization Agency (SNI 3144), 2015, which regulates the quality requirements for soybean tempeh, where the maximum water content limit set is 65%. Determining water content is very important. Water content determines the product's durability because it will affect the physical and chemical properties and activity of microorganisms in food (Hallsworth, 2022).

In the fat content testing results, the fat content was found to be 4.26%. These results indicate a discrepancy with the quality requirements for soybean tempeh in National Standardization Agency (2015), where the minimum tempeh fat content that has been set is 7%. This discrepancy, which can be caused by the long distribution and testing time interval, may have implications for the product's quality. During this time lag, fermentation by the mold will continue to occur where fatty acids become a source of energy for the mold to continue to grow, so the impact is a decrease in the fat content of tempeh (Fallo, 2022). Fat is a glycerol ester with fatty acids, where the fatty acids contained in tempeh can be seen in the test results using GCMS below.

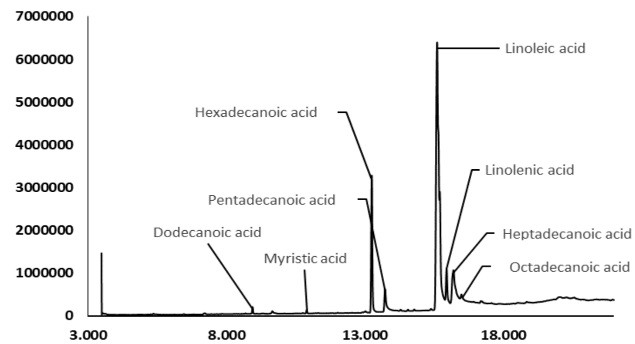

Figure 1. Chromatogram of fatty acids in tempeh.

Based on the chromatogram of FAME analysis results from tempeh fat extract, 10 types of fatty acids were detected. 10 types of tar fatty acids can be seen in Table 2.

Table 2. Names of tempeh fatty acid analysis results.

Peak	Area (%)	*R. time*	Compound Name
1	0.47	8.950	Dodecanoic Acid, methyl ester
2	0.17	9.668	1,2-Benzenedicaboxylic acid
3	0.26	10.908	Myristic acid, methyl ester
4	13.42	13.256	Hexadecanoic acid, methyl ester
5	3.12	13.734	Pentadecanoic acid
6	52.25	15.613	9,12-Octadecadienoic acid, methyl ester
7	13.86	15.710	9,12,15-Octadecatrienoic acid, methyl ester
8	5.01	15.956	Heptadecanoic acid, methyl ester
9	10.01	16.203	9,12-Octadecadienoic acid, methyl ester
10	1.43	16.492	Octadecanoic acid

From the results of fatty acid analysis in tempeh, it found that the highest fatty acid content was 9.12-Octadecadienoic acid, or what is usually called linoleic acid (omega 6). Previous research indicated that the highest fatty acid content in tempeh is linoleic acid, which amounts to 4.052 g/100 g of ingredient. Consuming linoleic acid can significantly reduce LDL levels while increasing HDL levels. Apart from that, the presence of the compound 9,12,15-Octadecatrienoic acid, or what is usually called linolenic acid (omega 3), was also detected. Omega 3 can act as an anti-inflammatory compound, prevent neurological disorders, influence cognitive development, and prevent cardiovascular disease (Rizal *et al*., 2022). Based on the analysis results, the protein content in tempeh was 20.29%. The results are based on the quality requirements for soybean tempeh as stipulated in the National Standardization Agency (2015), where the minimum tempeh protein content is at least 15%. The protein content will increase as the tempeh is cured for longer. This condition is because complex compounds will be digested by mold and produce simpler compounds, so during ripening, the amount of amino acid content in tempeh will increase (Maryati *et al*., 2019). Furthermore, based on analysis of the antioxidant activity of tempeh, the results were 49.65% in 1000 ppm. The antioxidants in tempeh come from the isoflavones contained in it. The isoflavones contained in tempeh are isoflavone aglycones in the form of genistein, daidzein, and glycitein (Syukri *et al*., 2018). Based on the analysis of daidzein levels, the concentration of daidzein compounds in the sample was obtained at 56.67 ppm. The isoflavone content in tempeh is 60.61 mg/g product or around 6.06%. However, other isoflavone aglycones were not tested in this study, so the total levels of the tempeh used as raw material have yet to be discovered. Daidzein in tempeh has other functions besides its role in warding off free radicals. In the body, daidzein is converted into equol, which binds to the hormone estrogen, which helps fight aging and osteoporosis (Sirotkin *et al*., 2021).

***Antioxidant Activity*:** Antioxidants are compounds that can reduce free radicals by donating electrons (Anggraini *et al*., 2021). The method used to analyze antioxidant activity is the DPPH method. The DPPH method is commonly used to analyze antioxidant activity because the working steps are simple and fast. DPPH will act as a free radical that accepts electron donors until the color changes from purple to light yellow. Antioxidant activity can be seen in Figure 3.

From the research results, it can be seen that the highest antioxidant activity is found in tempeh rendang, while the lowest antioxidant activity is found in tempeh gulai. This can be influenced by the amount of spices used. The use of spices in making rendang is more complex than making gulai. Where each spice mostly has compounds that act as antioxidants. In addition, the longer the cooking time, the more Maillard reactions will form. When heated at high temperatures and for a long time, the Maillard reaction is between the carbonyl group of reducing sugar and the amino group. The Maillard reaction will form melanoidin, which has antioxidant activity and can prevent lipid oxidation (Liu *et al*., 2022).

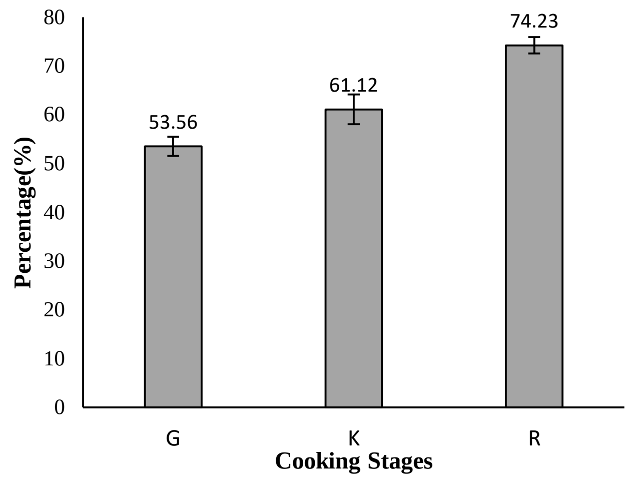

Figure 3. Antioxidant activity of gulai, kalio, and rendang tempeh.

***Fat Content*:** In the process of making gulai, kalio and rendang, one of the main ingredients used is coconut milk. Coconut milk is classified as an oil-in-water emulsion and has a fairly high fat content (Chen *et al*., 2023). Therefore, the fat content of gulai, kalio and tempe rendang was tested using the Soxhlet method. The results of the fat content analysis can be seen in Figure 4.

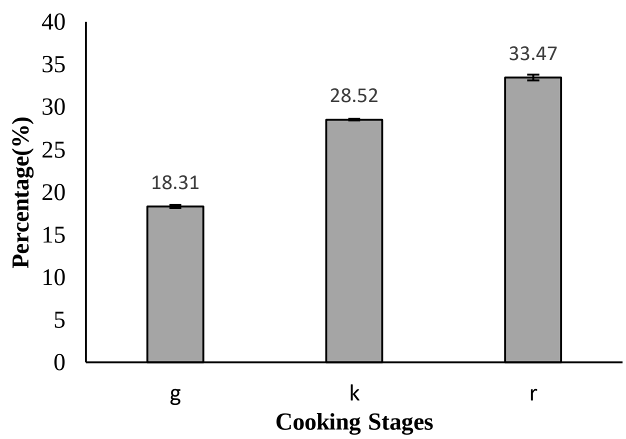

Figure 4. Fat content of gulai, kalio, rendang tempeh.

Based on the analysis, the results showed that tempeh rendang had the highest fat content, while tempeh gulai had the lowest fat content. This proves that the heating time is directly proportional to the increase in fat content. The heating process in making gulai, kalio, and rendang will cause water to evaporate. The longer the heating, the more water will evaporate. The more water that evaporates, the more the solid mass will decrease and cause the fat content in the spices to increase.

***Protein Content*:** Based on the source, protein is divided into two, namely animal protein and vegetable protein. Animal protein comes from food that comes from animals, while vegetable protein comes from food that comes from plants. Vegetable protein is found in many nuts, one of which is soybeans. Therefore, an analysis of protein levels was carried out in gulai, kalio and tempeh rendang to see changes in protein levels during cooking. The results of protein content analysis can be seen in Figure 5.

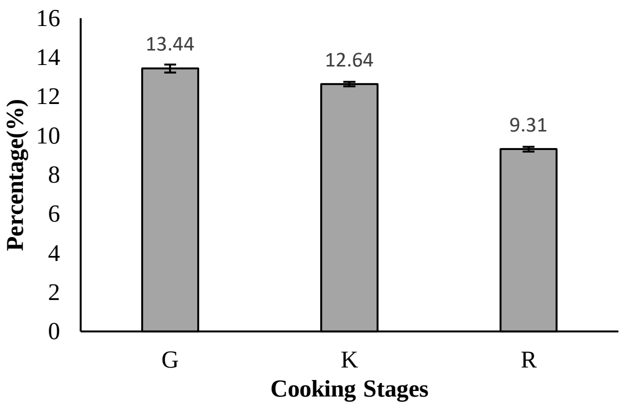

Figure 5. Results of protein analysis content in gulai, kalio, and rendang tempe.

Based on the analysis results, it can be seen that the highest protein content is found in tempeh gulai, and the lowest protein content is found in tempeh rendang. This can be influenced by the length of heating during cooking. Heating is one of the destructive food processing processes. Heating food can cause protein denaturation. At a temperature of 50oC the protein will begin to denature but has not been damaged, only structural changes occur, however, when heating continues and begins to reach a temperature of 80 ºC, the protein structure will begin to be damaged so that the protein will precipitate. Another factor that causes a decrease in protein content is the Maillard reaction between amino acids and reducing sugars (Kutzli *et al*., 2021).

***Fatty Acid Profile*:** Gulai, kalio, and rendang are food products that are prepared using coconut milk. Coconut milk is the result of coconut juice which has a fairly high fat content. Therefore, an analysis of the fatty acid profile was carried out in tempeh gulai sauce, tempe gulai, kalio tempeh seasoning, tempeh kalio, tempeh rendang seasoning, and tempeh rendang. This was done to determine the difference in fatty acid content between tempeh and the spices. The fatty acid analysis process in gulai, kalio, and rendang is carried out in the form of FAME (Fatty Acid Methyl Ester).

***Fatty Acid Profile of Tempeh Gulai*:** Based on the results of the analysis of the fatty acid profile in tempeh gulai and gulai broth in the form of FAME, eight compounds were detected in tempeh gulai and eight compounds in gulai broth. Based on the results of the analysis of fatty acids in tempeh gulai, it can be seen that the highest fatty acid in tempeh gulai is the same as raw tempeh, namely 9.12-Octadecanoic acid or what is usually called omega 6. This fatty acid is an essential fatty acid and is included in the acid group. unsaturated fat. Consuming omega 6 can significantly reduce LDL levels and increase HDL levels, thereby reducing the risk of coronary heart disease and several other degenerative diseases. At the gulai cooking stage, the content of 9,12,15-Octadecatrienoic acid or what is usually called linolenic acid (omega 3) was still detected. At this stage pentadecanoic acid and heptadecanoic acid were still detected. Based on previous research using in vivo methods, routine oral administration of pentadecanoic acid and heptadecanoic acid to mice was able to reduce glucose levels, reduce the risk of developing type 2 diabetes, reduce LDL levels, and stabilize the number of red blood cells, thereby reducing the potential for anemia (Venn-Watson *et al*., 2023). However, the analysis results also contained the compound cyclohexasiloxane. This compound most likely appeared due to an error in the column which contained silica in it. Based on data from the analysis of fatty acids in gulai broth, 7 of the 8 compounds detected were fatty acid compounds originating from coconut milk. These compounds include octanoic acid (caprylic acid), decanoic acid (capric acid), dodecanoic acid (lauric acid), myristic acid (myristic acid), hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid (linoleic acid), and 9-octadecenoic acid (oleic acid). Based on this data, it is suspected that the heptadecanoic acid (margaric acid) that appears in the chromatogram comes from tempeh which is used in making gulai.

***Kalio Tempeh*:** Based on the results of the fatty acid analysis carried out on Kalio tempeh, the compound with the highest levels was still found in linoleic acid (9,12-octadecadienoic acid). However, at the kalio cooking stage, the compound 9,12,15-octadecatrienoic acid or what is usually called linolenic acid (omega 3) is no longer detectable. This is because linolenic acid is an unsaturated fatty acid which has 3 double bonds. The more double bonds, the more unstable a fatty acid will be and have a lower boiling point compared to unsaturated fatty acids with the same number of chains but with fewer double bonds (Szabo *et al*., 2022). Based on the analysis results, the highest fatty acid contained in kalio sauce is myristic acid. When compared with gulai seasoning, the area of myristic acid in kalio seasoning increases quite

Table 3. Fatty acid content in gulai, kalio, and rendang tempeh (based on GCMS chromatogram area).

Fatty acid compounds	Tempe Gulai	Tempeh Kalio	Tempeh Rendang	Gulai Broth	Kalio Sauce	Rendang Paste
Pentadecanoic acid	159043	-	**-**	-	-	-
Linoleic acid	445641	38344927	**36727671**	703558	425036	375486
Linolenic acid	23537	-	**-**	-	-	-
Heptadecanoic acid	30035	105772	**3120335**	164533	166488	204535
Octanoic acid	-	88427	**441058**	77989	159663	173454
Decanoic acid	-	60824	**337951**	70310	114395	147753
Dodecanoic acid	-	454036	**2246182**	559691	850847	996624
Myristic acid	-	429509	**1012438**	169199	1000214	1209829
Hexadecanoic acid	-	8896055	**9899141**	907885	725430	1123751
Oleic acid	-	-	**-**	281966	356327	641833
10,13-Eicosadienoic acid	-	-	**76299**	-	-	-

significantly. This is thought to be due to the addition of nutmeg seeds in making kalio, where nutmeg seeds are not used when making gulai. Myristic acid is the fatty acid with the highest content found in nutmeg seeds, which is around 23% (Saini *et al*., 2021).

***Tempeh Rendang*:** Fatty acid analysis in tempeh was carried out on rendang tempeh and rendang paste respectively. Based on the results of fatty acid analysis in tempeh rendang, the detected compound components did not change much. However, there were additional compounds that were not previously detected in tempeh, tempeh gulai, or tempeh kalio. This compound is 10,13-eicosadienoic acid. This compound is the result of elongation of linoleic acid and acts as an anti-inflammatory (Pekkoh *et al*., 2011). The next point is the increase in linoleic acid levels in tempeh rendang. This increase in linoleic acid levels is thought to come from the use of various spices which also contain linoleic acid. Apart from that, ginger, cardamom, lemongrass and cloves used in making rendang also have activity to inhibit the oxidation of linoleic acid.

The compounds obtained from the analysis of fatty acids in tempeh rendang paste are not much different from those of kalio sauce. The highest fatty acid in rendang paste is myristic acid. A comparison of the content and area of fatty acids found in tempe gulai, gulai broth, tempeh kalio, kalio sauce, tempeh rendang, rendang paste can be seen in Table 3.

***Analysis of Daidzein Levels*:** In unprocessed soybeans, daidzein is an isoflavone compound that is not contained in much, because in raw soybeans isoflavones are found in the form of malonyl glycosides (malonyl genistin, malonyl daidzin, and malonyl glycitein) and isoflavone glycosides (daidzin, genistin, and glycitin) (Jung *et al*., 2020). The heating process of soybeans when making tempeh greatly influences the composition of the isoflavones produced. The boiling process produces malonyl glycoside levels which are inversely proportional to isoflavone glycoside levels. The hot temperature processing process reduces malonyl glycoside levels, but these isoflavones do not disappear, they only change form to isoflavone glycosides (Qu *et al*., 2021). When fermentation is carried out, the mold produces the enzyme β-Glucosidase which plays a major role in the hydrolysis process of isoflavone glycosides (Syukri *et al*., 2018). This hydrolysis process changes the form of isoflavone glycosides into isoflavone aglycones (genistein, daidzein, and glycitein). Tempeh contains more isoflavone aglycones because of this fermentation process. This type of isoflavone is more easily absorbed by the body compared to its glycoside form. In tempeh, the highest isoflavone aglycones are daidzein and genistein. In the pharmaceutical sector, daidzein is produced in tablet form with the claim of being able to replace the role of the estrogen hormone that is lost after menopause, thereby reducing the risk of osteopenia. Analysis of daidzein levels in tempeh was carried out on the extraction results of gulai tempeh, kalio tempeh and rendang tempeh using 70% ethanol. The extract was then analyzed using HPLC.

Based on the data from the analysis, the concentration of daidzein contained in gulai, kalio and rendang can be calculated. Sample concentrations can be seen in Table 4.

Table 4. Daidzein content in gulai, kalio, and tempeh.

Processing	Daidzein content (ppm)
Gulai	34.02
Kalio	25.27
Rendang	25.03

Based on the analysis results, it can be seen that the highest daidzein levels are found in gulai, while the lowest daidzein levels are found in rendang. Differences in processing of tempeh can cause different changes in isoflavone levels. Processing tempeh at hot temperatures can reduce the levels of isoflavone aglycones, because these compounds are susceptible to high temperatures. Boiling can cause a decrease in isoflavone aglycone levels by 18.20%, steaming by 13.30%, and frying by 39.15% (Raihanah *et al*., 2023).

Conclusion**:** Based on data from previous research, it can be concluded that the antioxidant activity of gulai, kalio, and tempe rendang was 52.56%, 61.12%, and 74.23%, respectively. The antioxidant activity of tempe gulai is lower than that of kalio and rendang, even though the cooking process is shorter. This is because the amount of spices used differs; melanoidin also influences it due to the Maillard reaction, which has antioxidant activity. The fat content increases with the length of cooking. The composition of the fatty acids that make up each gulai, kalio, and rendang is also different if seen from the fatty acid profile analysis results. Linoleic acid is the highest fatty acid in tempeh, whether unprocessed or gulai tempeh. Meanwhile, the linolenic acid in tempeh can only survive until the gulai stage. In contrast to fat, isoflavones in tempeh decrease with cooking time. This condition happens because isoflavones themselves are susceptible to heat. Likewise, with protein, the longer it is cooked, the more it will decrease.

Authors' contributions**:** Fachrur Rozi: wrote the manuscript and laboratory analysis, Wellyalina: data curator, Meisya Az-zahra Rachmayanti: laboratory analysis, Rini: data analysis, Rahadian Zainul: manuscript review**,** Daimon Syukri: conception the research and supervisor.

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