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Original Research

The Isoflavone Contents of Devon 1 Soybeans during Fermentation and Processing into Soybean-Tempeh Steamed Buns

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Abstract*—The soybean Devon 1 variety is a superior variety developed in Indonesia. The isoflavone content makes this soybean a top choice for a functional food. However, fermenting soybeans into tempeh and processing them into other products can affect the final isoflavone content. The objective of this study was to analyze the total isoflavone and its derivatives content in Devon 1 soybeans, soybean tempeh, soybean tempeh flour, and buns made from tempeh flour. The content of each isoflavone type was determined by high-performance liquid chromatography at λ249 and λ260 nm wavelengths. The buns were made from tempeh flour with variations of tempeh flour substitution of 0, 10, 20, and 30%. The results showed that the fermentation of Devon 1 soybeans and further processing of tempeh significantly altered the isoflavone components and content. The concentrations of daidzin and genistin in tempeh were decreased, while daidzein and genistein were increased. Further processing of tempeh into flour increased all isoflavone levels significantly, which might be caused by the isoflavone transformation during drying and enzymatic activity. Substitution of wheat flour with soybean tempeh flour increased these four isoflavones content of the buns. Therefore, tempeh flour made from Devon 1 soybeans has the potential to be used as a functional food ingredient rich in bioactive compounds that promote health.*

Keywords: *flour, heat, isoflavone aglycones, isoflavone glycoside, soybean tempeh*

Abstrak—Kedelai varietas Devon 1 merupakan varietas unggul yang dikembangkan di Indonesia. Kandungan isoflavonnya menjadikan kedelai ini sebagai pilihan utama untuk pembuatan pangan fungsional. Namun, proses fermentasi kedelai menjadi tempe, serta pengolahan lebih lanjut menjadi berbagai produk turunan dapat memengaruhi kandungan akhir isoflavonnya. Penelitian ini bertujuan untuk menganalisa kandungan total isoflavon dan turunannya pada kedelai Devon 1, tempe kedelai, tepung tempe kedelai, serta bakpao yang dibuat dari tepung tempe. Kandungan masing-masing jenis isoflavon diuji menggunakan kromatografi cair kinerja tinggi pada panjang gelombang λ249 dan λ260 nm. Adapun bakpao dibuat dari tepung tempe dengan variasi substitusi tepung tempe sebesar 0, 10, 20, dan 30%. Hasil penelitian ini menunjukkan bahwa fermentasi kedelai Devon 1 dan pengolahan tempe lebih lanjut secara signifikan mengubah komponen dan kandungan isoflavon. Kandungan daidzin dan genistin dalam tempe menurun, sedangkan kandungan daidzein dan genisteinnya mengalami peningkatan. Pengolahan tempe menjadi tepung lebih lanjut meningkatkan seluruh kandungan isoflavon secara signifikan, yang kemungkinan disebabkan oleh transformasi isoflavon selama proses pengeringan dan aktivitas enzimatis. Substitusi tepung terigu dengan tepung tempe meningkatkan kandungan keempat jenis isoflavon pada bakpao. Dengan demikian, tepung tempe kedelai Devon 1 berpotensi digunakan sebagai bahan pangan fungsional yang kaya akan senyawa bioaktif untuk menunjang kesehatan.

Kata kunci: *aglikon isoflavon, glikosida isoflavon, pemanasan, tempe kedelai, tepung*

INTRODUCTION

The Devon 1 soybean variety is one of the superior varieties developed in Indonesia through soybean breeding. This plant is superior to other local varieties. It is a commodity plant with high economic value due to its many advantages over other varieties. For example, its productivity is higher than that of most local soybeans (1), and it adapts fairly well to various agricultural environments. It also has good resistance to pests and diseases (2). Nutritionally, this variety stands out due to its high isoflavone content (3) of 221.97 mg/100 g (2) and genistein content of 13,081 µg/g, the third highest after the Anjasmoro (15,996 µg/g) and Argomulyo (14,175 µg/g) varieties (4).

Processing soybeans into tempeh can alter the nutritional composition of the final product. The tempeh fermentation process primarily involves a species of fungus known as *Rhizopus* sp. This process breaks down complex soybean compounds into simpler forms, thereby reducing the levels of antinutritional substances and increasing the bioavailability of isoflavones (5). The fermentation of soybean tempeh can affect the concentration of isoflavones and the bioconversion of isoflavone glycosides into aglycones, which is mediated by the activity of the β-glucosidase enzyme (6). During tempe fermentation, the content of genistin and daidzin decreased, while the content of genistein and daidzein increased (6). In soybean meal fermentation using *Saccharomyces cerevisiae*, aglycone isoflavone content increased by 5.5-7.8-fold (7). The isoflavones genistein and daidzein, along with their glycoside forms, genistin and daidzin, are the main types of isoflavones found in soybeans. Isoflavone aglycones are more easily absorbed by the body and are more biologically active than isoflavone glycosides (8). These compounds have been shown to have various health benefits, such as anticancer (9,10), antioxidant, and anti-inflammatory activities (11,12). Moreover, isoflavones are classified as phytoestrogens that have been studied as bioactive compounds in functional foods due to their potential to reduce the risk of chronic diseases such as cardiovascular disorders, osteoporosis, and hormone-related cancers (13). However, the content of each type of isoflavone in Devon 1 soybeans and tempeh before and after fermentation has not yet been determined.

From a functional food perspective, converting tempeh into flour enhances its stability and expands its application in various formulations such as bakery products, snacks, and beverages, thereby improving their nutritional and health value. This approach also overcomes the limitation of fresh tempeh, which has a short shelf life due to its high moisture content (55–65%), by reducing water levels, extending storage stability, and increasing versatility for further processing into value-added products (14). Flour of soybean tempeh has a longer shelf life and retains significant amounts of isoflavones, such as genistein (367.9 μg/g) and daidzein (291.6 μg/g) (15). In this study, soybeans were fermented into tempeh. The tempeh was then dried and crushed into flour. The flour was used as a raw material for making steamed buns. Some of these processing stages have potential effects on isoflavone content, especially the steaming and fermentation processes.

Processing soybeans into tempeh flour and other processed products can affect isoflavone levels. Another study proved that isoflavone composition changes with processing at high temperatures, such as steaming, presumably due to degradation (16). Additionally, steaming soybean tempeh for 10 to 30 minutes has been shown to decrease isoflavone content, though not significantly (17).

This study aims to analyze the isoflavone contents and its derivatives of the Devon 1 soybeans, tempeh, soybean tempeh flour, and buns made from soybean tempeh flour. By focusing on functional food development, this research provides evidence for the use of tempeh flour as an innovative ingredient that can contribute to healthier dietary patterns and support the prevention of chronic diseases.

METHOD

This research was conducted experimentally at the Purification and Molecular Biology Laboratory, as well as the Microorganism Biotechnology Laboratory of the University of Surabaya, from September to December 2022, using a Completely Randomized Design (CRD) with a single factor, namely the percentage of soybean tempeh flour substituting wheat flour (0%, 10%, 20%, and 30%).

The Soybean Tempeh Fermentation Process and the Production of Tempeh Flour

The fermentation process was carried out as follows: First, 100 grams of Devon 1 soybeans from Balitkabi in Malang, Indonesia, were washed to remove impurities. The soybeans were then soaked in water for nine hours, and the seed coat was peeled off. The uncoated seeds were steamed for 15 minutes. After steaming, tempeh yeast (Raprima) was added to equal 2% of the total soybean mass. The inoculated seeds were packed in plastic, which was then perforated using toothpicks, and incubated for two days at 25°C until tempeh was formed. The tempeh was subsequently processed into tempeh flour, for which the tempeh was cut into 0.5

cm pieces. The tempeh pieces were dried in a food dehydrator at 60°C for eight hours. After drying, the tempeh was pulverized in a blender and sifted through a 70-mesh sieve.

Preparation of Tempeh Buns

The bun filling and skin both were prepared from tempeh. The filling for the buns was prepared as follows: 75 g of fresh soybean tempeh was steamed for 25 minutes and then mashed using a blender. This paste was then mixed with 35 g of sugar (Gulaku), 20 mL of water, 15 mL of cooking oil (Sunco), 0.3 g of salt (Cap Kapal), and one teaspoon of vanilla paste (Koepoe Koepoe). The filling paste was cooked over low heat for five minutes until it was solidified, moldable, and not sticky. The dough used for the buns was composed of the components listed in Table 1. Soybean tempeh flour was incorporated into the mixture as a substitute for wheat flour, with concentrations of 0%, 10%, 20%, and 30%. The process of making bun dough was done by mixing wheat flour (Kunci Biru, Bogasari), tempeh flour, and baker's yeast (Fermipan) in a mixing bowl. After that, water and sugar are added gradually while stirring until evenly distributed. Margarine (Filma) and salt are then mixed into the dough, then the dough is kneaded until homogeneous and not sticky. A total of 20 grams of dough is then shaped round and flattened with a thicker center to prevent the dough from breaking when given the filling. Next, 20 grams of bun filling is put into the dough, then the dough is rounded again. The dough underwent a proofing process that lasted for 30 minutes. Following this, the filled buns were steamed for 12 minutes.

Table 1
Steamed buns formulation

Material	Unit	Treatment			
		F_0 (100:0)	F_1 (90:10)	F_2 (80:20)	F_3 (70:30)
Wheat flour	(g)	50	45	40	35
Soybean tempeh flour	(g)	0	5	10	15
Baker's Yeast	(g)	1.2	1.2	1.2	1.2
Sugar	(g)	6	6	6	6
Water	(mL)	30	30	30	30
Margarine	(g)	5	5	5	5
Salt	(g)	0.6	0.6	0.6	0.6
Total	(g)	92.8	92.8	92.8	92.8

Isoflavone Content Assay

The extraction of isoflavones was carried out in accordance to the following protocol: A total of 1 gram of the sample was mixed with 25 mL of 80% methanol (p.a. grade Merck) in an Erlenmeyer flask. Then, the mixture was homogenized in an ultrasonic bath for 15 minutes. The suspension was then centrifuged at 11,000 rpm for 10 minutes. The supernatant was transferred into a vial and stored at -18°C for further analysis.

HPLC separation and analysis were performed on a C18 column (Agilent RP ODS C18, Germany). Samples were injected into the HPLC device (Agilent ChemStation) as 25 uL. The mobile phase was acetonitrile (HPLC grade, Mallinckrodt) and acetic acid (Merck) 0.1%, with the composition ratio as presented in Table 2. The flow rate was set at 1 mL/min. Detection of compounds was performed with UV at wavelengths of 249 nm and 260 nm. The compounds genistein, genistin, daidzein, and daidzin (Adooq Bioscience, California) were used as standards. The data obtained were analyzed using statistical tests, namely one-way ANOVA followed by Tukey HSD test, using SPSS software with a significance level of 5%.

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Table 2
Mobile phase composition

Mobile Phase Composition (%)		Time (min)
Acetonitrile	Acetic acid 0.1%	
20	80	0-4
25	75	4-8
30	70	8-12
35	65	12-16
40	60	16-22

RESULT

The analysis showed that the daidzin and genistin contents in soybean seeds were 53.58 ppm and 54.09 ppm, respectively, while daidzein and genistein were not detected. After fermentation into soybean tempeh, daidzin and genistin levels decreased significantly, by 82.4% and 74.5%, respectively. In contrast, daidzein and genistein levels were increased by 6.97 ppm and 7.17 ppm, respectively. These results indicate the conversion from the glycoside to aglycone form. Further processing into soybean tempeh flour led to an increase in daidzin and genistin levels by 173.5% and 415.9%, respectively. Similarly, daidzein and genistein levels increased by 67.3% and 70.5%, respectively. Meanwhile, isoflavones were not detected in the wheat flour used in the bun formulation (Figure 1, Table 3).

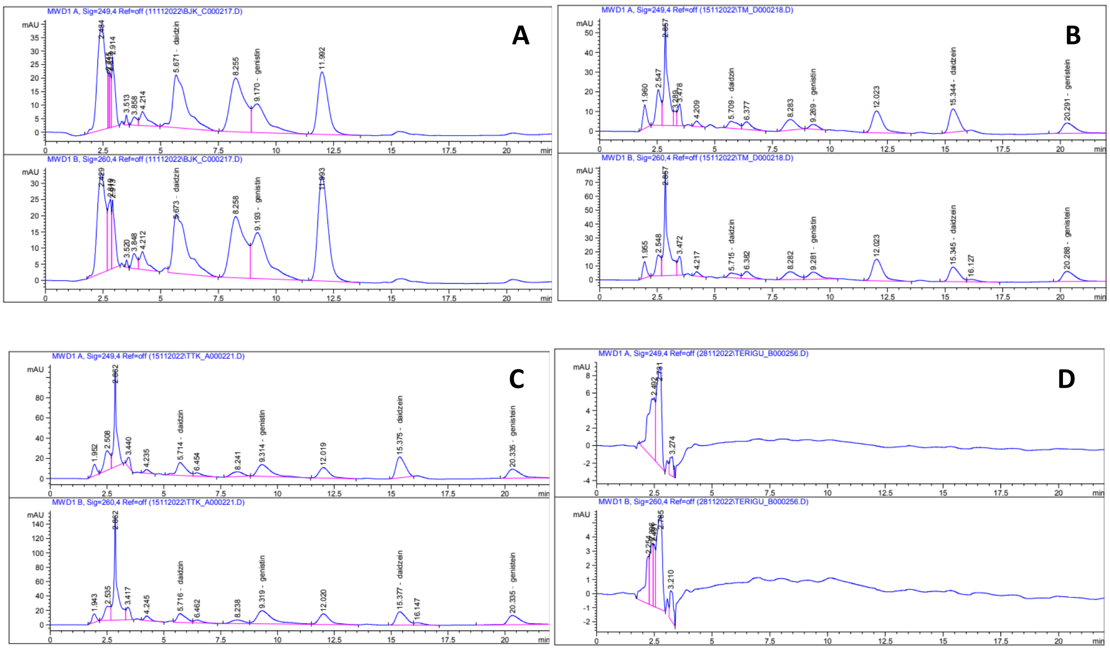
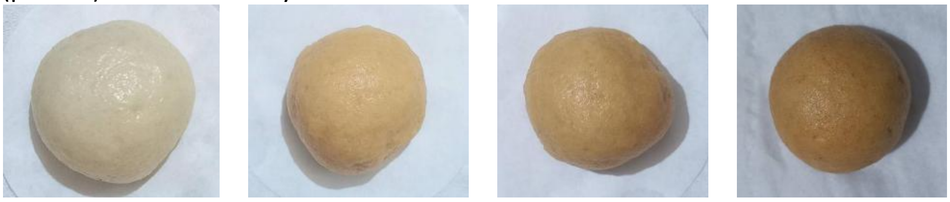


Figure 1. HPLC chromatogram of soybean seeds (A), soyben tempeh (B), soybean tempeh flour (C), and wheat flour (D).

Table 3
Isoflavone content of soybean seeds, soybean tempeh, soybean tempeh flour, and wheat flour

Types of Isoflavone	Isoflavone Content (ppm)			
	Soybean Seeds	Soybean Tempeh	Soybean Tempeh Flour	Wheat Flour
Daidzin	53.58±0.246	9.41±0.114	25.73±0.010	0.00±0.000
Daidzein	0.00±0.000	6.97±0.065	11.66±0.610	0.00±0.000
Genistin	54.09±0.235	13.79±2.291	71.14±0.084	0.00±0.000
Genistein	0.00±0.000	7.17±0.175	12.23±0.272	0.00±0.000
Total	107.67±0.480[b]	37.33±2.417[c]	120.76±0.788[a]	0.00±0.000[d]

Note: Values in the same row and followed by the different annotation are significantly different (p<0.05) based on Tukey HSD test



F_0 F_1 F_2 F_3

Figure 2. Visualization of tempeh buns.

The higher the percentage of soybean tempeh flour substituted for wheat flour in bun production, the higher the levels of daidzein, genistin, and genistein ($p<0.05$). Daidzin was not detected in buns without tempeh flour (F_0) and was first detected at a 30% substitution rate (F_3). Buns with a 30% substitution rate (F_3) showed the highest total isoflavone content (Table 4). These results indicate that substituting wheat flour with soybean tempeh flour directly increases the bun's isoflavone content. Therefore, this flour has the potential to be developed into a functional food.

Table 4

Isoflavone Content of Tempeh Buns

Types of Isoflavone	Isoflavone Content (ppm)			
	F_0	F_1	F_2	F_3
Daidzin	ND	ND	ND	6.44±0.226
Daidzein	2.82±0.097	3.18±0.000	3.76±0.049	4.03±0.301
Genistin	ND	7.60±0.092	10.55±0.067	12.75±0.642
Genistein	3.10±0.004	3.51±0.013	4.13±0.110	4.45±0.253
Total	5.91±0.101^{d}	14.29±0.105^{c}	18.44±0.092^{b}	27.66±1.404^{a}

Note: Values in the same row and followed by the different annotation are significantly different (p<0.05) based on Tukey HSD test. ND = not detected

DISCUSSION

The fermentation process of soybeans into tempeh significantly alters the isoflavone composition. It appears that the glycoside form may undergo bioconversion into aglycones. During fermentation, the glycoside bonds of daidzin and genistin are hydrolyzed by the enzyme β-glucosidase into daidzein and genistein, respectively (8). However, further bioconversion can occur during fermentation, whereby aglycone isoflavones can be converted into other compounds such as factor-2, which is found only in soybean tempeh (18). Factor-2 is formed through the hydroxylation of daidzein's C6 group, while genistein can undergo enzymatic dehydroxylation to form daidzein. Daidzein is then converted to factor-2 via enzymatic hydroxylation (19). These findings demonstrate that tempeh has a lower total isoflavone content than soybeans, but a higher concentration of aglycone isoflavones.

The isoflavone content could still be detected in flour and buns. Heating is one of the factors that influence the transformation of isoflavones in soy-derived products. Compared to soybean seeds, soybean tempeh flour showed significantly higher levels of all isoflavone types. Isoflavones are relatively stable at temperatures up to 100°C (17). The increase in isoflavone content during the tempeh drying at high temperatures is thought to be due to changes in the chemical structure of isoflavones (20). Isoflavones undergo bioconversion from malonylglycosides into glycosides, acetylglycosides, and aglycones via decarboxylation and deesterification mechanisms (21,22). These transformations contribute to the increase in total isoflavone levels, especially the aglycone form, in soybean tempeh flour.

The aglycone form has higher bioavailability than the glycoside form due to its lipophilic nature, making it easier to pass through cell membranes and be absorbed by villi in the small intestine (23). The stability of isoflavones in tempeh flour to heat is another advantage of using tempeh for derivative products. In addition, the antioxidant activity of soybean tempeh and tempeh flour further supports their role as functional food ingredients. According to the research of Wahjudi *et al.* (24), the scavenging activity of soybean tempeh reached 88.4%, while tempeh flour showed 80.7%, indicating that both retained strong antioxidant potential even after processing. Therefore, soybean tempeh flour is not only a promising source of isoflavones but also a functional ingredient with considerable antioxidant properties. Formulating food products with tempeh flour, can serve as an effective strategy to improve isoflavone intake and antioxidant status in the diet, thereby contributing to the prevention of degenerative and chronic diseases (25).

CONCLUSION

The fermentation and processing of tempeh products from Devon 1 soybeans significantly altered the isoflavone components and content. The content of aglycone and total isoflavone increased in tempeh flour, while the daidzin content decreased. Substitution of wheat flour with soybean tempeh flour increased the isoflavone content of the buns. Therefore, tempeh flour made from Devon 1 soybeans has the potential to be used as a functional food ingredient rich in bioactive compounds that promote health.

REFERENCES

1. Roswita R, Yohana, Abdullah S. PERTUMBUHAN DAN HASIL TIGA VARIETAS UNGGUL KEDELAI PADA LAHAN SAWAH TADAH HUJAN DI KABUPATEN PASAMAN, SUMATERA BARAT. Jurnal Pembangunan Nagari. 2020;5(2):225–34. Available from: https://ejournal.sumbarprov.go.id/index.php/jpn/article/view/186
2. Yulifianti R, Muzaiyanah S, Utomo JS. Kedelai sebagai Bahan Pangan Kaya Isoflavon. Buletin Palawija. 2018;16(2):84–93.
3. Krisnawati A. Kedelai sebagai Pangan Fungsional Kedelai sebagai Sumber Pangan Fungsional. Iptek Tanaman Pangan. 2017;12(1):57–65.
4. Hasanah SU, Sukrasno S, Hartati R, W DP. Perbandingan Kandungan Genistein Pada Berbagai Varietas Kedelai (Glycine max) Di Indonesia. Jurnal Penelitian Pertanian Tanaman Pangan. 2020;4(2):113–8.
5. Wu JY, Wee S, Ler SG, Henry CJ, Gunaratne J. Unraveling the impact of tempeh fermentation on protein nutrients: An in vitro proteomics and peptidomics approach. Food Chem. 2025;474:143154. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0308814625004042
6. Liu WT, Huang CL, Liu R, Yang TC, Lee CL, Tsao R, et al. Changes in isoflavone profile, antioxidant activity, and phenolic contents in Taiwanese and Canadian soybeans during tempeh processing. LWT. 2023;186:115207. Available from: https://www.sciencedirect.com/science/article/pii/S0023643823007867
7. Silva F de O, Miranda TG, Justo T, Frasão B da S, Conte-Junior CA, Monteiro M, et al. Soybean meal and fermented soybean meal as functional ingredients for the production of low-carb, high-protein, high-fiber and high isoflavones biscuits. LWT. 2018;90:224–31. Available from: https://www.sciencedirect.com/science/article/pii/S0023643817309295
8. Borges CWC, Carrão-Panizzi MC, Mandarino JMG, Silva JB da, Benedetti S, Ida EI. Contents and bioconversion of β-glycoside isoflavones to aglycones in the processing conditions of soybean tempeh. Pesqui Agropecu Bras. 2016;51(3):271–9. Available from: https://www.scielo.br/j/pab/a/7hCd8zKjZGZ57sqpGxfTZCf/
9. MIZUSHINA Y, SHIOMI K, KURIYAMA I, TAKAHASHI Y, YOSHIDA H. Inhibitory effects of a major soy isoflavone, genistein, on human DNA topoisomerase II activity and cancer cell

proliferation. Int J Oncol. 2013;43(4):1117–24. Available from: https://www.spandidos-publications.com/ijo/43/4/1117
10. Spagnuolo C, Russo GL, Orhan IE, Habtemariam S, Daglia M, Sureda A, et al. Genistein and Cancer: Current Status, Challenges, and Future Directions. Advances in Nutrition. 2015;6(4):408–19. Available from: https://www.sciencedirect.com/science/article/pii/S2161831322009012?via%3Dihub
11. Jheng H, Hayashi K, Matsumura Y, Kawada T, Seno S, Matsuda H, et al. Anti-Inflammatory and Antioxidative Properties of Isoflavones Provide Renal Protective Effects Distinct from Those of Dietary Soy Proteins against Diabetic Nephropathy. Mol Nutr Food Res. 2020;64(10). Available from: https://onlinelibrary.wiley.com/doi/10.1002/mnfr.202000015
12. Peng Y, Shi Y, Zhang H, Mine Y, Tsao R. Anti-inflammatory and anti-oxidative activities of daidzein and its sulfonic acid ester derivatives. J Funct Foods. 2017;35:635–40. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1756464617303444
13. Gómez-Zorita S, González-Arceo M, Fernández-Quintela A, Eseberri I, Trepiana J, Portillo MP. Scientific Evidence Supporting the Beneficial Effects of Isoflavones on Human Health. Nutrients. 2020;12(12):3853.
14. Mursyid, Astawan M, Muchtadi D, Wresdiyati T, Widowati S, Harnina Bintari S, et al. Evaluasi Nilai Gizi Protein Tepung Tempe yang Terbuat dari Varietas Kedelai Impor dan Lokal. PANGAN. 2014;23(1):33–42. Available from: https://www.jurnalpangan.com/index.php/pangan/article/view/48
15. Puspitasari A, Astawan M, Wresdiyati T. Pengaruh Germinasi Kedelai terhadap Komposisi Proksimat dan Komponen Bioaktif IsoflavonTempe Segar dan Semangit. PANGAN. 2020;29(1):35–44. Available from: https://www.jurnalpangan.com/index.php/pangan/article/view/460
16. Qu S, Kwon SJ, Duan S, Lim YJ, Eom SH. Isoflavone Changes in Immature and Mature Soybeans by Thermal Processing. Molecules. 2021;26(24):7471. Available from: https://www.mdpi.com/1420-3049/26/24/7471
17. Surya R, Romulo A. Steaming Process Does Not Affect The Antioxidant Activities of Tempeh Ethanol Extract. J Phys Conf Ser. 2020;1655(1):012023. Available from: https://iopscience.iop.org/article/10.1088/1742-6596/1655/1/012023
18. Istiani Y, Handajani S, Pangastuti A. Karakterisasi senyawa bioaktif isoflavon dan uji aktivitas antioksidan dari ekstrak etanol tempe berbahan baku koro pedang (Canavalia ensiformis). Biofarmasi. 2015;13(2):50–8. Available from: https://smujo.id/jnpb/article/download/2205/2062
19. Suharto KF, Soetjipto H, Martono Y. THE EFFECT OF TEMPE FERMENTATION TIME ON THE TOTAL PHENOLIC AND ISOFLAVONE GENISTEIN CONTENTS. ALCHEMY Jurnal Penelitian Kimia. 2017;13(2):228. Available from: https://jurnal.uns.ac.id/alchemy/article/view/5094
20. Zaheer K, Humayoun Akhtar M. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Crit Rev Food Sci Nutr. 2017;57(6):1280–93. Available from: https://www.tandfonline.com/doi/full/10.1080/10408398.2014.989958
21. An JH, Ko MJ, Chung MS. Thermal conversion kinetics and solubility of soy isoflavones in subcritical water extraction. Food Chem. 2023;424:136430. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0308814623010488
22. Xu B, Chang SKC. Total Phenolics, Phenolic Acids, Isoflavones, and Anthocyanins and Antioxidant Properties of Yellow and Black Soybeans As Affected by Thermal Processing. J Agric Food Chem. 2008;56(16):7165–75. Available from: https://pubs.acs.org/doi/10.1021/jf8012234
23. Hsiao YH, Ho CT, Pan MH. Bioavailability and health benefits of major isoflavone aglycones and their metabolites. J Funct Foods. 2020;74:104164. Available from: https://www.sciencedirect.com/science/article/pii/S1756464620303881

24. WAHJUDI M, TJAHYADI AL, ERAWATI CM, SUKWEENADHI J. Flour made from cacao-soybean tempeh fermented using soybean tempeh inoculum. Food Science and Technology. 2023;43.
25. Kim IS. Current Perspectives on the Beneficial Effects of Soybean Isoflavones and Their Metabolites for Humans. Antioxidants. 2021;10(7):1064. Available from: https://www.mdpi.com/2076-3921/10/7/1064