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Proximate Composition, Total Phenolic Content, and Sensory Analysis of Rice Bran Tempeh

Laras Cempaka[1], Naila Eliza[1], Ardiansyah[1], Dody D. Handoko[2], and Rizki Maryam Astuti[1]

1. Department of Food Science and Technology, Universitas Bakrie, Kawasan Epicentrum, Jakarta 12920, Indonesia
2. Indonesian Centre for Rice Research, Sukamandi, Subang 41256, Indonesia

E-mail: laras.cempaka@bakrie.ac.id

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Abstract

Tempeh is a fermented food prepared using the raw materials of soybean fermented by Rhizopus sp. This traditional food is fairly highly consumed in Indonesia. Soybean, which is the primary raw material used in the processing of tempeh, is still being imported due to its insufficient production in Indonesia. Rice bran can be used as a substitute for soybeans in tempeh processing to add to its (health) benefit. Rice bran has good nutritional value as it contains a high total content of phenolic compounds, dietary fiber, fat, and the amino acid lysine. Thus, rice bran provides an opportunity to use it as a product with functional properties. This study was conducted to determine the proximate characteristics, the total phenolic content (TPC), and the sensory properties of tempeh with the addition of rice bran, chitosan, and glucono delta-lactone (GDL). The addition of chitosan was used as a source of prebiotic, and GDL was required to accelerate the fermentation process. The chemical characteristics (protein, fat, and water content), the TPC, and the sensory properties (color, aroma, texture, and overall acceptability) of rice bran tempeh were analyzed. Results showed that the tempeh sample with the addition of 20% (w/w) rice bran showed the best sensory analysis. It consisted of 57.23% of water content, 37.42% of protein content, 19.72% of fat content, and 83.98 mg GAE/100 g of TPC.

Abstrak

Komposisi Proksimat, Kandungan Total Fenolik dan Analisis Sensori Tempe Bekatul. Tempe adalah makanan fermentasi yang terbuat dari kacang kedelai dengan bantuan kapang Rhizopus sp. Panganan tradisional ini merupakan makanan yang cukup tinggi dikonsumsi di Indonesia. Kedelai yang merupakan bahan baku utama dalam pengolahan tempe masih harus diimpor ketersediaannya, karena produksi kedelai yang belum mencukupi. Bekatul dapat digunakan sebagai pengganti kedelai dalam pengolahan tempe untuk menambah manfaat kesehatannya. Bekatul diketahui memiliki kandungan total senyawa fenolik yang tinggi, serat pangan, lemak, asam amino lisin. Penelitian ini bertujuan untuk mengetahui karakteristik proksimat, kandungan fenolik total (TPC), dan sifat sensori tempe dengan penambahan bekatul, kitosan, dan glucono delta-lactone (GDL). Penambahan kitosan digunakan sebagai sumber prebiotik dan GDL diperlukan untuk mempercepat proses fermentasi. Karakteristik kimia (protein, lemak dan kadar air), kandungan fenolik total, dan sifat sensori (warna, aroma, tekstur dan keseluruhan) tempe bekatul telah dianalisis. Hasil penelitian menunjukkan bahwa tempe dengan penambahan bekatul 20% (b/b) memiliki analisis sensoris terbaik memiliki kadar air 57,23%, kadar protein 37,42%, kadar lemak 19,72% dan kadar fenolik total 83,98 mg GAE/100 g.

Keywords: chitosan, glucono delta-lactone (GDL), rice bran, tempeh, total phenolic content

Introduction

Tempeh is a traditional fermented food of Indonesia that has been recognized globally and is prepared by the fermentation of soybean with a culture starter. The consumption of tempeh in Indonesia has been reported to be about 1.64 million tons/year, despite the availability of soybeans being 963,183 tons/year [1, 2]. Due to its good nutritional value, tempeh is used as a daily diet and a meat substitute. In addition, the presence of phenolic compounds in tempeh may help improve its functional properties. Currently, soybeans are being imported from other countries due to the insufficient production of soybean in Indonesia. The dependence on imported soybean can lead to higher cost of raw materials, which could affect the production of tempeh. Therefore, it is extremely important to identify potential alternative raw materials that can be used as substrate. For better food security, it is necessary to diversify the raw materials for tempeh, e.g., using rice bran. Rice bran is a by-product of rice milling, which is known to possess bioactive food components that are beneficial to health. Rice bran has good nutritional value as it contains a high total content of phenolic compounds, dietary fiber, fat, and the amino acid lysine, which are useful for the human body [3]. Thus, rice bran provides an opportunity to be used as a product possessing good functional properties. In this study, there is not only the addition of rice bran but also the addition of chitosan as fortification. The addition of rice bran and chitosan to tempeh results in new functional properties; it serves as a prebiotic with antihypercholesterolemic properties. The mechanism underlying this property is related to the role of chitosan as a bioadsorbent binding to macromolecules such as fat Prebiotics are nondigestible food ingredients that could provide beneficial effects to the host. They can stimulate the growth of colonic bacteria by increasing the number and activity of probiotics. This study was conducted to investigate the chemical characteristics, the total phenolic content (TPC), and the organoleptic properties of rice bran tempeh. Proximate analysis was used to compare the chemical characteristics of rice bran tempeh, while TPC analysis was conducted to determine the differences in the phenolic content of tempeh after the addition of rice bran. Sensory analysis was used to assess the level of preferences of rice bran tempeh among the consumers.

Materials and Methods

Soybean was obtained from the Sukamandi traditional market. Yeast tempeh Raprima was produced commercially by PT. Aneka Fermentasi Industri (AFI) in Bandung, Indonesia. The fungus *Rhizopus oligosporus* was the mold type present in the starter culture of Raprima. Rice bran was obtained from PT. Indo Beras Unggul- TPS Food. Chitosan from shrimp shells was obtained from the Faculty of Fisheries and Marine Science, Bogor Agricultural Institute.

Rice Bran Stabilization. Rice bran stabilization was conducted by the extrusion method using a twin screw extruder [4]. The temperature of the beginning, middle, and the end of the extruder was set at 120 °C. The parameters used for the stabilization of the combination of rice bran were a screw speed of 12 Hz and a feed rate of 10 Hz. Rice bran coming out of the extruder was placed in a dry container and sieved with a sieve size of 40 mesh. Then, the rice bran was stored in plastic containers.

Preparation of Tempeh. Tempeh was prepared according to the traditional method. The soybean was soaked in distilled water (1:7 w/v) for 24 h [5]. The dehulled beans were washed a few times and boiled in water (1:6 w/v) for 30 min at 100 °C, drained, and cooled to room temperature. Then, 2% chitosan solution (w/v) was added [6] and drained. Next, rice bran was added according to the formulation and stirred until evenly mixed. Cooked beans (0.1 g) were inoculated into the starter culture and packed in perforated polyethylene bags. *Rhizopus oligosporus* was the mold type that was present in the starter culture of Raprima [7]. The inoculated beans were aerobically incubated at room temperature (30 °C) for 48 h.

Chemical Analysis. The proximate analysis of rice bran tempeh was performed using the standard methods of the Association of Analytical Communitie [8]. The protein content was analyzed using the Kjeldahl method (AOAC method 991.20). A protein conversion factor of 5.71 was used to calculate the percentage of protein from nitrogen determined by the Kjeldahl method. Fat content was determined by ether extraction using the AOAC method 989.05. The moisture content was determined in a hot air oven using the AOAC method 925.45.

Determination of TPC. The TPC of the extracts was determined using the Folin–Ciocalteu method [9]. First, 10 g of tempeh was cut into small pieces, 20 mL of hot water was added, and then it was crushed using a mortar and pestle until it became smooth and homogeneous. Then, the sample was heated and filtered with a filter cloth. An aliquot of 80 µl was mixed with 200 µl of 0.25 N Folin–Ciocalteu phenol reagent. After 3 min, 1 mL of saturated Na_2CO_3 solution was added, and the sample was allowed to stand for 2 h before reading the absorbance of the reaction mixture at 725 nm. All samples were analyzed in triplicates, and the average of the results was calculated. The TPC of the rice bran tempeh extract was calculated using a gallic acid calibration curve (five different concentrations within the range of 1.7–3 mM, $R^2 = 0.98$) and expressed as milligram of gallic acid equivalent per gram of fresh weight (mg GAE/100 g).

Sensory Evaluation. Sensory evaluation was conducted using 30 untrained panelists. Each panelist was given three different samples, i.e., (a) rice bran tempeh A, (b) rice bran tempeh B, and (c) rice bran tempeh C, along with mineral water to rinse their mouth. This sensory evaluation test was carried out according to the 9-point hedonic scale [10], where the points ranged from "dislike very much" to "like very much." The scores and preferences are shown in Table 1. The panelists were asked to taste the sample and give the score accordingly. Five sensory attributes were selected for evaluation, which were color, aroma, taste, texture, and overall acceptability.

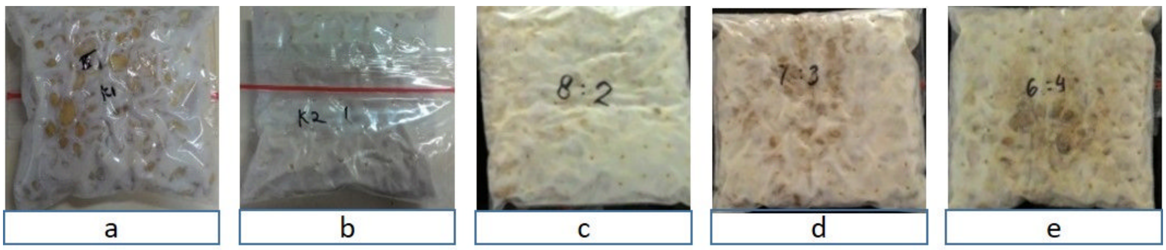
Statistical Analysis. The results of the chemical characteristics of rice bran tempeh are the average of three independent (triplicate) experiments (batches) and triplicates of analysis. The results of rice bran tempeh samples that were also obtained from triplicate independent experiments were statistically analyzed using ANOVA, and the significant differences, if present, were evaluated using Duncan's multiple range test (DMRT). In the sensory analysis, the overall acceptability was tested using XLSTAT.

Table 1. Scores and Preferences of the 9-point Hedonic Scale

Score	Preferences
1	Dislike Very Much
2	Dislike Much
3	Dislike Moderately
4	Slightly Dislike
5	Neither Like Nor Dislike
6	Slightly Like
7	Like Moderately
8	Like Much
9	Like Very Much

Results and Discussion

As shown in Figure 1, rice bran tempeh B and C samples are not covered by intact mycelia when compared with rice bran tempeh A. During the fermentation process, the enzymatic digestion of substrates leads to an increase in the amount of free amino acids, water-soluble nitrogen compounds, and free fatty acids and to the development of a characteristic flavor. A large range of water-soluble high-molecular-weight oligosaccharides are liberated by the enzymatic degradation of polysaccharides. The major carbohydrases of *R. oligosporus* in tempeh include poly-galacturonase, endocellulase, xylanase, and arabinose, and during enzymatic maceration, the arabinogalactan and pectin fractions of soybean are predominantly solubilized. Initially, the starter cultures were grown on a simple medium. This is because when *Rhizopus* sp. is grown on a more complex substrate, it takes a longer time to digest the substrate due to the process of enzymatic activation to break up the substrate. This confirms that the addition of rice bran causes the fungi to take a longer time to digest the substrate.



* Tempeh = only soybean,
Tempeh with chitosan = soybean + 2% chitosan (w/v),
Rice bran tempeh A = soybean:rice bran = (8:2) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh B = soybean:rice bran (7:3) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh C = soybean:rice bran (6:4) + 2% chitosan (w/v) + 0.4% GDL (w/w).

Figure 1. Tempeh after Fermentation (a. Tempeh, b. Tempeh with Chitosan, c. Rice Bran Tempeh A, d. Rice Bran Tempeh B, and e. Rice Bran Tempeh C)

Table 2. Chemical Composition of Rice Bran Tempeh

Sample	Moisture (%)	Protein (%)	Fat (%)
Tempeh	60.61 ± 0.33^{bc}	46.75 ± 0.54^{c}	17.69 ± 1.30^{a}
Tempeh with chitosan	64.04 ± 0.80^{c}	44.31 ± 1.81^{c}	20.37 ± 2.87^{a}
Rice bran tempeh A	57.23 ± 5.98^{abc}	37.42 ± 1.81^{b}	19.72 ± 2.58^{a}
Rice bran tempeh B	53.87 ± 4.43^{ab}	34.06 ± 0.92^{a}	16.64 ± 2.09^{a}
Rice bran tempeh C	50.57 ± 4.71^{a}	32.23 ± 2.00^{a}	17.15 ± 2.21^{a}

*The numbers in the same column followed by different letters show significant differences ($p < 0.05$).
Tempeh = only soybean,
Tempeh with chitosan = soybean + 2% chitosan (w/v),
Rice bran tempeh A = soybean:rice bran = (8:2) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh B = soybean:rice bran (7:3) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh C = soybean:rice bran (6:4) + 2% chitosan (w/v) + 0.4% GDL (w/w).

Determination of Chemical Composition. The chemical characteristics of the rice bran tempeh samples are shown in Table 2. The addition of rice bran had a significant effect on the moisture content of tempeh (the different letters in Table 2 show significant differences). Tempeh with the addition of rice bran has lower moisture content than that of soybean tempeh. The fermentation of rice bran may cause a decrease in moisture levels associated with loss of dry matter in the rice bran [11].

Dry matter loss occurs because of the conversion of the material by mold activity for growth [12]. Dry matter overhauled will be converted into energy and other molecules (CO_2 and H_2O) and then gets evaporated [11, 13]. Fermentation is known to increase the levels of amino acids in soybean. The increase in the amino acid content in soybean is due to the hydrolysis reaction carried out by *Rhizopus* sp. The increase in the content of essential amino acids in soybean has been reported to be about 85%, whereas that of non-essential amino acids 33% [14]. Rice bran tempeh C showed the lowest protein content because the protein from soybeans was substituted with the portion of rice bran. During the fermentation process, an interaction occurs between some normal bacterial flora and yeasts [15]. Yeast is known to synthesize a protein nitrogen source derived from its substrate. Furthermore, a decrease in the levels of protein in soybean with the addition of rice bran was caused due to a decrease in the proportion of soybean used in the manufacture of tempeh. Therefore, the smaller the proportion of soybeans used in the manufacture of tempeh, the more the decrease in the protein content of the final product. As shown in Table 2, there were no significant differences in the fat content among the three treatments. An earlier study also reported that the addition of brown rice bran had no significant effect on the fat content of tempeh sausage [16]. Therefore, based on the results obtained, it can be concluded that the addition of chitosan and rice bran had no effect on the levels of fat in the soybean produced ($p > 0.05$).

Determination of TPC. Phenolic compounds are one of the most effective antioxidative constituents that contribute to the antioxidant activity and play a role in free radical-scavenging capacities [17]. The TPC of the rice bran tempeh samples was determined by the Folin–Ciocalteu assay, and the results are shown in Table 3.

Statistical analysis of the TPC data of the extracts revealed that there was a significant effect of formulation and method of extraction ($\alpha = 0.05$) on the TPC. As shown in Table 3, the highest TPC was obtained from rice bran tempeh C (110.05 ± 21.98 mg GAE/100 g). A higher composition of rice bran results in a higher amount of TPC.

Sensory Analysis. In this study, five sensory attributes of color, aroma, taste, texture, and overall acceptability were focused among the physical characteristics of rice bran tempeh samples. The level of preference of soybean tempeh ranged from neither like to slightly like. Tempeh with the addition of chitosan was not significantly different compared with tempeh without the addition of chitosan. The level of preference of each attribute of tempeh with the addition of rice bran ranged from dislike much to slightly dislike. This result shows that rice bran tempeh was not favored by the consumers in terms of sensory evaluation. Aroma is one of the important parameters in seasoning. The rice bran tempeh A sample showed the highest level of preference in the sensory evaluation. Tempeh with the addition of rice bran showed a slightly sour aroma. It has been reported that the aroma is caused by the presence of the compound 1-nonanal that contributes to the citrus aroma and the fatty odor in the bran [18]. Regarding taste attributes, there were no significant differences ($p < 0.005$) between all the tempeh samples (Table 4). However, the most preferred flavor was for rice bran tempeh A. As shown in Table 4, the results of color, aroma, taste, and texture attributes indicate that rice bran tempeh A has the highest score. Furthermore, the addition of rice bran to the raw material for preparing tempeh makes the color dark, and the product has a distinctive aroma of rice bran. Only the result of texture attributes was statistically different between rice bran tempeh A and rice bran tempeh B samples.

Table 3. Total Phenolic Content of Rice Bran Tempeh Samples

Sample	Total phenolic content (mg GAE/100 g)
Tempeh	65.88 ± 7.65^{ab}
Tempeh with chitosan	56.93 ± 4.08^{a}
Rice bran tempeh A	83.98 ± 12.58^{bc}
Rice bran tempeh B	96.28 ± 14.66^{cd}
Rice bran tempeh C	110.05 ± 21.98^{d}

*The numbers in the same column followed by different letters show significant differences ($p < 0.05$).
Tempeh = only soybean,
Tempeh with chitosan = soybean + 2% chitosan (w/v),
Rice bran tempeh A = soybean:rice bran = (8:2) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh B = soybean:rice bran (7:3) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh C = soybean:rice bran (6:4) + 2% chitosan (w/v) + 0.4% GDL (w/w).

Table 4. Sensory Characteristics of Rice Bran Tempeh with Various Rice Bran Compositions

Sample	Color	Aroma	Taste	Texture
Tempeh	5.83 ± 0.87[c]	5.70 ± 0.95[c]	6.20 ± 0.71[b]	5.67 ± 0.88[b]
Tempeh with chitosan	5.87 ± 0.82[c]	5.70 ± 0.84[c]	5.87 ± 0.90[b]	5.27 ± 1.20[b]
Rice bran tempeh A	3.40 ± 1.33[b]	3.17 ± 1.32[b]	3.30 ± 1.34[a]	3.97 ± 1.61[a]
Rice bran tempeh B	2.43 ± 0.90[a]	2.50 ± 1.22[a]	2.60 ± 0.97[a]	3.90 ± 1.40[a]
Rice bran tempeh C	2.13 ± 0.94[a]	2.47 ± 1.14[a]	3.10 ± 1.49[a]	3.33 ± 1.58[b]

*The numbers in the same column followed by different letters show significant differences ($p < 0.05$).
Tempeh = only soybean,
Tempeh with chitosan = soybean + 2% chitosan (w/v),
Rice bran tempeh A = soybean:rice bran = (8:2) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh B = soybean:rice bran (7:3) + 2% chitosan (w/v) + 0.4% GDL (w/w),
Rice bran tempeh C = soybean:rice bran (6:4) + 2% chitosan (w/v) + 0.4% GDL (w/w).

Table 5. Proximity Value of Overall Acceptability Attributes with Multidimensional Scaling

	Var1	Var2	Var3	Var4	Var5
Var1	1	0.999	1.000	1.000	1.000
Var2	0.999	1	0.998	0.999	0.999
Var3	1.000	0.998	1	1.000	1.000
Var4	1.000	0.999	1.000	1	1.000
Var5	1.000	0.999	1.000	1.000	1

Var 1 = Tempeh; Var 2 = Tempeh with chitosan; Var 3 = Rice bran tempeh A; Var 4 = Rice bran tempeh B; Var 5 = Rice bran tempeh C

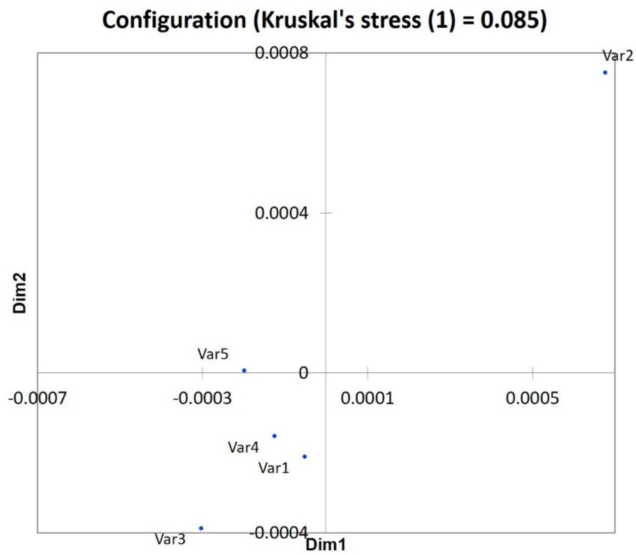


Figure 2. Perceptual Map of Five Variations of Rice Bran Tempeh. Var 1 = Tempeh; Var 2 = Tempeh with Chitosan; Var 3 = Rice bran Tempeh A; Var 4 = Rice Bran Tempeh B; Var 5 = Rice Bran Tempeh C

Figure 2 shows that all rice bran tempeh variations were similar in terms of the overall acceptability in the sensory analysis. It can be observed that all variations are in the same quadrant, except tempeh with the addition of chitosan; however, this difference was not significant because the distance between the samples is very small. These results are also shown in Table 5. The numbers listed in Table 5 show the proximity value approaching 1, implying that there are similarities between all variations.

In general, consumers tend to prefer tempeh without the addition of rice bran and chitosan. However, the addition of rice bran increases not only the TPC but also the fiber content. Fill the fiber every 100 g of rice bran to 7–11 g [18]. High fiber content in rice bran provides an opportunity to be utilized as a fiber-containing product. This can be concluded that by the observation that consumer acceptance of rice bran tempeh A was more significant than that of rice bran tempeh B and C. In contrast to the texture and overall attributes, there were significant differences between rice bran tempeh B and rice bran tempeh C. Rice bran tempeh C received a higher score than rice bran tempeh B, which confirms that it is not always the addition of rice bran that reduces the texture and overall acceptability. Among all the treated samples, rice bran tempeh A showed the highest level of preference, although not significantly different. This result confirms that the tempeh with the addition of 20% rice bran is quite acceptable by the panelists.

Conclusions

The addition of rice bran, chitosan, and GDL was directly proportional to the increase in the TPC, which amounted to 110.05 mg/100 g of the body weight. The addition of rice bran, chitosan, and GDL decreased the moisture content and protein content of soybean, but there was no significant effect on the fat content of rice bran tempeh. The moisture content of rice bran tempeh was in line with the SNI quality score, implying that it fulfills the quality requirement. As the protein from soybeans was substituted with the portion of rice bran, the protein content was decreased in the rice bran tempeh. However, the addition of rice bran increased the TPC, which was due to the fermented rice bran content. Rice bran tempeh A [soybean:rice bran (8:2) + chitosan 2% (w/v) GDL + 0.4% (w/w)] received the highest preference level compared with rice bran tempeh B [soybean:rice bran (7:3) + chitosan 2% (w/v) GDL + 0.4% (w/w)] and rice bran tempeh C [soybean:rice bran (6:4) + chitosan 2% (w/v) GDL + 0.4% (w/w)] in terms of overall acceptability.

Acknowledgments

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