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Development of Functional Beverage with Antioxidant Properties using Germinated Red Rice and Tempeh Powder Mixture

Widya Agustinah*, Joshua Yuandi
Food Technology Study Program, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta 12930, Indonesia

ABSTRACT

The objectives of this study were to develop a functional beverage containing Germinated Red Rice (GRR) and tempeh powders and analyze the total soluble phenolic content, phenolics profile, in vitro antioxidant activity, and sensory evaluation. A mixture of GRR and tempeh powder at 1:2, 1:1, and 2:1 w/w ratios were mixed with water at 6% w/v concentration. The total soluble phenolic content and the antioxidant activity of the samples increased significantly ($p<0.05$) with the increasing level of GRR powder and the decreasing level of tempeh powder, whereas 2:1 w/w ratio of GRR and tempeh powder showed the highest total soluble phenolic content (79.79±12.10 µg/ml GAE) and in vitro antioxidant activity (68.84±1.56%). However, a control beverage containing only GRR powder and only tempeh powder had the highest and lowest total soluble phenolic content and antioxidant activity, respectively. Ferulic acid was detected in all samples containing GRR, while daidzein was not detected and genistein was only detected in 1:2 and 1:1 sample ratios. All formulated samples in lemongrass sugar solution were accepted by the panelists (score 5 out of 7). In conclusion, GRR was responsible to increase the total soluble phenolic content and antioxidant activity of the beverage. A loss of isoflavone in the tempeh-containing beverage samples suggested that optimizing the dose and processing method were important to achieve the optimum health benefits of the ingredients.

Keywords: daidzein, ferulic acid, genistein, HPLC, phenolic compounds

INTRODUCTION

Germination and parboiling of whole grain red rice could modify the texture of whole grain rice resulting in softer rice texture, higher bound phenolics content, and better palatability (Hu *et al.* 2017). The main bound phenolics in red rice were ferulic acid, syringic acid, trans-p-coumaric acid, and quercetin, while the main free phenolics were catechin, protocatechuic, and caffeic acids (Sumczynski *et al.* 2016). During germination, various biochemical and enzymatic reactions occurred which could increase the nutritional value and digestibility, release the bound phytochemicals in the substrate, and induce the biosynthesis of phenolic compounds potentially contributed to many beneficial biological activities, such as scavenging free radicals and protecting against oxidative-stress related diseases (Maksup *et al.* 2018). Germinated Red Rice (GRR) was usually consumed as steamed rice to replace white rice or processed into powder, cereal drink, and baby food.

Tempeh is an Indonesian indigenous food made from soybean fermentation using *Rhizopus spp.* mold (Ahnan-Winarno *et al.* 2021). Tempeh provides higher nutrition and isoflavones aglycone content than soybean due to its biochemical degradation during fermentation process (Astawan *et al.* 2015). Tempeh flour that was processed using steam blanching and oven drying at 60°C for 8 h still exhibited a high content of genistein, daidzein, and a high antioxidant activity (Astawan *et al.* 2020). Tempeh is seldom found in a beverage form. It is proposed that the addition of tempeh, instead of other soy products, in the GRR beverage could enrich not only the protein and vitamin B12, but also the isoflavone content (Ahnan-Winarno *et al.* 2021; Sethi *et al.* 2016).

Dietary polyphenols can be served in a beverage form (Shahidi & Ambigaipalan 2015). A plant-based beverage can be a milk alternative for those who have lactose intolerance, milk allergy, or are vegan. The design of a plant-based beverage, which is based on the cereal and legume mixture, that can provide

*Corresponding Author: tel: +628129555236, email: widya.agustinah@atmajaya.ac.id
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complementary nutrition and various phenolic bioactive compounds is hypothetically able to provide a better delivery of phenolic compounds and enhance. This can be attained through the modification of the phenolic compounds bound to the food matrices. The enzymatic hydrolysis occurred during germination could liberate the ferulic acid and other phenolic acids that were bound to the bran layer of the cereals (Xu *et al.* 2020). The aglycone form of isoflavones were the most bioavailable phenolic compounds in tempeh (Ribas-Agusti *et al.* 2018). Based on the hypothesis above, this study was aimed to develop a functional beverage containing a mixture of GRR and tempeh powder and further analyze the total soluble phenolic content, phenolics profile, in vitro antioxidant activity, and sensory evaluation of the mixed beverage.

METHODS

Design, location, and time

The experimental study used a completely randomized design. The study was conducted in the Laboratory of Food Processing, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, started from September 2018 until June 2019.

Materials and tools

The main ingredients for the beverage, such as organic red rice (*Cap Kenari*, Jakarta, Indonesia), fresh lemongrass, and fresh tempeh (*Karya Abadi*, Tangerang, Indonesia) were purchased from the market at *Bumi Serpong Damai* area, South Tangerang. The reagents used for the chemical analysis were purchased from Merck (Germany) distributor in Jakarta, Indonesia. The standard chemicals for High Performance Liquid Chromatography (HPLC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay were purchased from Sigma-Aldrich (Singapore) distributor in Jakarta, Indonesia. The tools used were drying oven (Memmert, Germany), centrifuge (Eppendorf, Germany), pH meter (Mettler Toledo, USA), UV-Vis spectrophotometer (Thermo Fischer Scientific, USA), and HPLC with a UV-Vis detector (Agilent 1100 Series, USA).

Procedure

Preparation of GRR powder. Red rice (100 g) was rinsed with running tap water, drained, and soaked in sterile distilled water at 1:3 w/v ratio at room temperature (25°C) for 24 h, then drained and rinsed again with sterile distilled water. The rice was placed on a tray layered with a damp tissue paper and covered with a damp clean cloth. The germination process was performed in the dark at room temperature for 48 h following Anawachkul and Jiamyangyuen (2009) method. The covering cloth was sprayed with sterile water every 4 h to maintain humidity. GRR was oven-dried at 60°C for 12 h, then ground into powder using a food processor and sieved at 60 mesh. The GRR powder was stored at room temperature in a plastic zipped bag added with a silica gel sachet and used in the beverage formulation within 2 weeks.

Preparation of tempeh powder. Tempeh powder preparation followed the Bastian *et al.* (2013) method with a modification in drying time. Fresh bought tempe was sliced to 0.5–1 cm thickness, then blanched in hot water at 90°C for 15 min to reduce the beany flavor and inactivate oxidizing enzymes. Tempeh was oven-dried at 70°C for 12 h. Dried tempeh was ground into powder using a food processor and sieved at 60 mesh. The tempeh powder was packed in a plastic zipped bag added with a silica gel sachet and stored at a room temperature for use within 2 weeks.

Beverage formulation. The mixture of GRR and tempeh powder were rehydrated in water to reach 6% w/v powder concentration as having the best solubility and stability in the preliminary study. The formulation used three different ratios of GRR and tempeh powder mix, i.e. 1:2, 1:1, and 2:1 w/w. A plain formulation was used for the chemical analysis and phenolics profiling. A sugar formulated beverage was used for the sensory evaluation by adding 10% v/v lemongrass syrup to help masking the beany flavor of the beverage. As many as 1 kg of table sugars were dissolved in 500 ml of boiling water, then 8 lemongrass stalks were brewed into a sugar solution for 15 min and filtered out to make the lemongrass-infused syrup. Both the plain and sugar added beverage were subjected to sensory evaluation.

Sample preparation and pH analysis. Each formulation was sterilized with an autoclave at 121°C, 1 atm for 15 min. Samples of each formulation were centrifuged at 4,000×g for 7 min. The supernatant was collected, measured for pH using a pH meter, and stored in a freezer at -20°C prior to analysis.

Analysis of total soluble phenolics content. The total soluble phenolics content was measured using a method described by Agustinah *et al.* (2016). The absorbance of each sample was measured using a UV-Vis spectrophotometer at 725 nm wavelength. The absorbance was converted into total phenolics concentration and expressed in µg gallic acid equivalent per mL sample (µg/ml GAE).

Analysis of in vitro antioxidant activity. The antioxidant activity was measured using DPPH radical scavenging assay (Agustinah *et al.* 2016) with a modification of DPPH concentration at 0.2 mM in 96% ethanol. The absorbance (A) was read at 517 nm wavelength using a UV-Vis spectrophotometer. The antioxidant activity was expressed as % inhibition of DPPH radical formation and calculated using a formula:

$$\%Inhibition = \frac{A\ reaction\ control - A\ sample}{A\ reaction\ control} \times 100\%$$

Phenolics profiling by HPLC. The phenolics profile of each sample was determined using HPLC protocols as described by Agustinah *et al.* (2016) for phenolic acids and Lee *et al.* (2008) for isoflavones. A volume of 2 ml sample was mixed with methanol for ferulic acid profiling or acetonitrile for genistein and daidzein profiling at 2:1 v/v ratio, then centrifuged at 10,000×g for 5 min. The supernatant was diluted with HPLC-grade water at 1:1 v/v ratio, then filtered through a 0.2 µm syringe filter. Twenty microliters of filtered sample were injected into HPLC with a UV-Vis detector. The analytical column used was Zorbax XDB-C18 4.6x150 mm with packing material of 5 µm particle size.

The profiling of ferulic acid was conducted at a flow rate of 1 ml/min for 25 min with a gradient elution consisting of (A) 100% methanol and (B) 10 mM phosphoric acid (pH 2.5). The solvent system (%A/ %B) was run at 8 min (60/40), 7 min (100/0), 3 min (0/100), and 7 min (0/100). The chromatogram was recorded at 225 nm during each run.

The profiling of isoflavones was performed at a flow rate of 0.5 ml/min for 25 min with a gradient elution consisting of (A) 0.1% v/v glacial acetic acid in water and (B) 0.1% v/v glacial acetic acid in acetonitrile. The solvent system (%A/ %B) was run at 0 min (85/15), 5 min (70/30), and 20 min (35/65). The chromatogram was recorded at 254 nm during each run. Pure standards of ferulic acid, genistein, and daidzein were used to calibrate the standard curves and retention times.

Sensory evaluation. An affective test using a 7-scale hedonic score, i.e.: 1 (disliked very much); 2 (disliked); 3 (quite disliked); 4 (neutral); 5 (quite liked); 6 (liked); 7 (liked very much), was conducted to evaluate the acceptance (liking) of the taste, color, aroma, texture, and aftertaste of each beverage sample. There were 30 semi-trained panelists who were selected from the students of Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia and trained once on the specific sensory evaluation method that was used in this study. The acceptance test was done in two rounds, the first round used plain samples and the second round used the formulated beverage with lemongrass-infused syrup.

Data analysis

The experiment was performed in duplicate, except for phenolics profiling and sensory evaluation that were performed once. All analysis was done in triplicate, except for HPLC (phenolics profiling) and sensory evaluation which were done in duplicate. The data were presented in mean±standard deviation, further analysis of mean difference was done using one-way ANOVA and Duncan post-hoc test in Statistical Package for the Social Science (SPSS) software version 24. The confidence level set was 95% and the p-value less than 0.05 was considered as significant.

RESULTS AND DISCUSSION

Total soluble phenolics content and pH

The formulation of GRR and tempeh powder beverage was provided in Table 1. As was shown in Figure 1, the total phenolics content of all 6% w/v mix beverage sample was at the same level which ranged between 63.65±7.81 to 79.79±12.10 µg/ml GAE. Tempeh as a protein-loaded food was reported to contain high amount of isoflavones aglycone, such as genistein and daidzein, with high antioxidant activity (Astawan *et al.* 2020). However, in this study tempeh powder had the lowest total phenolics content. The interaction between protein and phenolic compounds, via non-covalent or covalent processes, in tempeh could affect the detectability and bioavailability of phenolic

Table 1. Formulation of GRR and tempeh mix beverage

Ingredients	1:2 Mix	1:1 Mix	2:1 Mix	GRR	Tempeh
GRR powder (g)	4	6	8	12	0
Tempeh powder (g)	8	6	4	0	12
Water (ml)	188	188	188	188	188
Total (g)	200	200	200	200	200

GRR: Germinated Red Rice

compounds in tempeh (Ahnan-Winarno *et al.* 2021). Such protein-phenolics interaction could reduce protein solubility and digestibility and mask phenolics bioavailability and antioxidant capacity (Zhang *et al.* 2020).

The total phenolics content in a single GRR beverage sample was almost 1.8 fold higher than that in a single tempeh beverage sample. However, the increasing ratio of GRR powder and the decreasing ratio of tempeh powder did not change the total phenolics content in the mix beverage. Moreover, the total phenolics content of the 1:2 mixed sample (with higher tempeh powder ratio) was similar to that of single tempeh beverage sample. It was also shown in 1:1 and 2:1 mix samples, respectively, that by replacing 50% (6 g) and 67% (8 g) of tempeh powder with GRR powder, which contained approximately 0.44–0.59 mg/ml GAE, it could increase the total phenolics content of the mix sample to 1.5 to 1.6-fold as compared to a single tempeh beverage sample or reach the same level of phenolics content as in the single GRR beverage sample. This finding was higher than the study conducted by Sęczyk *et al.* (2017) that exhibited

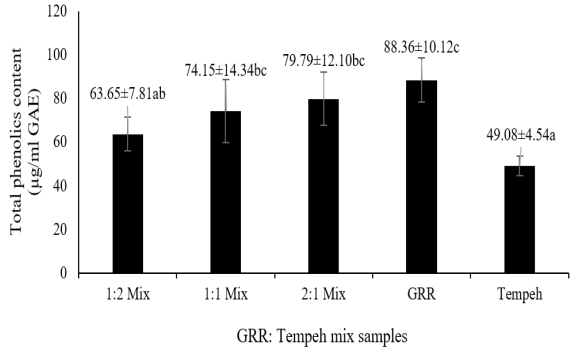

Figure 1. The total soluble phenolics content of beverage sample consisted of GRR and tempeh powder mix. Bars with different letters are significantly different ($p<0.05$)

only 1.2-fold increase of total phenolics content in the soymilk fortified with green coffee extract containing 0.5 mg/ml GAE of total phenolics content. The combination of various phenolics from two different substrates, such as in fruit juices, suggested a possible synergistic interaction which could increase the content and solubility of the phenolic compounds (Agustinah *et al.* 2016).

Based on the calculation from Figure 1, each 1:2, 1:1, and 2:1 mix beverage sample should contain the estimated 62, 69, and 75 µg/ml GAE, respectively. The experimental value was slightly higher than the estimated value of the total phenolics content. This result indicated a potential effect of mixing GRR and tempeh powder in the beverage in increasing the total phenolics content. However, further optimization study was required to increase the ingredients concentration to above 6% and solve the problem of powder solubility and stability in the beverage. The beverage which consisted of dissolved GRR and tempeh powder mix in water showed a mean pH of 6. The pH data was not shown as it was not significant among all samples.

Germination of cereal seeds was one of the non-thermal processing which resulted in the dynamic changes of various types of phenolic compounds (Xu *et al.* 2020). The total phenolics in this study was considered as soluble free phenolic compounds since ethanol was used in the method as a solvent (Agustinah *et al.* 2016). The increase of soluble free phenolic compounds could be resulted from the breakdown of soluble bound phenolic compound from the macromolecule complex in the endosperm through the activation of endogenous hydrolytic enzymes during germination (Xu *et al.* 2020). Additionally, heat treatment on the GRR, such as parboiling (Hu *et al.* 2017) or potentially oven-drying in this study, could also release the bound phenolics from the cell wall which resulted in the increasing of soluble free phenolics content. However, this study did not characterize the

bound phenolic compounds in the mixture; thus, indicating the need of further confirmation study.

Antioxidant activity (DPPH free radical scavenging activity)

Following the pattern of the total soluble phenolics content, the antioxidant activity of the mix beverage was also improved by the increasing ratio of GRR powder that reached 68.84±0.08% in 2:1 GRR: tempeh mix beverage (Figure 2). The GRR single beverage had the total phenolics content and antioxidant activity as high as in the 2:1 mix and higher than that of a single tempeh beverage. There was a potential positive relationship between the total phenolics content and the antioxidant activity of the plant samples as was also reported by Wahyuni *et al.* (2020) in the leaves extrats of Sundanese traditional salad.

Both the total phenolics content and the DPPH free radical scavenging activity in the mix beverage showed an increasing trend as the GRR powder content increased and the tempeh powder content decreased. The addition of tempeh powder did not seem to provide any additional benefits in the total phenolics content and antioxidant activity of the mix beverage, in contrast to our hypothesis. It was the GRR that contributed more to the total phenolics content and antioxidant activity of the mix beverage. There were some bioactive compounds in the ungerminated and germinated rice extracts, such as phenolic acids, flavonol, tannin, Gamma-amino Butyric Acid (GABA), and a-tocopherol (Kaur *et al.* 2017). The phenolic acids content such as ferulic acid, p-coumaric acid, 2,5-dihydroxybenzoic acid, sinapic acid, vanillic acid, and syringic acid was correlated with the antioxidant activity (Shao *et al.* 2018).

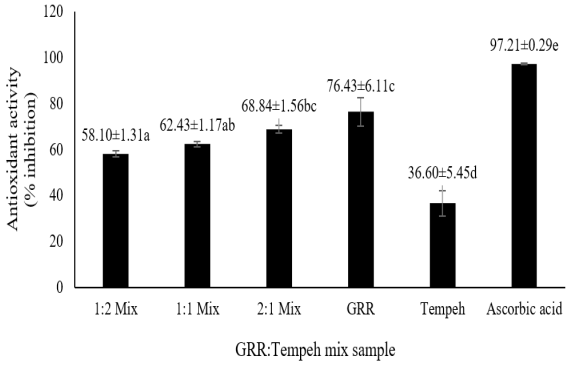

Figure 2. The antioxidant activity of beverage sample consisted of GRR and tempeh powder mix. Bars with different letters are significantly different ($p<0.05$)

The antioxidant activity of tempeh powder-only beverage was the lowest among all samples (Figure 2). Tempeh powder in this study was made on the same day when the commercial fresh tempeh was purchased from the supermarket, which was about 2 days after the production date. Tempeh that was fermented for 24–60 h (IC_{50} of 1 mg/ml) had a stronger antioxidant activity than that of 72 h (IC_{50} of 2 mg/ml) (Athaillah *et al.* 2019). The tempeh-only beverage in this study was made with 6% w/v (60 mg/ml) tempeh powder from approximately 48 h-fermented tempeh and only showed 36% antioxidant activity. It indicated that the powdering process could give negative impact to the antioxidant activity of tempeh powder by degrading or modifying the structure of the compounds that contributed to tempeh antioxidant activity, such as isoflavones, low molecular weight peptides (<3 kDa), several amino acids, particularly hydrophobic amino acids (Astawan *et al.* 2020), and 3-Hydroxyanthranilic Acid (HAA) as an intermediate metabolite of tryptophan (Ahnan-Winarno *et al.* 2021). The loss of isoflavone in tempeh-only beverage as was shown in this study (Table 2) and a low isoflavone content in another tempeh flour (0.62 mg genistein/g db) as was reported by Astawan *et al.* (2020) also supported this finding. The content of peptides, amino acids, and HAA were not analyzed in this study. Further, tempeh antioxidative properties might not derived from a high dose of single compounds, but rather a mixture of other bioactive compounds in a much lower dose (Ahnan-Winarno *et al.* 2021).

The addition of tempeh powder could still contribute to other nutritional content in the mix beverage. Tempeh powder provided 51% db protein, 5% db crude fiber, and high essential amino acids content with hypoglycemic and insulinotropic properties, such as arginine, alanine, phenylalanine, isoleucine, and leucine (Astawan *et al.* 2020). Further study on the nutritional content and other bioactive compounds, such as peptides and polysaccharides, could be conducted to elucidate the other health benefits of the GRR and tempeh mix beverage.

Phenolics profile

Ferulic acid, genistein, and daidzein were detected in the HPLC chromatograms at 2.1,

Table 2. Ferulic acid, genisten, and daidzein content in beverage samples

Sample (GRR:Tempeh)	Ferulic acid (µg/ml)	Genistein (µg/ml)	Daidzein (µg/ml)
1:2 Mix	239.03	2.99	ND
1:1 Mix	315.62	0.44	ND
2:1 Mix	233.73	ND	ND
GRR	163.85	ND	ND
Tempeh	ND	ND	ND

GRR: Germinated Red Rice; ND: Not Detected

14.0 and 15.8 min retention times, respectively (Figure 3 and Figure 4).

Ferulic acid could mainly be found in cereals, and to the lesser extent, legumes (Shahidi & Ambigaipalan 2015). Table 2 showed that ferulic acid was only detected in the GRR-containing beverage. The lowest and highest content of ferulic acid was found in the GRR only-containing beverage (163.85 µg/ml) and 1:1 GRR: tempeh mix beverage (315.62 µg/ml), respectively. Although the ferulic acid content in the 1:1 mix beverage sample increased by almost two-fold from the single GRR beverage, which could possibly suggest the transformation of the phenolics in the GRR and tempeh, the antioxidant activity of the 1:1 mix beverage sample was lower than the single GRR beverage sample (Figure 2).

Ferulic acid was reported to provide many physiological functions, such as antioxidant, antimicrobial, antiinflammatory, and anticancer properties, which could reduce the risk of several chronic oxidative-linked diseases (Shahidi & Ambigaipalan 2015). However, other bioactive compounds in GRR, instead of ferulic acid, could also contribute to the high antioxidant activity of the single GRR beverage sample, such as other phenolic compounds, γ-oryzanol, GABA, tocopherol, and tocotrienol (Kaur *et al.* 2017; Widyawati *et al.* 2014).

Tempeh fermentation could enhance the release of water-soluble phenolics, especially the isoflavone aglycones, which was due to the microbial hydrolysis action (Athaillah *et al.* 2019). However, genistein and daidzein were not detected in some tempeh powder-containing beverage samples. Genistein was only detected in the 1:2 and 1:1 mix beverage samples, while daidzein was not detected in all samples. Genistein was reported for its antioxidant, phytoestrogen, and cancer cell growth suppression activities at a low concentration (Athaillah *et al.* 2019), while daidzein was found to have antioxidant and antiviral activities by inhibiting the proteolytic activity of SARS-CoV-3C like protease (Lammi & Arnoldi 2021).

In contrast to this finding, Astawan *et al.* (2020) showed that tempeh flour which was made from ungerminated soybean contained 62 mg genistein and 54 mg daidzein per 100 g db. The beverage in this study was made with 12 g of tempeh, GRR, or mix powder in 200 ml of water. This would suggest that a very low concentration of genistein and daidzein might be present in the beverage which was below the detection capability of the profiling method. Therefore, increasing the tempeh powder concentration to above 6% w/v might increase the isoflavone aglycones content as well, but it would result in the decreased solubility that would require further formulation of the beverage. Moreover, the low concentration of genistein and daidzein in the soybean and commercial tempeh might also potentially contribute to the very low

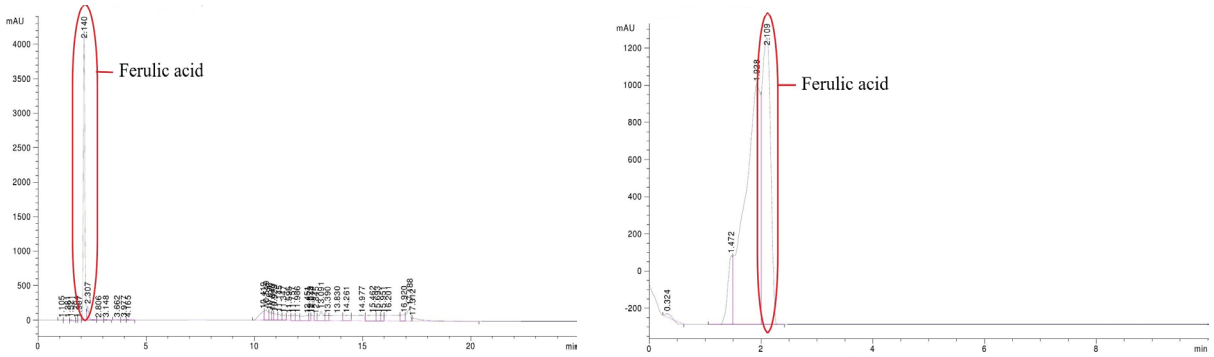

Figure 3. Chromatogram of (a) 400 µg/ml ferulic acid standard solution and (b) 1:2 GRR: Tempeh mix beverage sample

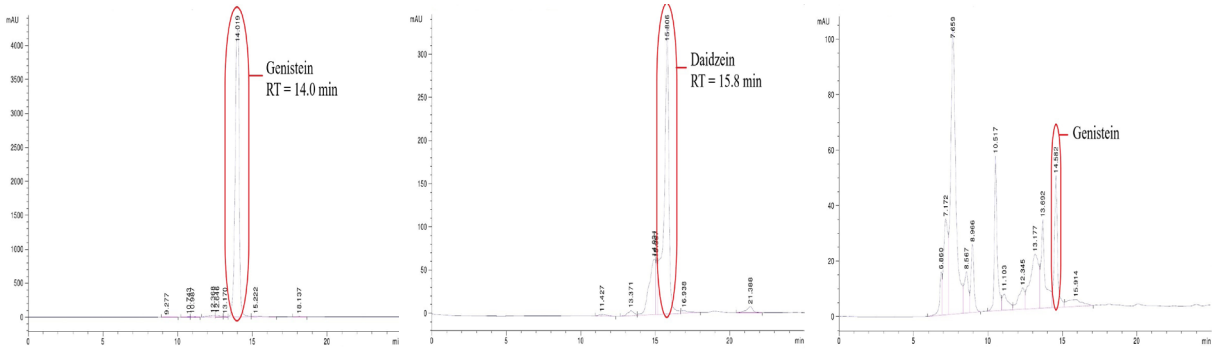

Figure 4. Chromatogram of (a) 100 µg/ml genistein standard solution, (b) 50 µg/ml daidzein standard solution, and (c) 1:1 GRR: Tempeh mix beverage sample

concentration of both compounds in the beverage samples. A selection of soybean variety with high isoflavones content might increase the isoflavone aglycones content in tempeh because soybean variety was the dominant contributor of the isoflavone aglycones content in tempeh (Athaillah *et al.* 2019).

A solid substrate fermentation, as was applied in tempeh production using *Rhizopus* spp., served as a good strategy to increase the total phenolics content and improve the antioxidant activity of the substrate, as was also observed in chickpea fermentation (Sánchez-Magana *et al.* 2014). Isoflavone aglycones, such as genistein and daidzein, were abundant in tempeh as compared to soybean which contributed to the higher bioavailability and antioxidant activity (Santos *et al.* 2018). Tempeh fermentation could initially transform the glycosides form of isoflavones to their aglycone form, then further cause the biotransformation of the isoflavones to polyhydroxylated isoflavones through microorganisms' action during tempeh fermentation (Chang 2014) which could result in the reduction of genistein and daidzein content.

Protein could protect the soy isoflavones from a thermal degradation (Malaypally & Ismail 2010). However, the heat treatment applied during tempeh powder production and beverage formulation in this study could degrade the protein, thus, exposing the isoflavones to the thermal degradation. Genistein and daidzein began to degrade at 95°C and drastically declined at a temperature above 200°C (Huang *et al.* 2006). The reduction and optimization of heat treatment in the production of functional beverage was, therefore, essential to maintain the stability of phenolic compounds with targeted health benefits.

Sensory evaluation

The acceptance test scores on the plain beverage samples were not different significantly among all samples (Table 3) which ranged from 2.90 to 4.27. Formulation of the beverage with lemongrass-infused syrup improved the acceptance particularly for the taste, texture, and aftertaste attributes which ranged from 4.93 to 5.63 (Table 4). However, such result was also not different significantly for each sensory attribute among all samples.

The taste, color, aroma, and aftertaste of GRR-containing beverage was more liked than tempeh single beverage in both plain and sugar added beverage. The beany flavor of

Table 3. The acceptance test result on plain beverage sample with a 7-scale hedonic score

Sample (GRR:Tempeh)	Taste	Color	Aroma	Texture	Aftertaste
1:2 Mix	2.90±1.42a	4.53±1.28a	4.27±1.48a	3.77±3.77a	3.07±3.07a
1:1 Mix	3.33±1.42a	4.50±1.22a	4.23±1.33a	3.97±1.47a	3.37±1.59a
2:1 Mix	3.40±1.59a	4.17±1.46a	4.08±1.46a	3.77±1.50a	3.73±1.46a
GRR	3.23±1.52a	4.83±1.39a	4.03±1.27a	4.27±1.51a	3.50±1.48a
Tempeh	3.07±1.64a	4.90±1.45a	4.23±1.45a	4.10±1.49a	3.23±1.43a

Values with similar letters within a column are not significantly different ($p>0.05$); GRR: Germinated Red Rice

Table 4. The acceptance test result on formulated beverage sample (after addition of lemongrass-infused syrup) with a 7-scale hedonic score

Sample (GRR:Tempeh)	Taste	Color	Aroma	Texture	Aftertaste
1:2 Mix	5.63±0.96a	5.07±1.17a	4.87±1.11a	5.13±1.28a	5.07±1.14a
1:1 Mix	5.07±1.46a	4.93±1.20a	4.60±1.25a	4.93±1.26a	5.00±1.49a
2:1 Mix	5.43±1.22a	4.97±1.38a	4.73±1.31a	5.37±1.19a	5.50±1.17a
GRR	5.60±1.25a	5.43±1.17a	5.13±1.07a	5.23±1.17a	5.37±1.19a
Tempeh	5.20±1.03a	5.30±1.18a	4.83±1.23a	5.40±1.04a	4.93±1.41a

Values with similar letters within a column are not significantly different ($p>0.05$); GRR: Germinated Red Rice

tempeh powder was detected and not liked by some panelists. The tempeh single beverage still exhibited beany flavor although it had been blanched and oven-dried during the tempeh powdering process. The beany flavor of soy-derived product was the result of volatile compounds that was released during the heating process and/or oxidation reaction, such as short chain fatty acids, sterols, and sulfur compounds (Sethi *et al*. 2016). Blanching could inactivate the lipoxygenase enzyme in tempeh; however, it was more effective to deodorize the beany flavor of soybean when it was applied in the soybean soaking step (Sethi *et al.* 2016). The increasing content of GRR and the decreasing content of tempeh in 2:1 mix beverage showed the highest score for the taste and aftertaste in the plain beverage. The lemongrass flavor and sugar addition was suitable to improve the sensory properties of the GRR and tempeh powder mix or single beverage by masking the beany flavor of tempeh powder. Lemongrass contained flavoring compounds, such as β-myrcene, α-pinene, and geraniol which could interact with the macromolecules in the beverage to entrap the beany flavor of tempeh (Natisri *et al.* 2014).

CONCLUSION

Mixing both GRR and tempeh powder at 6% w/v concentration as a beverage could be a good strategy to achieve the potential health benefits in terms of the phenolics and antioxidant activity. The 2:1 GRR : tempeh powder mix beverage could generate a total phenolic content and a DPPH radical scavenging activity as high as a single GRR powder beverage and 1.6-fold higher than that of a single tempeh powder beverage. GRR contributed to the increase of total phenolic content, ferulic acid content, and antioxidant activity of the beverage samples. A lemongrass syrup addition was suitable to improve the taste and aftertaste of the beverage formulation. A loss of isoflavone in the tempeh-containing beverage samples suggested that optimizing the dose and minimizing heat treatment during processing were important to achieve the optimal health benefits from both ingredients.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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