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Research Article
The Acceptability of a *Tempe* and Rice Bran Flour Combination Formula for Anti-hyperlipidemia and Antioxidative Stress

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Abstract

Background and Objective: Dyslipidemia is a lipid metabolic disorder caused by complex interactions between genetic factors, lifestyle and decreased estrogen hormones. The decrease in endogenous estrogen hormones correlates with changes in the distribution of body fat and central obesity. Thus, the decrease in estrogen causes oxidative stress. Antioxidants are compounds that can prevent the occurrence of free radicals through the prevention of oxidation reactions. The best exogenous antioxidant is vitamin E (Tocopherol). One of the natural and safe Tocopherol sources is rice bran. Another antioxidant that has hypolipidemic properties is isoflavone, which is found in soybean *tempe*, can increase the superoxide dismutase (SOD) level. This study aimed to find the most preferred combination of *tempe* and rice bran flour. **Materials and Method:** This study was conducted through a quasi-experiment using Mono-factorial Completely Randomized Design (CRD). The Friedman method and the Wilcoxon test were used for the analyses of the organoleptic test data. **Results:** Based on the results of the hedonic test, which was based on the combination of raw materials, the panelists preferred the formula of yellow soybean flour: white rice bran flour, which resulted in the following values for color (2.98), aroma (3.14), viscosity (3.30) and taste (3.25) on a scale of 1-4. Based on the results of the hedonic test, which was based on variations, the composition formula of yellow soybean flour and white rice bran flour at a 1:1 ratio was most preferred by panelists according to the results of the parameters of color (3.21), aroma (3.18), viscosity (3.29) and taste (3.05). The Wilcoxon test results showed a significant difference ($p \leq 0.05$) for each variation in the raw materials and each combination of the formula ratios. **Conclusion:** The variation in raw materials selected by the panelists was the combination of yellow *tempe* flour and yellow rice bran at a composition of 1:1.

Key words: Anti-hyperlipidemia, flour combination formula, oxidative anti-stress, Rice Bran, *tempe*, white rice bran flour, yellow *tempe* flour

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dyslipidemia is a lipid metabolism disorder characterized by levels of total cholesterol >200 mg dL^{-1}, low density lipoprotein (LDL) cholesterol >130 mg dL^{-1}, triglyceride >150 mg dL^{-1} and high-density lipoprotein (HDL) cholesterol <40 mg dL^{-1}. This disorder is due to the complex interactions between genetic and unhealthy lifestyle factors[1-3]. One of the contributing factors of lipid profile change, especially in women over 50 years of age, is due to a decrease in estrogen (menopause)[3]. The decrease in endogenous estrogen correlates with changes in the body fat distribution including an increased total mass of fat and central obesity. Thus, the decrease in estrogen leads to oxidative stress[4-6]. The prevalence of dyslipidemia, according to Indonesia Basic Health Research (RISKESDAS) data in 2013, exceeds to 50.7%[7-9].

The main prevention of dyslipidemia can be achieved by control of serum cholesterol levels within normal limits, control of weight, a low cholesterol diet, regular exercise, pharmacological therapy using hypolipidemic drugs and consumption of food containing potent antioxidant compounds to decrease serum cholesterol levels[10]. Medicamentous therapy using hypolipidemia/lipid-lowering drugs has side effects such as elevated fasting blood glucose, dementia and mild cognitive impairment and psychiatry[11,12]. Therefore, both endogenous and exogenous antioxidant compounds are required to improve the lipid profile without any side effects for postmenopausal women with dyslipidemia[13-15].

Antioxidants are compounds that can prevent the occurrence of free radicals through the prevention of oxidation reactions. The results of studies conducted by Zaidi and Banu[16] and McQuillan *et al.*[17] show that the best exogenous antioxidant is vitamin E (Tocopherol) because it has the best effect when compared to vitamin A and vitamin C in reducing fat peroxidation. The addition of Tocopherol to diet can reduce the risk of coronary heart disease (CHD) by 34%[18,19].

One of the natural and safe Tocopherol sources is rice bran. Rice bran is one of the byproducts of the rice milling process in addition to white rice itself[20]. Based on the Central Bureau of Statistics (known in Indonesia as BPS) data in 2015, rice production in Indonesia is 75.36 million tons and results in rice bran at a total of 6-7.54 million tons from rice mills[21]. The types of rice bran vary depending on rice varieties such as white rice bran, red rice bran and black rice bran. Rice bran, in addition to containing vitamin E, also contains vitamin B complex (B1, B2, B3, B5 and B6), essential fatty acids, fiber, amino acids, g-oryzanol, polyphenols and phytosterol[22].

In addition to vitamin E (Tocopherol), another type of antioxidant that has hypolipidemic properties is isoflavone found in soybeans[23]. According to Hall *et al.*[24] and Geller and Studee[25], the isoflavone in soybeans (genistein and daidzein) acts similarly to estrogen because it is an antioxidant that neutralizes free radicals that cause damage to cells. Types of soybeans vary depending on the varieties such as yellow soybeans and black soybeans. One processed soybean product is *tempe* (a fermented soybean product in Indonesia). The biological activities of isoflavones in soybean *tempe* increase by 222.5% compared to that in soybeans and a decrease in phytic acid by 65% occurs due to phytic enzyme reactions produced by *Rhizopus oligosporus*. It is expected that *tempe* is related to superoxide dismutase (SOD) enzyme activities formed during the 24-60 h fermentation process and these activities were previously not found in soybeans and, therefore, increase SOD levels in the body[25]. Another alternative to extend the shelf life of *tempe* is by processing it into *tempe* flour[26].

The compound contained in *tempe* flour is isoflavone and the compound in rice bran is tocopherol, which acts as a chain breaker antioxidant; when combined, these compounds will better inhibit LDL oxidation. The results of Nunes *et al.*[27] shows that the addition of isoflavone has no effect; however, when combined with synthetic vitamin E, it is able to inhibit LDL oxidation by 45.6% compared to the atherogenic group ($p<0.05$). *Tempe* and rice bran flour also contain dietary fiber, which can inhibit the absorption of cholesterol in the small intestine to reduce the cholesterol concentration in the plasma and increase cholesterol synthesis by the liver, bile synthesis and cholesterol excretion through feces[28].

This study aimed to identify the variation of the formulation of raw materials most preferred by panelists from the combination of soybean *tempe* flour, black soybean *tempe* flour and yellow rice bran and red rice bran based on physical and organoleptic properties. In addition, this study also aimed to determine the composition most preferred by the panelists.

MATERIALS AND METHODS

Materials: This study received ethical approval from the Ethics Committee of the Faculty of Public Health, University of Muhammadiyah Semarang No. 073/KEPK-FKM/UNIMUS/2018. Yellow soybean *tempe* and black soybean *tempe*, white rice bran, red rice bran, non calorie sugar and food flavoring

Methods: This study was conducted through a quasi-experiment using a Mono-factorial Completely Random Design (CRD), namely, a *tempe*: rice bran flour formula.

- **Stage 1:** Hedonic and organoleptic tests of raw material variations for a *tempe*-rice bran formula
- **Stage 2:** Hedonic and organoleptic tests of a *tempe*-rice bran formula in accordance with the test results of Stage 1 and based on composition variations

***Tempe* flour production:** Yellow soybean and black soybean *tempe* are blanched by steaming for 15 min. Then, these components are chopped to a thickness of 1 cm and dried in the sun. Finally, they were extracted into flour and sifted using a 100 mesh sieve.

Rice bran flour production: The byproducts (secondary products) of white rice and red rice yielded from rice mills are then heated using a cabinet dryer to a temperature of 100 for 20-30 min. Then, they were sifted using a 100 mesh sieve.

The making of the raw material variations of the *tempe* flour: rice bran flour formula: The combinations of yellow soybean *tempe* flour and white rice bran flour, (A) Yellow soybean *tempe* flour and red rice bran flour, (B) Black soybean *tempe* flour and white rice bran flour and (C) Black soybean *tempe* flour and red rice bran flour (D), Each with a composition of 1:1 and a weight of 112 grams, were mixed together. A total of 3 g of non calorie sweetener and 3 g of food flavorings were added and then mixed together. After they were mixed, each of the combinations was dissolved in cold water at 250 cc.

Organoleptic testing procedure Stage 1: This procedure includes color, taste, aroma and viscosity. The organoleptic test was conducted using a hedonic test method with a total of 25 well-trained panelists. The test was randomly presented in the form of drinks and with 4 kinds of formulae; then, the panelists were asked to taste the formulae provided and assess them according to the assessment rubric created by the presenters. The selected formula was then tested to select the most accepted composition of materials for making the formula.

The making of the formula composition variations: The raw materials were mixed together in a combined formula of the selected *tempe* and rice bran flour in the previous organoleptic test with a composition of (A) 224 g: 112 g/2: 1, (B) 112 g: 112 g/1: 1 and (C) 112 g: 224 g/1: 2 with 3 g of non calorie sweetener and 3 grams of food flavorings each. The raw materials were mixed together by using a standard food mixer and then blended. After mixing, each combination was dissolved in cold water at 250 cc and then the organoleptic test was conducted.

Analysis of the obtained organoleptic test data: The data were tabulated and analyzed using the nonparametric statistics of the Friedman method and continued with a Wilcoxon test.

RESULTS

Table 1 and 2 shows the combinations of yellow and black soybean flour with red and white rice bran flour to determine a formula with the best flour combination based on the initial stage preference:

Based on Table 1 and according to the results of the Friedman test of preference score, the most preferred treatment is yellow soybean *tempe* flour+white rice bran flour.

Based on Table 2 and on the Freidman test, the most preferred treatment scoring result is yellow *tempe* flour+white rice bran flour. The results of the hedonic test based on the variations of the composition of raw materials are presented in Table 3.

Based on Table 3 and on the Friedman test results, the most preferred composition is 1: 1.

The test results of the color scores of the yellow soybean *tempe* flour and white rice bran flour formula in Table 4.

Table 1: Hedonic test/preferred formula based on the combinations of raw materials (scale 1-4)

	Parameters			
Treatment (flour)	Color	Aroma	Viscosity	Taste
Yellow soybean *tempe* flour+red rice bran flour	2.43^b	2.21^b	2.21^b	2.81^b
Yellow soybean *tempe* flour+white rice bran flour	2.98^a	3.14^a	3.30^a	3.25^a
Black soybean *tempe* flour+red rice bran flour	1.17^d	1.33^c	1.22^d	1.08^c
Black soybean *tempe* flour+white rice bran flour	1.56^c	1.84^b	1.40^c	1.22^d

*Different letter notation shows Wilcoxon test results with significant differences (p<0.05), Scale 1: Dislike extremely, 2: Dislike, 3: Like and 4: Like extremely

Table 2: The formula scoring test based on the combinations of raw materials (scale 1-5)

Treatment (flour)	Parameters Color	Aroma	Viscosity	Taste
Yellow soybean *tempe* flour+red rice bran flour	2.72[b]	2.64[b]	2.64[b]	2.48[b]
Yellow soybean *tempe* flour+white rice bran flour	3.88[a]	3.88[a]	3.80[a]	3.80[a]
Black soybean *tempe* flour+red rice bran flour	1.48[d]	1.84[d]	1.60[d]	1.28[d]
Black soybean *tempe* flour+white rice bran flour	2.16[c]	2.36[c]	2.20[c]	2.08[c]

*Different letter notation shows Wilcoxon test results with significant differences (p<0.05), Color; 1: Dark black, 2: Light black, 3: Brownish black, 4: Dark brown and 5: Light brown, Aroma; 1: Extremely not fragrant, 2: Not fragrant, 3: Slightly fragrant, 4: Fragrant, 5: Extremely fragrant, Viscosity; 1: Extremely viscous, 2: Viscous, 3: Slightly viscous, 4: Not viscous, 5: Extremely not viscous, Taste; 1: Extremely not sweet, 2: Not sweet, 3: Slightly sweet, 4: Sweet, 5: Extremely sweet

Table 3: Hedonic test/preferred formula based on raw material compositions (scale 1-4)

Treatment (comparison)	Parameters Color	Aroma	Viscosity	Taste
Yellow soybean flour: white rice bran flour (2:1)	2.98[b]	2.37[b]	2.27[b]	2.85[b]
Yellow soybean flour: white rice bran flour (1:1)	3.21[a]	3.18[a]	3.29[a]	3.05[a]
Yellow soybean flour: white rice bran flour (1:2)	2.71[b]	2.44[b]	1.33[c]	2.82[b]

*Different letter notation shows Wilcoxon test results with significant differences (p<0.05), Scale 1: Dislike extremely, 2: Dislike, 3: Like and 4: Like extremely

Table 4: Test scores of the *tempe* flour and rice bran flour formula based on compositions of raw materials (scale 1-5)

Treatment (comparison)	Parameters Color	Aroma	Viscosity	Taste
Yellow soybean flour: white rice bran flour (2:1)	3.52[b]	3.44[b]	3.24[b]	3.44[b]
Yellow soybean flour: white rice bran flour (1:1)	3.84[a]	3.64[a]	3.52[a]	3.72[a]
Yellow soybean flour: white rice bran flour (1:2)	2.28[c]	2.84[c]	2.36[c]	2.52[c]

*Different letter notation shows Wilcoxon test results with significant differences (p<0.05), Color; 1: Light brown, 2: Dark brown, 3: Brownish black, 4: Dark brown and 5: Light brown, Aroma; 1: Extremely not fragrant, 2: Not fragrant, 3: Slightly fragrant, 4: Fragrant and 5: Extremely fragrant, Viscosity; 1: Extremely not viscous, 2: Not viscous, 3: Slightly viscous, 4: Viscous and 5: Extremely viscous, Taste; 1: Extremely not sweet, 2: Not sweet, 3: Slightly sweet, 4: Sweet and 5: Extremely sweet

Based on Table 4, the most preferred result of the composition scoring of the Friedman test is 1:1.

DISCUSSION

Variations in the formula raw materials

Color: Treatment B was the panelists-selected treatment with a score of 2.98 and falls into the "like" category on a scale of 1-4; the highest color score chosen by the panelists was dark brown with a score of 3.88. The brown color is caused by phytochemical compounds contained in rice bran and *tempe* made from yellow soybeans. The wet heat treatment increases the brown color components and decreases the yellow color[29]. The color that was not chosen by the panelists was Treatment C with a score of 1.17, which falls into the "extremely dislike" category on a scale of 1-4 and has a color score of 1.48 that is dark black on a scale of 1-4. The black color on the mixture of the black *tempe* and red rice bran formula is due to the accumulation of anthocyanin, a chlorophyll resulting in a black pigment on black soybean skin and red rice[30] and that makes the color unappealing. The panelists' preference level tends to decrease with a blacker color of the formula[30]. The black color in *tempe* is also caused by Rhizopus oryzae, which has a high amylase protease activity and breaks down starch from grains into simple sugars that ferment into organic acids that produce undesired flavors[31].

Aroma: Treatment B was the panelists' most preferred aroma with a score of 3.14 on a scale of 1-4 and the highest aroma score was 3.88, which falls into the "extremely fragrant" category. This aroma is caused by the typical aroma of tocopherol oil (a volatile component) in rice bran. The process of heating the rice bran is expected to increase Maillard's reaction as a source of flavor[32]. The least preferred treatment was Treatment D with a score of 1.33 on a scale of 1-4, which has an aroma score of 1.84 and falls into the "not fragrant" category. The aroma of Treatment D was arises because black soybean *tempe* has a beany flavor caused by the lipoxygenase enzyme activities found in soybeans. The occurrence of the beany flavor arises mainly during processing and after the mixing of lipoxygenase in soybean fat. Based on the results of the study, the compound that produces the most beany flavors is ethyl phenyl ketone, whereas red rice bran has unstable properties that make it easier to form off-flavor odors due to damage of oil content in rice bran and because of lipase enzyme activities. These enzymes hydrolyze triglycerides and produce free fatty acids that are easily oxidized. Rice bran has a fat content of 20%, which is rich in unsaturated fatty acids (70-90%), especially oleic and linoleic acids. The processing of rice bran should be conducted within a short time and in no more than 24 h to avoid a rancid odor[22,29].

Viscosity: Treatment B was preferred panelists-selected viscosity with a score of 3.30, which falls into the "like" category. This treatment also had a viscosity score of 3.80 that falls into the "not viscous" category. The least selected treatment by the panelists was Treatment D with a score of 1.22, which falls into the "dislike extremely" category and had a viscosity score of 1.60 on a scale of 1-4 that falls into the "extremely viscous" category. Black soybeans *tempe* and red rice bran have high anthocyanin and dietary fiber that make the consistency seem more viscous and unappealin[30].

Taste: The hedonic test results show that the panelists, in terms of taste, selected Treatment B with a score of 3.25, which falls into the "like" category. This treatment also had a taste score of 3.80, which falls into "sweet" category. The treatment that was not selected by the panelists was Treatment D with a score of 1.08 that belongs to the "dislike extremely" category. The score of taste that was not selected by the panelists was Treatment C, which was in the "extremely not sweet" category. Factors that make the panelists not select other tastes may be due to the bitter taste and chalky taste in soybeans caused by the presence of glycoside compounds such as soyasaponin and sapogenol[33]. The bitter aftertaste can also be caused by the hydrolysis of amino acids occurring in the Maillard reaction during *tempe* flour processing. There are several amino acids that cause a bitter taste such as lysine, arginine, proline, phenylalanine and valine. Lysine is an amino acid that has the most bitter taste compared to the other bitter amino acids[26]. In addition, the reasons why the panelists did not select other tastes may be due to the use of rice bran that was stored for a very long period of time because of the rapid damage during postmilling, which causes a bitter taste[34]. This bitter taste is caused by the process of fat hydrolysis (triglycerides) that produces free fatty acids because of the activity of the in situ lipase enzyme[35]. The estimated speed of free fatty acid formation results from oil hydrolysis in rice bran, which reaches 5-10% per day and approximately 70% per month[36].

On average, the formula made from yellow soybean *tempe* flour can produce better color, aroma, viscosity and taste than that of black soybean *tempe* flour. This difference is due to the accumulation of anthocyanin, chlorophyll and the combination of several compounds that produce black pigment on black soybean skin[35]. According to Astawan[34], poor quality *tempe* is characterized by the existence of black spots on the *tempe*. This finding is also supported by a study conducted by Wihandini *et al*.[37] who indicated that the panelists tend to prefer yellow soybean *tempe* to black soybean tempe.

The results of this study are in accordance with the results of the study by Saraswati (2017), which is based on sensory results showing that soybean cakes and rice bran are more preferred by consumers than bran soybean cake with extracts of dates and cocoa powder.

Variations in formula compositions: In the second stage of this study, an advanced organoleptic test was conducted on a formula made from a combination of yellow soybean flour and white rice bran flour. This test aims to determine the optimal formula composition by comparing yellow soybean *tempe* flour with white rice bran flour. The performed organoleptic test consists of hedonic and scoring tests. Table 3 shows that there is a significant difference ($p \leq 0.05$) in each treatment of the formula composition. The panelists tend to prefer the formula with the ratio of yellow soybean flour: white rice bran flour (1:1) with a preference level that falls into the "like" category for each parameter of color (3.21), aroma (3.18), viscosity (3.29), or taste (2.82) on a scale of 1-4.

Color: The results of the color scoring test of the variation in formula composition of yellow soybean *tempe* and white rice bran is shown in Table 4.

Table 4 shows that there is a significant difference ($p \leq 0.05$) in color scores between each treatment of the formula composition of yellow soybean *tempe* flour and white rice bran flour. The best ratio between yellow soybean *tempe* flour and white rice bran flour was one to one (1:1) (Table 4) with a formula color of light black (3.84 out of 5). The panelists tend to prefer a dark-colored formula to a brightly colored formula. Increasing the formulation of white rice bran flour causes a brighter color of the formula. This color is due to the basic color of the white rice bran flour, which tends to be bright white.

Aroma: The results of the aroma scoring test of the formula composition variation of yellow soybean *tempe* flour and white rice bran can be seen in Table 4.

The formula of yellow soybean *tempe* flour and white rice bran flour with a formula of 1:1 has a distinctive fragrant aroma formula compared to other formulae. Rice bran has unstable properties caused by hydrolytic and oxidative damage to the oil that exists in rice bran. This condition makes the rice bran smell unpleasant or rancid. The unpleasant or rancid smell of the rice bran comes from the lipase that hydrolyzes fat into fatty acid and glycerol. Then, free fatty acid that is oxidized by the lipoxygenase enzyme into peroxide, ketone and aldehyde forms so that the rice bran becomes rancid. Finally, this influences the sensory acceptability of rice bran as a raw material of the formula[33].

Viscosity: The results of the formula viscosity scoring test of the yellow soybean *tempe* flour and white rice bran flour is shown in Table 4.

The viscosity of the formula is affected by the level of dissolved protein present in the formula[34]. The heating of the formula to a temperature of 800 causes the texture of the formula to be more stable because whey protein forms a gel when denaturing due to heating[35]. The protein content of yellow soybean *tempe* flour reaches 49.6%[35] while that of rice bran flour only reaches 16.5%[36].

Taste: The results of the taste scoring test of the yellow soybean *tempe* flour and white rice bran flour is shown in Table 4.

The formula of yellow soybean *tempe* flour and white rice bran flour with a formula of one to one (1:1) has a sweet taste with a score of 3.72 on a scale of 1-5. Increasing the addition of white rice bran flour causes a reduction in the sweetness of the formula.

Rice bran has a dietary fiber content of 25.3%, while its simple sugar content is only 5%[36]. This condition is suspected to cause a reduction in the sweet taste of the formula because of the high fiber content in the rice bran flour.

The limitation of this research is that there is only one variety of flavors, namely, the original taste of tempe and rice bran.

CONCLUSION

The variation in raw materials chosen by the panelists was a combination of yellow *tempe* flour and yellow rice bran. The composition of the yellow *tempe* flour and white rice bran formula chosen by the panelists was one to one (1:1).

SUGGESTION

The results of this study can be used as a foundation for further research in menopausal women with dyslipidemia to improve their lipid profile and reduce oxidative stress.

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