Journal of Halal Science and Research (JHSR)
Vol. 5, No. 1, Month February, Year 2024, Page 30 – 38
doi: 10.12928/jhsr.v5i1.9480

P-ISSN: 2715-6214
E-ISSN: 2964-4909

Antioxidant and color properties of black soybean tempe beverage with the addition of sappan wood and cinnamon

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ABSTRACT

Black soybean was fermented into Tempe to increase its functional properties and bioavailability. To develop black soybean tempe into a beverage for practical consumption, sappan wood and cinnamon were added to improve the appearance and functional properties. This research aims to determine the effect of sappan wood and cinnamon addition on antioxidant levels, phenolic levels, and brightness levels of black soybean tempe beverages. The research used a Completely Randomized Design (CRD) with a ratio of sappan wood and cinnamon at 0:0, 3:0.5, 2.5:1, and 2:1.5 (%w/w). Beverage made from commercial yellow soybean tempe was used as control. This research started by making tempe using Yogyakarta's traditional method. Beans were dehulled, boiled for 30 minutes, soaked in boiling water for 36 hours, boiled again for 30 minutes, inoculated with RAPRIMA, and incubated for 40 hours at room temperature. Tempe beverage was made by milling tempe and 80°C water at 1:3 (w/w) and the barks at the mentioned ratio, followed by filtration. The beverages were then analyzed for their Ascorbic Acid Equivalent Antioxidant Capacity (AEAC), total phenolics using the Folin-Ciocalteu method, and brightness levels using the CIE method. The results showed the antioxidant properties ranged between 82.67-86.93 mg AEAC/ml, total phenolics: 53.43-66.53 mg GAE/ml, color brightness parameter L*: 33.82-34.49, a *: 1.97-2.01, and b*: 4.09-5.05. It can be concluded that adding sappan wood and cinnamon improved the antioxidant levels and appearance of black soybean tempe beverages. The highest antioxidant properties and brightness level were obtained by adding 3% and 0.5% of sappan wood and cinnamon bark, respectively.

Keywords: Antioxidant, Black soybean beverage, Cinnamon, Sappan

INTRODUCTION

Black soybean is one of the functional foods known for its nutritional content. It contains protein (40 – 50%), fat (20 – 30%), and carbohydrates (26 – 30%), with polyphenol content (Varnosfaderani et al., 2019) as one of the varieties of soybean, which is differentiated by peel color, such as yellow, black and green, black soybean can be distinguished by the total content of bioactive compounds. They all contain polyphenols and flavonoids, but only the black ones contain anthocyanins (Nurisyah et al., 2019). The total phenolic content of black soybeans is known to be higher (1.78 RE mg/g) than yellow (0.57 RE mg/g) and green soybeans (0.47 RE mg/g) (Ren, 2012).

Fungal fermentation of beans using *Rhizopus oligosporus* into Tempe is a process widely used in Indonesia to improve digestibility and palatability. The process significantly reduces antinutrients, such as phytates, cyanogenic glycosides, oxalates, saponins, lectins, and inhibitors of enzymes such as alpha-amylase, trypsin, and chymotrypsin (Reddy & Pierson, 1994), while at the same time increase protein hydrolysis and produce beneficial compound, such as vitamin B12 (Kustyawati et al., 2020). Fermentation of black soybeans into tempeh increases the nutritional value of essential amino acids.

P-ISSN: 2715-6214
E-ISSN: 2964-4909

Rhizopus oligosporus can produce protease enzymes to hydrolyze proteins into simpler amino acids (Purry & Rafiony, 2018). As the benefits of tempe are superior to soybeans, Tempe can be developed further into a black soybean tempeh beverage. However, it was reported that fresh tempeh has a distinctive aroma that comes from the breakdown of fat involving protease and lipase enzymes during mold growth, which mixes with amino acids in the formation of mycelium during the fermentation process (Jeleń et al., 2013). However, fermentation might produce a dislikable ammoniac aroma, as previously reported with soybean tempeh beverage had a hedonic score of 3 on a scale of 1 – 5 (Kusmanto & Hidayati, 2011). In order to improve the quality and likability of black soybean tempeh beverages, black soybean tempeh beverages can be formulated with other additional ingredients, such as herbs or spices. Among spices that are commonly used in traditional beverages in Indonesia are sappan wood, locally known as *secang* and *kayumanis* (Fauziah et al., 2023). Sappan wood (*Caesalpinia sappan wood* L.) contains antioxidants and natural pigments. The antioxidant content in sappan wood includes brazilin, brazilein, methyl brazilin, sappanin, chalcone, and sappan wood chalcone (Harijono et al., 2021). According to previous research, the antioxidant activity value of sappan wood was 60.03% (Hastuti & Rustanti, 2014). Another natural ingredient of antioxidants is cinnamon. Cinnamon (*Cinnamomum burmannii*) contains antioxidants in the form of essential oils, phenols, and polyphenols (flavonoids, tannins) (Ervina et al., 2016). Cinnamon extract has antioxidant activity of 14.36% (Hastuti & Rustanti, 2014). Though below sappan wood, cinnamon contributes to improved functional properties and better flavor of traditional drinks (Harijono et al., 2021).

As soymilk is one of consumers' popular vegan protein beverages, tempe beverages might be unfamiliar, especially in terms of beany flavor. The addition of sappan and cinnamon, often mixed in Indonesian traditional beverages, such as *wedang secang* and *wedang uwuh* (Fauziah et al., 2023; Sabila et al., 2021), is expected to improve black soybean tempe beverage flavor, familiarity with the consumer, as well as functionality for health. There is still limited information on adding sappan wood and cinnamon to beverages made from fermented beans, especially made from black soybean tempe. Black soybean tempe beverages with sappan wood and cinnamon can be an alternative functional drink that can increase antioxidant levels. In this case, researchers will study the formulation of soybean tempeh beverage with differences in the addition of sappan wood and cinnamon and test the antioxidant content, phenolic content, and brightness level of the product.

RESEARCH METHOD

Materials

Materials used in this research were black soybeans (*Glycine max* var. mallika) obtained from the Mekar Mas cooperative, Kulon Progo, Yogyakarta, tempe mold (Raprima), sappan wood, cinnamon bark, and crystal palm sugar obtained from Yogyakarta traditional markets, distilled water (Chemmix laboratory), DPPH, methanol, ascorbic acid, Follin reagent, Na-tetraborate, Na_2CO_3 and gallic acid (Sigma Aldrich). All chemicals used for analyses were pro-analysis grade.

Methods

1. Preparation of Black Soybean Tempe Beverage

   Approximately 1000 g of black soybeans were washed with running water, boiled for 30 minutes, then soaked for 48 hours; the water was changed every 24 hours. After peeling, the seeds were separated, and the beans were steamed for 60 minutes and drained at room temperature. After inoculation using 2 g of tempeh fungi powder, around 50 grams of beans each were wrapped in banana leaves and incubated at room temperature for 42 hours.

   Sappan wood and cinnamon bought from the market were washed, drained, and sun-dried for 6 hours under a dark cloth cover. Dried materials were ground into powder using a dry blender (Philips HR-2115) for 3 minutes and kept in a closed container before utilization. To make a beverage, black soybean tempeh was steamed for 30 minutes and weighed; then, for each formula, 25 g of tempeh was mashed in a blender with the addition of 75 g of water at 80°C, 5 g of crystalline palm sugar, sappan wood, and cinnamon, for 3 minutes. Finally, the beverage was filtered from the pulp using a filter cloth, and the filtrate was boiled for 5 minutes. There were four formulations and one control (Table 1).

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Table 1. Sappan wood and cinnamon bark addition.

Sample	Weight (gram/100 g of tempeh beverage)	
	Sappan wood	**Cinnamon**
F1	3	0.5
F2	2.5	1
F3	2	1.5
F4	0	0
C	Beverage made from commercial yellow soybean tempe without sappan wood and cinnamon bark	

2. Analysis of antioxidant level

   The antioxidant level of the beverage was measured using Ascorbic Acid Equivalent Antioxidant Capacity (Leong & Shui, 2002). After making 0.4 mM DPPH (1.1-diphenyl-2picrillhydrazyl) solution, an ascorbic acid standard curve was made at 0 to 200 ppm. Antioxidant compounds were measured as 1 ml of sample was added with 7 ml of methanol and 2 ml of DPPH solution. The solution was then vortexed and incubated for 30 minutes in a dark room. The control contained 8 ml methanol and 2 ml DPPH solution. Measurements were made using a spectrophotometer (Optima SP-300) at 520 nm. Then the results were substituted into the standard curve equation for ascorbic acid to determine AEAC (Ascorbic Acid Equivalent Antioxidant Capacity).
3. Total Phenolic Content

   Total Phenolic Content (TPC) determination was performed using the previous method (Lin et al., 2012) with slight differences. First, the standard curve of gallic acid was made at 0 to 200 ppm. Approximately 0.2 ml of sample was added with 1 ml of Folin-Ciocalteu, vortexed, and incubated for 8 minutes. Then 0.8 ml of 7.5% Na_2CO_3 solution and 3 ml of distilled water were added. After 30 minutes of incubation at room temperature, measurement was carried out using a spectrophotometer (Optima SP-300) at 753 nm. The results were substituted into the gallic acid standard curve equation to determine GAE (Gallic Acid Equivalent).
4. Analysis of Color Brightness Levels

   The measurement method refers to the International Commission on Color Illumination (French Commission Internationale de L'ecairage), often known as CIE. The principle used in measuring the brightness level of color is measuring color differences through the reflection of light on the surface. The sample was prepared in a beaker and then measured using a Konica Minolta CR 400 Chromameter using a tristimulus, and results were obtained with the following parameters:

   (L) Lightness between 0 and 100 is white.
   (a) Redness between 0 and 60 is red, and between 0 and -60 is green.
   (b) Yellowness between 0 and 60 is yellow, and between 0 and -60 is blue.

Data Analysis

A completely Randomized Design was applied in all experiments, triplicate for each test. Fixed variables were tempeh formulation, water, and palm sugar, and independent variables were formulations of sappan wood and cinnamon of F1 (3g:0.5g), F2 (2.5g:1g), F3 (2g:1.5g), F4 (without sappan wood and cinnamon), and C (control, beverage made from commercial tempe made using yellow soybean). The dependent variables were antioxidant activity, total phenolic content, and color brightness level. The test results data were analyzed using the Microsoft Excel 2019 and SPSS 25.0 programs with the One Way ANOVA (Analysis of Variance) test analysis method and continued with DMRT (Duncan Multi Range Test) analysis.

RESULT AND DISCUSSION

Antioxidant content

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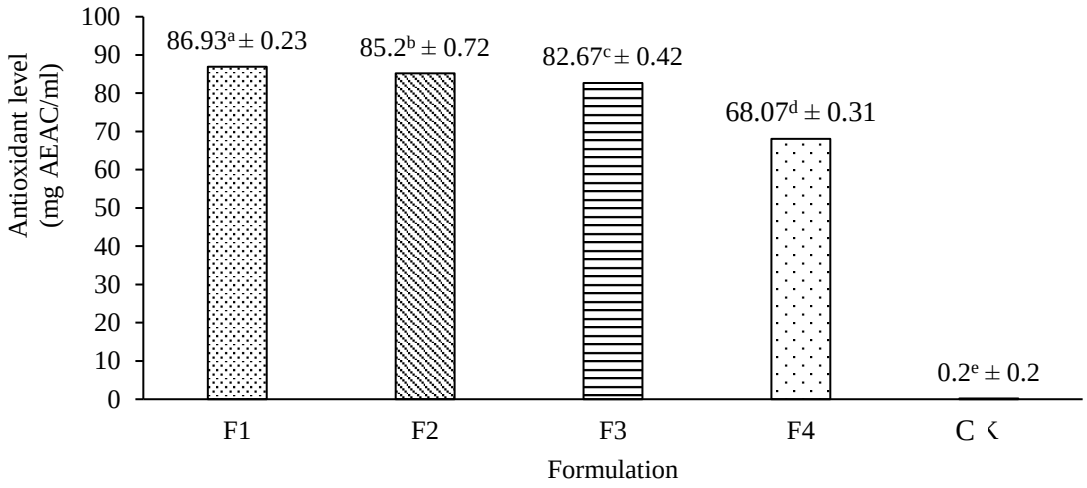
E-ISSN: 2964-4909

Antioxidants are a group of compounds that can soak up free radicals and have the benefit of breaking up unpaired chemical molecules or free radicals. Free radicals in the body have reactive properties that can undergo oxidation reactions, so they can be dangerous for the body (Verrananda et al., 2016). The 1-1 diphenyl-2-picrilhydrazyl (DPPH) method is often used due to its simplicity, speed, and lack of large sample size (Setyawati, 2020). Antioxidant compounds donate one electron to stabilize unpaired compounds so that they react with DPPH, which only has one electron. After the two compounds pair, the DPPH concentration of the solution will decrease, causing the intensity of the DPPH color to decrease from purple to yellow (Naspiah et al., 2013). Methanol solvent has semi-polar, non-polar (-CH_3), and polar (-OH) properties so that it can attract bioactive components that have polar and non-polar properties, thus very suitable when used in dilution (Mukti et al., 2016). Determination of RSA (Radical Scavenging Activity) was carried out to determine the percentage of the sample's ability to inhibit free radicals; then, the results were substituted with the standard curve equation to determine the value of antioxidant compound levels and expressed in units of mg AEAC (Ascorbic Acid Equivalent Antioxidant Capacity)/ml of sample.



Results ± SD; numbers followed by different superscript indicate significant difference; F1: sappan wood 3%, cinnamon 5%; F2: sappan wood 2.5%, cinnamon 1%; F3: sappan wood 2%, cinnamon 1.5%; F4: without sappan wood and cinnamon; C: commercial yellow soybean beverage

Figure 1. An antioxidant level of black soybean beverage is added with sappan wood and cinnamon bark.

Commercial soybean beverages had significantly lower levels of antioxidants at 0.2 mg AEAC/ml compared to black soybean tempeh beverages. According to research by Tambunan (2018), the adequate Vitamin C per day for productive age (16-64) is 75 to 90 mg/day. The addition of sappan wood and cinnamon to the Tempe beverage had a significant effect on antioxidant levels (Figure 1). Black soybean tempeh beverage without the addition of sappan wood and cinnamon had a value of 68.07 mg AEAC/ml, which was the lowest value compared to soybean tempeh beverage with the addition of sappan wood and cinnamon. This is because sappan wood and cinnamon contain high antioxidant compounds (Ervina et al., 2016; Ulma et al., 2018). Sappan wood contains the antioxidant compound brazilin (Rina, 2013), while cinnamon has flavonoids and tannins (Ervina et al., 2016).

The test results for the levels of antioxidant compounds in black soybean tempeh beverage with the addition of 3 g of sappan wood and 0.5 g of cinnamon were the highest at 86.93 mg AEAC/ml compared to the other two formulations with a 21.70% difference from black soybean tempeh beverage without sappan and cinnamon addition. The beverage with 2.5 g of sappan wood and 1 g of cinnamon was followed at 85.2 mg AEAC/ml with a 20.11% difference from the control. The beverage with 2 g sappan wood and 1.5 g cinnamon had the lowest result of 82.67 mg AEAC/ml, or with a 17.66 %

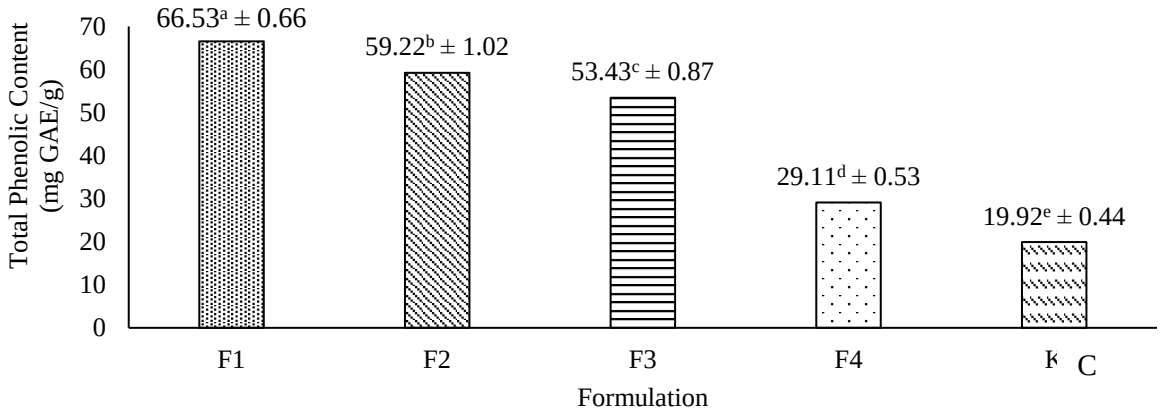
P-ISSN: 2715-6214
E-ISSN: 2964-4909

difference from the control. Antioxidant compound levels increased with increased sappan wood and a lower amount of cinnamon. This was due to sappan wood's higher antioxidant level than cinnamon (Hastuti & Rustanti, 2014). This was also supported by research by Sugiyanto (2011), which states that the levels of the sappan wood are much higher than those of the synthetic antioxidant compounds BHA and BHT.

In addition, processing soybeans fermented with *Rhizopus* sp into tempeh products increases the antimicrobial and antioxidant value (Endrawati & Kusumaningtyas, 2017). The antioxidant compound content of black soybean beverage was 46.48 mg AEAC/ml, lower than that of black soybean tempeh beverage without the addition of sappan wood and cinnamon.

Total Phenolic Content

Total phenolic content testing was done with a Folin-Ciocalteu (FC) reagent solution made from phosphowolframic acid and phosphomolybdic acid. After oxidizing the phenolic compound, these two compounds will form a blue complex compound. The total phenolic content in black soybean tempeh and commercial soybean beverages is expressed in mg GAE (Gallic Acid Equivalent) / ml sample units. GAE is used as a reference to measure the amount of phenolic compounds contained in a material. Gallic acid is often used as a standard for testing phenolic compounds because it has high stability, is easy to obtain in pure form, is often found in food product matrices, and has a lower price than other standards (Pereira et al., 2018). AEAC is used as a reference to measure the amount of antioxidant compounds contained in a material. Ascorbic acid, also known as vitamin C, is water soluble, with white crystals forming and susceptible to oxidation when exposed to air (Tareen et al., 2015). Additionally, ascorbic acid is also known as a strong source of antioxidants and can donate one hydrogen atom to form relatively stable free radicals.



Results ± SD; numbers followed by different superscripts indicate significant difference; F1: sappan wood 3%, cinnamon 5%; F2: sappan wood 2.5%, cinnamon 1%; F3: sappan wood 2%, cinnamon 1.5%; F4: without sappan wood and cinnamon; C: commercial yellow soybean beverage.

Figure 1. Total phenolic content of black soybean beverage added with sappan wood and cinnamon bark.

Based on the results (Figure 2), black soybean tempeh beverage without sappan wood and cinnamon addition was 29.11 mg GAE/g, significantly lower than the other three formulations. This is followed by black soybean tempeh beverage with the addition of 2.5 g of sappan wood and 1 g of cinnamon, amounting to 50.85% of black soybean tempeh beverage without the addition of sappan wood and cinnamon. Furthermore, soybean tempeh beverage with the addition of 2 g of sappan wood and 1.5 g of cinnamon was 45.52% of black soybean tempeh beverage without the addition of sappan wood and cinnamon, due to the addition of sappan wood and cinnamon to black soybean tempeh beverage. During processing into tempeh, soybean is fermented with *Rhizopus* sp, which is known to

P-ISSN: 2715-6214
E-ISSN: 2964-4909

increase the value of total phenolic content (Suharto et al., 2017). This is supported by previous research by Rahayu & Astuti (2017), which stated that the total phenolic content value in black soybean beverage was 20.06 mg GAE/g, lower than black soybean tempeh beverage but higher than the control commercial yellow soybean beverage—amounted to 19.92 mg GAE/g. This is because black soybeans have a higher total polyphenol content in the form of flavonoids and anthocyanins than yellow soybeans (Nurisyah et al., 2019).

Adding sappan wood and cinnamon to soybean tempeh beverage significantly affected the total phenolic content value (Figure 2). Black soybean tempeh beverage with the addition of 3 g sappan wood and 0.5 g cinnamon has a value of 66.53 mg GAE/g, which is the highest value compared to the other two formulations, followed by black soybean tempeh beverage with the addition of 2.5 g sappan wood and 1 g cinnamon amounted to 59.22 mg GAE/g. The lowest total phenolic content value was in black soybean tempeh beverage with the addition of 2 g sappan wood and 1.5 g cinnamon amounting to 53.43 mg GAE/g. Total phenolic content showed a higher increase along with the increase in the addition of sappan wood and the decrease in the addition of cinnamon, as did the increase in the value of the antioxidant compound levels. This was due to the content of active compounds contained in the ingredients for adding sappan wood and cinnamon, which are affected by antioxidant compounds, namely in the form of flavonoids, which donate one electron so that they can stabilize free radical compounds so that the higher the flavonoid content in the ingredients which causes an increase in total phenolic levels will be followed by with an increase in levels of antioxidant compounds (Dewi et al., 2018). The active compounds in sappan wood are primarily flavonoids that prevent and convert free radicals and prevent their chain reactions, such as brazilin, brazilein, 3'-O-methyl brazilin, sappanin, chalcone, and sappan-chalcone (Rina, 2013). Meanwhile, the active compounds in cinnamon are in the form of essential oils, including phenols and polyphenols in flavonoids and tannins.(Ervina et al., 2016).

Brightness level of black soybean tempe beverage

Color brightness level testing includes three parameters: L*, a*, and b*. The L point indicates lightness or the brightness level of the sample with a range of 0 (black) to 100 (white). A higher L value indicates a whiter sample, and vice versa. The color parameter a, namely redness, shows the level of redness or greenness of the sample with a range of +60 (red) and -60 (green); the higher the value of parameter a, the sample was closer to red, while the lower the value of parameter a, the sample was closer to green. The color parameter b, namely Yellowness, shows the level of Yellowness or bluishness with a range of +60 (yellow) and -60 (blue); the higher the value of parameter b, the sample was closer to yellow, while the lower the value of parameter b, the sample was closer to blue (Table 2).

Table 2. Brightness level of black soybean tempe beverage

Sample	Parameter		
	L*	**a***	**b***
F1	$34.49^{a} \pm 0.79$	$1.97^{b} \pm 0.12$	$4.89^{c} \pm 0.09$
F2	$33.82^{a} \pm 0.05$	$2.01^{b} \pm 0.15$	$5.05^{c} \pm 0.24$
F3	$34.01^{a} \pm 0.03$	$1.98^{b} \pm 0.22$	$4.09^{a} \pm 0.04$
C	$66.94^{b} \pm 0.10$	$-1.94^{a} \pm 0.01$	$4.53^{b} \pm 0.15$

Results ± SD; numbers followed by different superscript indicate significant difference; F1: sappan wood 3%, cinnamon 5%; F2: sappan wood 2.5%, cinnamon 1%; F3: sappan wood 2%, cinnamon 1.5%; F4: without sappan wood and cinnamon; C: commercial yellow soybean beverage

The addition of sappan wood and cinnamon to black soybean tempeh beverage had no significant effect on brightness but had a significant effect on commercial soybean beverage (Table 2). Based on the results of brightness analysis of the L point, black soybean tempeh beverage formulation 1 with the addition of 3 g of sappan wood and 0.5 g of cinnamon has the highest brightness level of the L parameter of 34.49 compared to the other two formulations. This is due to the addition of cinnamon in small quantities. Continuing with the black soybean tempeh beverage, formulation 3, with the addition of 2 g of sappan wood and 1.5 g of cinnamon, has a lower or darker brightness value of 34.01 compared to

P-ISSN: 2715-6214
E-ISSN: 2964-4909

formulation 1 due to the content of the cinnamaldehyde compound produced by cinnamon. The addition of cinnamon in large quantities can produce a darker color (Yulianto & Widyaningsih, 2013).

Furthermore, the black soybean tempeh beverage, formulation 2, with the addition of 2.5 g of sappan wood and 1 g of cinnamon, had the smallest or darkest value of 33.82 among the other two formulations. This is because sappan wood can be completely oxidized at a high pH. According to research by Hastuti & Rustanti (2014), the pH value of cinnamon is 8.5, which is included in the alkaline group; an increase of 0.5g of cinnamon in the sample is thought to be able to increase the atmosphere of the sample to become alkaline. The control test results of commercial soybean beverage had the highest brightness level, namely 66.94, when compared with black soybean tempeh beverage. This was due to the difference in raw materials used for yellow and black soybeans.

The results showed that the addition of sappan wood and cinnamon to black soybean tempeh beverage had no significant effect, as shown in alphabetical order superscript in the same column, but had a significant effect on commercial soybean beverage. Black soybean tempeh beverage formulation 2, with the addition of 2.5 g of sappan wood and 1 g of cinnamon, had the highest or red value of 2.01 compared to the other two formulations. According to previous research by Rina (2012), sappan wood will oxidize and produce a red color in an alkaline environment. The alkaline atmosphere in black soybean tempeh beverage is thought to be the result of adding water and cinnamon, as well as the effect of fermentation during tempeh making. The pH value for drinking water quality is following the requirements of SNI 3553-2015, namely in the range of 6-8.5, and the pH value for cinnamon is 8.5, which is included in the alkaline group. During fermentation, protein will be degraded into free amino acids, increasing the pH value (Nurrahman et al., 2012). Continuing with black soybean tempeh beverage formulation 3 with adding 2 g of sappan wood and 1.5 g of cinnamon, the highest value was 1.98 compared to black soybean tempeh beverage formulation 2. This can be expected due to the decrease of 0.5 g of sappan, so the content sample was dominated by the cinnamaldehyde content produced by cinnamon.

Furthermore, black soybean tempeh beverage formulation 1, with the addition of 3 g of sappan wood and 0.5 g of cinnamon, had the lowest value of 1.97 compared to the other two formulations. The higher amount of sappan wood in black soybean tempeh beverage reduced the pH of the sample to become acidic, thus preventing the oxidation of the brazilin in sappan. According to research by Hastuti & Rustanti (2014), the pH value of sappan wood is 4.5-5, an acid group. Meanwhile, the brazilin compound in sappan wood will be red at a pH of 6-7 and purplish red at a pH of 8.(Hastuti & Rustanti, 2014). The control test results of commercial soybean beverage had a lower level of parameter a of -1.94 compared to black soybean tempeh beverage, which was shown by the original color of commercial soybean beverage, without the addition of other ingredients such as sappan wood and cinnamon. Parameter b results show that adding sappan wood and cinnamon in formulations 1 and 2 significantly affects formulation three and commercial soybean beverage controls in the same column (Table 2). This shows that the difference in adding sappan wood and cinnamon affected the level value of parameter b for black soybean tempeh beverage. Based on the results, black soybean tempeh beverage was added with 2.5 g of sappan wood, and 1 g of cinnamon has the highest or yellowest value of 5.05 compared to the other two formulations. This was due to the higher sappan wood compared to cinnamon. Increasing cinnamon by 0.5 g increased the sample pH to alkaline, hence the yellow color, as oxidized brazilin was combined with the yellow color produced by the cinnamaldehyde in cinnamon. Formulation 1, with the addition of 3 g of sappan wood and 0.5 g of cinnamon, has a lower value of 4.89 compared to formulation 2. This can be due to the low cinnamaldehyde compound in cinnamon, thus brazilin oxidation, which is a lower amount. Formulation 3, with the addition of 2 g of cinnamon and 1.5 g of cinnamon, had the lowest value of 4.09 compared to the other two formulations. This can be expected because the cinnamaldehyde compound is high in cinnamon and the low brazilin compound in sappan, so the cinnamaldehyde compound content will dominate the color of the sample more than the brazilin compound. Commercial soybean beverage had a lower value than that of formulations 1 and 2 but higher than formulation three due to the natural color content of yellow soybeans.

CONCLUSION

P-ISSN: 2715-6214
E-ISSN: 2964-4909

Sappan wood and cinnamon bark addition to black soybean tempeh beverage had a significant effect on antioxidant levels of 82.07-86.93 mg AEAC/ml and total phenolics of 53.43 to 66.53 mg GAE/ml with higher sappan wood increased the level. Brightness parameter L*: 33.82-34.49, a*: 1.97-2.01, b*: 4.09-5.05 for black soybean tempe beverage products. The highest antioxidant properties and brightness level were obtained by adding 3% and 0.5% of sappan wood and cinnamon bark, respectively.

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