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The addition of crude gambir extract in the production of functional robusta coffee powder

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Abstract

This study aims to improve the functional properties of robusta coffee by adding crude gambir extract. A completely randomized non-factorial design with 5 treatments is used and each treatment was repeated three times. The treatments consist of: robusta coffee powder (%): crude gambir extract (%) F1 = 100:0, F2 = 95:5, F3 = 90:10, F4 = 85:15, and F5 = 80:20. The results showed that the addition of crude gambir extract has a significant effect on increasing the total phenol value from 10.65 to 101.20 mg/mL GAE and the antioxidant activity with an IC₅₀ value of 583.06 to 40.10 µg/mL, acidity level (5.43-5.51), and the solubility percentage of 27.55-31.15%. Furthermore, the addition of crude gambir extract have no significant effect on the taste, color, and aroma of the functional coffee produced.

Keywords: antioxidant; crude gambir extract; functional; gambir; robusta coffee.

Practical Application: Mixed powder as separately powder of coffee powder at one package and crude gambir extract on the other package and it will be mixed a minute before serve coffee drink.

1 Introduction

Functional and sensory properties are the two most important factors that influence consumer attractiveness to coffee drinks. The functional properties of coffee are influenced by chlorogenic acid while the sensory properties are influenced by caffeine levels. Chlorogenic acid and caffeine are the most dominant components in coffee and both affect the health of the human body. However, high caffeine level has a negative impact on human health. Chlorogenic acid and caffeine in robusta coffee are higher than in Arabica coffee (Chu, 2012). Jeszka-Skowron et al. (2020) added that chlorogenic acid is the main bioactive compound with antioxidant properties. According to Wolska et al. (2017), the antioxidant compounds in robusta coffee are higher than Arabica, namely 43.63% and 36.18%, respectively. Herawati et al. (2019) and Bobková et al. (2020) stated that roasting coffee has a significant impact on reducing antioxidant properties. The study conducted by Kuncoro et al. (2018) showed that coffee roasted at temperatures of 100, 110, and 120 °C decreases the caffeine content by 13, 18, 25% and chlorogenic acid by 37, 50, 59%, respectively.

Many studies have been carried out in recent years to maintain the antioxidant properties of coffee. Isac-Torrente et al. (2020) explained that processing coffee using the capsule method reduces antioxidant activity and overall phenol content. According to Jeszka-Skowron et al. (2021), in addition to the roasting process to preserve the chlorogenic acid and total phenol in coffee, other special treatment processes that can be carried out are steaming, decaffeination, or natural fermentation (luwak coffee). Haile

& Kang (2020) carried out a spontaneous fermentation using *Wickerhamomyces anomalus* (KNU18Y3) strain on green coffee beans. Similarly, Cheng et al. (2019) showed that coffee processing using the vacuum drying method with the aid of a microwave can maintain the phenolic compounds in green coffee. Furthermore, Microwave-roasted coffee beans have a lower reducing power in IC₅₀ and total phenol compared to unprocessed coffee beans (Salamatullah et al., 2021). Samsonowicz et al. (2019) explained that the addition of bioactive compounds from cereal herbs increases the antioxidant and total phenols in coffee drinks and Bajaj & Ballal, (2021) reported that instant coffee incorporated with *Ganoderma lucidum* extract powder has a large number of anti-tumor and anti-oxidative properties because it contains triterpenoids as the main bioactive compounds. Rashidnejad et al. (2021) explained that the addition of milk to coffee can reduce its functional properties due to the interaction of milk components with the phenolic compounds in coffee.

A previous study showed that coffee contains bioactive compounds, which interact with each other to produce several beneficial effects when combined with other bioactive compounds. One of the bioactive compounds potentially used is catechin derived from the gambir plant. This plant has high antioxidants, namely (+)-catechins (Yeni et al., 2014). Santoso et al. (2018, 2019) added that the inclusion of gambir catechin extract in canna-based edible films increases antioxidant and antibacterial properties, respectively. Pambayun et al. (2019) stated the addition of gambir catechin extract in the formulation of marshmallow

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candy inhibits the growth of the bacterium *Streptococcus mutans*, which causes human dental plaque. Kamsina & Firdausni (2018) reported that the addition of gambir catechin extract to yam cake can increase the shelf life by inhibiting the growth of *Escherichia coli* and *Salmonella* bacteria with an inhibitory value of 11 mm and 15 mm, respectively. The incorporation of gambir catechin extract of about 600 ppm in margarine can reduce free fatty acids in these products (Aini et al., 2020).

24 2 Material and methods

2.1 Tools and materials

The tools used consist of a blender (Philips, Holland), hot plate, Whatman No 1 filter paper, analytical balance (Kenko, Japan), drying oven, pH meter (Eutech, Malaysia), rotary vacuum evaporator, 80 mesh filter, and a spectrophotometer (A and E Lab, USA). The various materials used are robusta coffee powder with medium to dark roast type from gadRaye Coffee Pagar Alam, South Sumatra; gambir powder from Toman Village, Musi Banyuasin, South Sumatra; tannic acid; 2,2-diphenyl-1-picrylhydrazyl (DPPH); ethanol, methanol, gallic acid, pH 4 buffer, pH 7 buffer, and Folin-Ciocalteu from the chemical laboratory of agricultural products, Sriwijaya University.

2.2 Study design

A non-factorial completely randomized design was used in this study. A total of five treatments are carried out using the percentage ratio of robusta coffee powder with crude gambir extract (F1 = 100:0, F2 = 95:5, F3 = 90:10, F4 = 85:15, and F5 = 80:20) with 3 repetitions. In addition, 15 samples were used for each parameter in such a way that the total samples used for the 5 parameters were 75. Furthermore, the data were analyzed using an analysis of variance with the SAS Windows 9 program. The parameters observed consist of total phenol, antioxidant activity, acidity level (pH), solubility percentage (Gontard et al., 1993), and the sensory test was conducted using the hedonic method of taste, color, and aroma on a scale (1 = dislike very much, 2 = dislike, 3 = like, and 4 = very much like) (Pratama, 2018).

a. Total Phenol

The total phenol level was determined based on Murni et al. (2015) which have been modified are as follows: 50 g of coffee powder were weighed, 500 mL of distilled water were added and stirred until homogeneous. 100 mg of the extract are then dissolved to 10 mL with distilled water to obtain a concentration of 10 mg/mL. The concentration of 10 mg/mL was pipetted into 1 mL and diluted to 10 mL with distilled water and the concentration of the extract was 1 mg/mL. Pipette 0.2 mL of extract, add 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, shaken and allowed to stand for 8 minutes. Add 3 mL of 10% Na₂CO₃ to the mixture, leave the solution for 2 hours at room temperature. The absorption was measured using a UV-Vis spectrophotometer with an absorption wavelength of 765 nm. The phenol content was obtained as mg gallic acid equivalent/g sample and created a calibration curve with the regression equation $y = ax + b$, where X is the concentration and Y is the absorbance.

Preparation of gallic acid calibration curve with Folin-Ciocalteu. Reagent: 50 mg of gallic acid was weighed, 1 mL of 96% ethanol was added, distilled water was added until the final volume was 50 mL in such a way that a concentration of 1 mg/mL was gained as the mother liquor. The mother liquor was pipetted to 1 mL, 1.25 mL, 1.5 mL, 1.75 mL, and 2 mL, respectively and then diluted with distilled water to a final volume of 10 mL at concentrations of 100, 125, 150, 175, 200 ppm gallic acid. Furthermore, a 0.2 mL pipette each concentration of the gallic acid solution was added, then 15.8 mL distilled water and 1 mL Folin-Ciocalteu reagent are added and the mixture was stirred until homogeneous and allowed to stand for 8 minutes. 3 mL of 10% Na₂CO₃ solution was added, shaken homogeneously, and then allowed to stand for 2 hours at room temperature, and the absorption was measured at an absorption wavelength of 765 nm.

b. Antioxidant Activity (IC₅₀)

The antioxidant activity test was carried out by calculating the value using the DPPH method (1.1 diphenyl 2 picrylhydrazyl) according to Association of Official Analytical Chemists (2005), which has been modified: The combined ground coffee sample weighed ± 0.1 g and then dissolved with 100 mL of methanol (1000 ppm). The sample solution was formulated into 5 concentration series, namely 100 ppm, 80 ppm, 60 ppm, 40 ppm, and 20 ppm. A series of 100 ppm dilution was created from 0.5 mL of sample added to 4.5 mL of methanol in a test tube and homogenized. The 80 ppm dilution series was composed from 0.4 mL of the sample, 4.6 mL of methanol was added, placed in a test tube, and homogenized. A series of 60 ppm dilutions were produced from 0.3 mL of sample added to 4.7 mL of methanol in a test tube and homogenized. A 40 ppm dilution series was prepared from 0.2 mL of sample added to 4.8 mL of methanol in a test tube and homogenized. A series of 20 ppm dilutions were prepared from 0.1 mL of sample added to 4.9 mL of methanol in a test tube and homogenized. In addition, 0.2 mL of each concentration was taken and 2 mL of DPPH solution (0.0038 g DPPH plus 50 mL methanol) was added and homogenized with a vortex. The DPPH solution was placed into a cuvette and the absorbance value was measured using a spectrophotometer (wavelength 517 nm) and was recorded as absorbance blank (A_{blank}). The solution that has been vortexed was left in a dark room for 30 minutes and then placed into a cuvette and absorbance value was measured using a spectrophotometer (wavelength 517 nm) and recorded as sample absorbance (A_{sample}). Antioxidant capacity (% inhibition) can be calculated using the following formula: Percent Inhibition (%) = (A_{blank} - A_{sample}) / A_{blank} × 100%. The value of antioxidant capacity (% inhibition) at each concentration was used to find a linear equation. The linear regression equation ($y = ax + b$) was obtained to determine the IC₅₀ value. The value of y = 50 in such a way that the value of x can be obtained as the value of the antioxidant activity.

c. Acidity Level (pH)

According to Kumesan et al. (2017), the pH value was determined using a modified pH meter as follows: The pH meter must first be calibrated to the sensitivity of the pointer with a pH 7 buffer solution. The sample of about 10 g was weighed

and homogenize with 20 mL of distilled water for 1 minute, and pour into a 10 mL beaker. The electrode was immersed in the sample and waited for a while until the pH is stable. The pH value can be directly read on the pH meter scale, after which the electrodes were removed and rinsed with distilled water.

d. Sensory Test

A sensory test was carried out on functional coffee using the hedonic method with semi-trained panelists. A panel of 25 students of the Agricultural Products Technology Study Program, Sriwijaya University who had previously been trained in testing the properties of ground coffee and had studied plantation plant processing technology courses, especially coffee processing were used in this study. The functioning of the hedonic test is based on (Pratama, 2018), where panelists are asked to provide responses regarding the level of likes or dislikes of the sample presented. Samples are presented one at a time, then the panelists assess the sample based on the level of preference for color, aroma, and taste. Based on available value standards. Score scale: strongly dislike = 1, dislike = 2, like = 3, and like very much = 1).

2.3 Procedure

This study was carried out in 2 different stages, namely the production of crude gambir extract and functional robusta coffee.

a. The Production of Crude Gambir Extract

The crude gambir extract was produced using a modified maceration method (Damanik et al., 2014) as the 80% crude extract of gambier produced: dry gambir powder, pulverized in a blender and sieved through an 80 mesh sieve. A total of 100 g of dry gambir powder was added to the Erlenmeyer flask, and then 300 mL of 70% ethanol was poured into it and the maceration process as performed for 24 hours. The macerated powder was filtered using Whatman No.1 filter paper to obtain gambir filtrate. The filtrate was evaporated using a rotary vacuum evaporator at a temperature of 85 °C until the ethanol evaporates. Furthermore, the process continued with the drying using a drying oven at a temperature of 85 °C until the crude gambir extract was obtained. The extract was mashed with a blender and filtered using an 80 mesh sieve and was placed in an airtight and light-tight bottle.

b. The Production of Functional Coffee

Functional coffee was produced based on a predetermined formulation of robusta coffee powder and crude gambir extract. Furthermore, functional coffee of 30 g with a powder size of 80 mesh was placed in a beaker, and 250 mL of coffee was added at 80 °C and stirred for 15 seconds using a magnetic stirrer. Functional coffee drinks are filtered using a bleached paper filter and can be analyzed according to predetermined parameters.

3 Results and discussion

3.1 Solubility percentage

The measurement of the solubility percentage was carried out on the conventional coffee powder. Functional robusta coffee

powder has a solubility percentage of 27.55-31.15% with the lowest F1 treatment and the highest F5 treatment. The average solubility percentage is shown in Figure 1.

The percentage comparison of the coffee powder with crude gambir extract has a significant influence on the percentage solubility of the functional coffee produced. The solubility percentage increases with the higher concentration of crude gambir extract (Figure 1). This is influenced by the polarity nature of the catechin compounds in crude gambir extract. Pambayun et al. (2007b) stated that gambir powder extracted using the maceration method with a mixture of water and ethanol as a solvent with polarity index of 7.7 produced the highest catechin extract. Based on these results, it can be concluded that the catechin extract is polar. Furthermore, polar compounds only dissolve polar solvents such as ethanol, methanol, butanol, and water, while non-polar compounds only dissolve in non-polar solvents, such as ether, chloroform, and n-hexane (Leksono et al., 2018). Yeni et al. (2017) states that catechin compounds from gambir powder dissolve well in hot water.

These results are similar to a study performed by Budiyanto et al. (2021), which states that the solubility of kirmanan and juremian clones at different degrees of roasting ranged from 21.67-45.00%. Azizah et al. (2019) and Siregar et al. (2020) explained that arabica coffee processed by the fermentation method using *Saccharomyces cerevisiae* and lactic acid bacteria results in a solubility value of 30.35-30.74% and 4.26-4.56%, respectively.

3.2 Total phenol

The total phenol functional coffee ranges from 10.65-101.20 mg/mL GAE. The highest total phenol is found at the F5 treatment at 101.20 mg/mL GAE and the lowest with the F1 treatment at 10.65 mg/mL GAE. The average total functional phenol of coffee is shown in Figure 2.

The honest significance difference test (Figure 2) shows that the total functional coffee phenol produced increases significantly with the increase in the concentration of crude

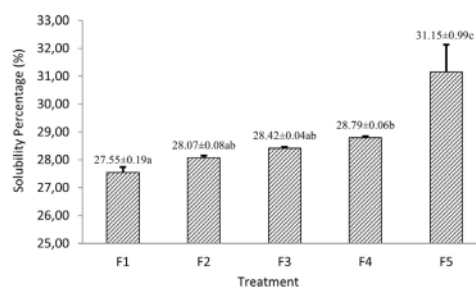


Figure 1. The average solubility percentage of functional robusta coffee powder. Description: F1 = 100% robusta coffee powder: 0% crude gambir extract. F2 = 95% robusta coffee powder: 5% crude gambir extract. F3 = 90% robusta coffee powder: 10% crude gambir extract. F4 = 85% robusta coffee powder: 15% crude gambir extract. F5 = 80% robusta coffee powder: 20% crude gambir extract.

The addition of crude gambir extract in robusta coffee powder

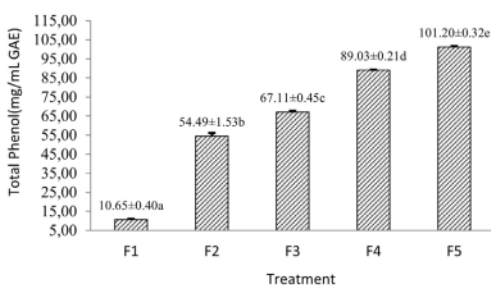


Figure 2. The average total phenol of functional robusta coffee powder.

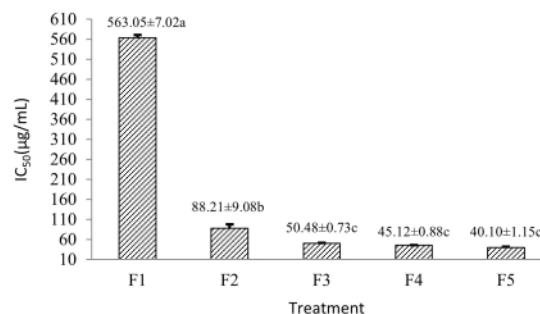


Figure 3. The average IC₅₀ value (µg/mL) of functional coffee.

gambir extract. In addition, it can be explained that the extract contains antioxidants. Pambayun et al. (2007b) and Rauf et al. (2010) explained that gambir powder contains catechin with a total phenol of 50.96% and 62.13%, respectively. Kamsina et al. (2020) report that adding gambir catechin extract increases total phenol in wet noodles by 84%.

The total phenol produced from coffee was similar to that of Nichmah et al. (2019), which shows that cinnamon coffee bags contain a total phenol of 34.46 mg/mL GAE. This functional coffee has a higher total phenol content than oven-roasted coffee, namely 16.66 mg/mL GAE (Alkaltham et al., 2020). The well-known branded coffee that is circulating in Indonesia is 46.27 mg/mL GAE (Lelyana & Cahyono, 2015) and roasted arabica coffee is 49.90 mg/mL GAE (Odžaković et al. 2016). Compared to the study by Gornas et al. (2016), unroasted green robusta coffee contains 208.89 mg/mL GAE of total phenol and 119.22 mg/mL GAE in roasted coffee.

3.4 Antioxidant activity

The antioxidant activity of this functional coffee uses IC₅₀. The resulting IC₅₀ values range from 40.10-583.06 µg/mL with the highest value in F1 treatment and lowest in F5. The average IC₅₀ value of the functional coffee produced is shown in Figure 3.

The IC₅₀ value decreases along with the concentration of the crude gambir extract (Figure 3). This means that the antioxidant activity of functional coffee increases with the higher concentration of crude gambir extract, and this increase is due to its antioxidants. Gambir (*Uncaria gambir* Roxb) is a plant that contains derivatives of polyphenolic compounds, namely: catechins, tannins, epicatechin, quercetin epigallocatechin, and others. Most of the catechin are found in gambir, hence the plant is known as antioxidants and antibacterials (Aditya & Ariyanti, 2016). Kurniatri et al. (2019) explained that gambir extract contains 92.45% of catechin compounds.

Compared to the study by Haile & Kang (2020), green coffee beans fermented with *Wickerhamomyces anomalus* (Strain KNU18Y3) have an IC₅₀ of 25.51 ppm. Desai et al. (2019) cover the antioxidant compounds of green coffee using the 12.78 µg/mL microencapsulation method, the results are lower than that in this study. This was higher than that of Bobková et al. (2020), which stated that low-quality roasted green coffee powders are about 69.08-78.55 µg/mL and Isindar et al. (2017). Masek et al.

(2020) reported that robusta coffee and robusta green coffee contain antioxidants with an IC₅₀ of 2210 µg/mL and 81.6 µg/mL, respectively.

3.5 Acidity level (pH)

The functional coffee has a pH ranging from 5.43-5.51 with the lowest reported in the F5 treatment and the highest in F1. The average pH is shown in Figure 4. The average pH is shown in the figure below.

The acidity level (pH) of functional coffee decreases along with the increase in the concentration of crude gambir extract (Figure 4). This is because the extract is a weak acid and stable under acidic conditions. These results agree with Pambayun et al. (2007a) according to which the catechin in the gambir extract has many hydroxyl groups (characteristic of Arrhenius base compounds) because it binds directly to the phenolic ring and forms acidic compounds. Yeni et al. (2017) respectively stated that the antioxidant activity of gambir catechin extract increased and catechin is a weak acid that can be easily oxidized at a neutral pH value (pH 6.9) and stable at a low pH value (pH 2.8 and 4.9). The pH value of functional coffee hardly differs from robusta coffee, which is around 5.47 (Suwarni et al., 2017), fermented robusta coffee 5.25-5.37 (Budi et al., 2020), brewed robusta coffee 5,16-5,69 (Aditya & Ariyanti, 2016), and instant powder beverage with mangosteen-peeled coffee ranged from 5,26-5,63 (Apriani et al., 2016).

The relationship between the parameters of solubility percentage, total phenol, antioxidant activity (IC₅₀), and pH is closely related to the content of catechin compounds in crude gambir extract. It is known that catechin compounds are polar, classified as phenolic compounds, antioxidants, and stable at acidic pH conditions, so that the higher the crude gambir extract content, the higher the solubility level and total phenol, the lower the IC₅₀ and pH values.

3.6 Sensory test

The method used to sensory test the taste of functional coffee taste, flavor, and the color is hedonic using 25 semi-trained panelists. The test results are presented in Figure 5.

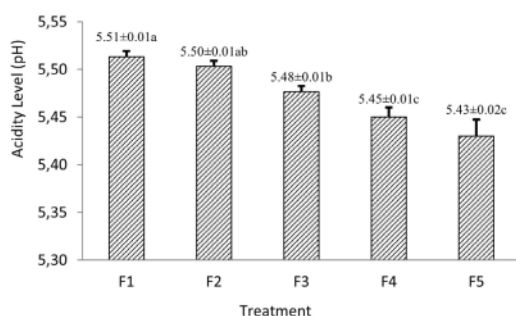


Figure 4. The average acidity level (pH) of functional robusta coffee.

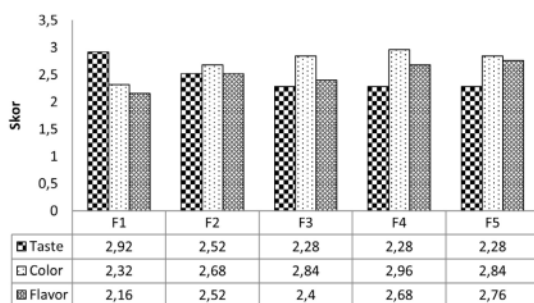


Figure 5. Sensory test on taste, color, and flavor of functional robusta coffee.

The average hedonic scales for the taste, color, and flavor of functional coffee are 2.64-3.38; 2.8-3.16; and 2.8-3.4, respectively (Figure 5). The results of the Friedman-Conover test show that the combined treatment of robusta coffee powder with crude gambir extract has no significant influence on the taste, color, and flavor of the functional coffee produced. Furthermore, it is interpreted that the addition of crude gambir extract in a concentration of 20% (w/w) does not change the parameters mentioned. Furthermore, The sensory test also showed that the score for taste, color, and flavor of functional coffee is above 3, which means that this coffee is preferred by the members of the panel.

4 Conclusion

The addition of crude gambir extract in robusta coffee powder improves the antioxidant properties of functional robusta coffee with a sensory preference of the panelists. Robusta coffee which is incorporated with crude gambir extract has total phenol of 10.65-101.20 mg/mL GAE, antioxidant activity (IC_{50} = 583.06-40.10 µg/mL), acidity level (5.43-5.51), and the solubility percentage of 27.55-31.15%.

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