

Phenolic content and α -glucosidase inhibition of tubruk-brew kalosi coffee processed by different post-harvest processing

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ABSTRACT

Brewed coffee contains phenolic compounds, bioactive compounds known for inhibiting the α -glucosidase enzyme. This study evaluated the total phenolic content and α -glucosidase inhibitory activity of tubruk-brewed prepared from Kalosi Enrekang Arabica coffee processed by different post-harvest processing. Coffee beans were processed using natural, honey, and full-washed methods, roasted to a medium level, and brewed using the tubruk method. Results revealed that coffee brewed from beans processed using the natural method exhibited the highest phenolic content (3.02 ± 0.11 g GAE/100 g) and α -glucosidase inhibitory activity ($18.48 \pm 1.31\%$), compared to those processed with honey and full-washed methods. These findings highlight that post-harvest processing significantly impacts total phenolic content and bioactivity. A higher phenolic content in brewed coffee corresponds to an enhanced ability to inhibit the α -glucosidase enzyme. Thus, post-harvest processing methods play an important role in determining the functional properties of coffee.

Key words: Arabica Enrekang Coffee; bioactive compound; natural processing; full-washed processing; honey processing.

1 INTRODUCTION

Kalosi Enrekang Arabica coffee is one of Indonesia's certified Geographical Indication products (Ministry of Law and Human Rights of The Republic of Indonesia, 2024). This coffee has a complex flavor and aroma (Yulianti et al., 2024). Differences in post-harvest processing of this Arabica coffee affect the flavor and aroma of the brewed coffee (Yulianti et al., 2023a, 2024). Moreover, post-harvest processing also influences the chemical composition (Sunarharum; Williams; Smyth, 2014). Currently, there are various post-harvest processing methods, but the most commonly applied are natural, honey, and full-washed processing (Várady et al., 2022). These three methods involve different stages. In the natural process, harvested ripe coffee cherries are dried directly without undergoing skin removal and fermentation (Duguma; Chewaka, 2019; Kim et al., 2022). The cherries are de-pulped, fermented, and dried in the honey process. In the full-washed process, the cherries are de-pulped, soaked in water for 8-24 hours, washed to remove mucilage, and dried (Sanz-Urbe et al., 2017). These differences in processing stages result in variations in the chemical composition and bioactivity of the brewed coffee (Yulianti et al., 2024). Understanding how these processing methods influence bioactive compounds such as phenolic content and their related bioactivities could contribute to optimizing processing techniques for functional beverage development.

The chemical composition of brewed coffee is influenced by various factors, one of which is post-harvest processing. According to Yulianti et al. (2024), brewed coffee derived from

natural, honey, and full-washed processes has different chemical compositions, with each process producing characteristic compounds. Additionally, post-harvest processing also affects the concentration of bioactive components in green beans and roasted beans (Yulianti et al., 2022). One of the bioactive components in brewed coffee is phenolic compounds. According to Várady et al. (2022), post-harvest processing affects the concentration of phenolic compounds in green beans. Arabica coffee roasted at a light level and brewed using the cold brew technique has a higher total phenolic compound concentration compared to hot brew (Pan et al., 2023). However, medium roasting is the most optimal level for producing a complex aroma (Bhumiratana; Adhikari; Chambers, 2011). Gloess et al. (2013) stated that the brewing technique correlates with the chemical composition of the coffee. In Indonesia, the *tubruk* method is a popular brewing technique (Sunarharum; Farhan, 2020). This method is also used in *specialty coffee* evaluation (Specialty Coffee Association of America - SCAA, 2015). The effect of post-harvest processing on the phenolic concentration in coffee brewed using the tubruk method remains insufficiently studied, necessitating further comprehensive research. This study sheds light on the connection between distinct post-harvest processing techniques and the health-beneficial phenolic content in tubruk coffee.

The bioactivity of brewed coffee is linked to the presence of bioactive components. According to Yulianti et al. (2024), Kalosi Enrekang Arabica coffee brews contain various bioactive components, particularly phenolic compounds,

which play a role in antioxidant activity. Additionally, brewed coffee has potential in preventing type 2 diabetes by inhibiting the α -glucosidase enzyme (Herawati et al., 2019). Research conducted by Herawati et al. (2024) revealed that Arabica coffee brews from different regions and brewed using three different brewing methods exhibited varying levels of α -glucosidase enzyme inhibition. Numerous studies have explored the potential of coffee as an α -glucosidase enzyme inhibitor (Alongi; Anese, 2018; Herawati et al., 2019; Ni et al., 2024). This study specifically investigates the effects of three post-harvest processing (honey, natural, and full-washed) on the phenolic content of coffee brewed using the tubruk method. It seeks to assess how distinct post-harvest processes influence the total phenolic content of tubruk coffee and its capacity to inhibit the α -glucosidase enzyme. The findings from this research are anticipated to contribute to a deeper understanding of optimizing post-harvest practices to enhance the functional properties of Indonesian specialty coffees.

2 MATERIAL AND METHODS

2.1 Materials

The material used was Arabica Kalosi Enrekang coffee, processed using three post-harvest methods : natural, full-washed, and honey. Other materials include pro-analysis ethanol (Merck, KGaA), Folin-Ciocalteu reagent (Merck, KGaA), sodium carbonate (Na_2CO_3), and gallic acid (Sigma-Aldrich).

2.2 Sample Preparation

The green beans were roasted process lasted for 12 minutes using a roasting machine from PT Kemenady Industri Mandiri (IKRI, Jember, Indonesia), with an initial temperature of 155°C and a final temperature of 196°C. Roasted beans ground using a coffee grinder (Gemilai CRM905, China). The coffee powder 8.25 g was brewed using 150 mL of hot water at 95°C. The brewed coffee was steeped for 10 minutes and filtered using a V60 filter (Hario, China). The brewed coffee was then ready for analysis.

2.3 Coffee Brew Color

The color of the brewed coffee was analyzed in terms of L^* (lightness), a^* (red-green), b^* (yellow-blue), and hue using

a chromameter (Konica Minolta CR400, Konica Minolta Inc., Japan)(Herawati et al., 2018).

2.4 Total Phenolic Content

Modifying Shetty et al.'s method (1995) quantified the total phenolic content. A 1 mL sample of brewed coffee was added to 5 mL of distilled water, 1 mL of 95% ethanol, and 0.5 mL of 50% Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 minutes, after which 1 mL of 5% Na_2CO_3 was added. The solution was homogenized and incubated for 60 minutes. The absorbance was measured using a UV-Vis spectrophotometer (UV-2450-Shimadzu Corp, Japan) at a wavelength of 725 nm. A calibration curve of gallic acid with concentrations ranging from 20 to 140 ppm ($R^2=0.99$) was used.

2.5 α -Glucosidase Inhibition

The analysis of α -glucosidase enzyme inhibition was conducted by modifying the method of Sancheti, Sancheti and Seo (2009). A reaction mixture was prepared consisting of 50 μL of 0.1 M phosphate buffer (pH 7), 25 μL of 0.5 mM p-nitrophenyl α -D-glucopyranoside dissolved in 0.1 M phosphate buffer (pH 7), ten μL of brewed coffee, and 25 μL of α -glucosidase solution. The mixture was incubated at 37°C for 30 minutes. The reaction was terminated by adding 100 μL of 0.2 M sodium carbonate solution. The absorbance was measured using a microplate reader (BioTek Epoch; Agilent Technologies, Inc.) at 410 nm. Acarbose was used as a standard comparator in this study.

2.6 Data Analysis

The data were analyzed using ANOVA, followed by Duncan's post-hoc test ($p < 0.05$). Prior to ANOVA, normality and homogeneity tests were performed (data not shown). Additionally, Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC) were conducted using XLSTAT software (Lumivero, France).

3 RESULTS

3.1 Brewed Coffee Color

Table 1 shows that post-harvest processing does not affect the color of the brewed coffee. The L^* value of the

Table 1: Color of tubruk brew from Kalosi- Enrekang Arabica coffee beans processed with different post-harvest processing.

Post-harvest Processing	L^*	a^*	b^*	Hue
Natural	37.25±0.01 ^a	7.49±0.01 ^a	11.53±0.07 ^a	56.97±0.14 ^a
Honey	36.66±0.01 ^a	7.46±0.01 ^a	11.58±0.01 ^a	57.21±0.05 ^a
Full-washed	36.95±1.82 ^a	7.32±1.36 ^a	11.46±1.78 ^a	57.22±7.20 ^a

Notes : L^* : lightness, a^* : red-green, and b^* :yellow-blue. Values are the average values of 3 replications and standard deviations. Values followed by different superscript letters are stated to be significantly different ($p < 0.05$)

brewed coffee ranged from 36.66 to 37.25. The a^* value of the brewed coffee was positive, ranging from 7.32 to 7.49, indicating a reddish hue in the brew. Similarly, the b^* value was positive (11.46–11.58), suggesting a brownish or yellowish tone. The hue value of the brewed coffee ranged from 56 to 57, indicating a reddish-brown dominant color.

3.2 Total Phenolic Content

Figure 1 shows that coffee brews from natural processed beans had a higher total phenolic content (3.02 ± 0.01 g GAE/100 g) than honey (2.32 ± 0.06 g GAE/100g) and full-washed processed (2.23 ± 0.07 g GAE/100g). The total phenolic content of tubruk brewed coffee is influenced by post-harvest processing ($p < 0.05$). This suggests that the coffee from natural processed beans

may offer greater to total phenolic content compared to other processing methods.

3.3 α -Glucosidase Enzyme Inhibition

The study showed that post-harvest processing significantly influenced the α -glucosidase inhibition activity in brewed coffee ($p < 0.05$) (Figure 2). Among the processing methods, coffee processed using the full-washed method exhibited the lowest inhibition activity ($15.74 \pm 0.14\%$) compared to those processed using the natural ($18.48 \pm 1.31\%$) and honey methods ($18.30 \pm 1.38\%$). However, despite these differences, the inhibition capacity of brewed coffee remains relatively low compared to Acarbose, which exhibits an inhibition rate of 98.13%. These findings highlight the potential of selecting specific post-harvest processing methods to enhance α -glucosidase inhibitory activity.

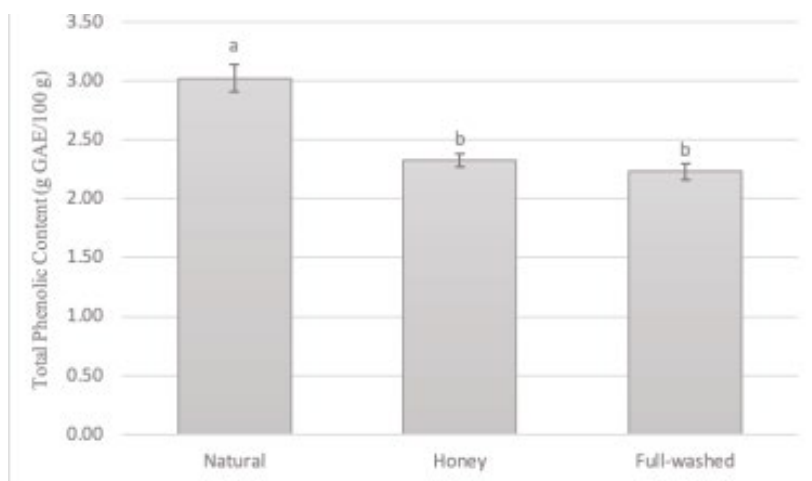


Figure 1: Total phenolic content in tubruk brew from Kalosi-Enrekang Arabica Coffee with different post-harvest processing methods (g GAE/100 g). Values are the average values of 3 replications and standard deviations. Values followed by different superscript letters are stated to be significantly different ($p < 0.05$).

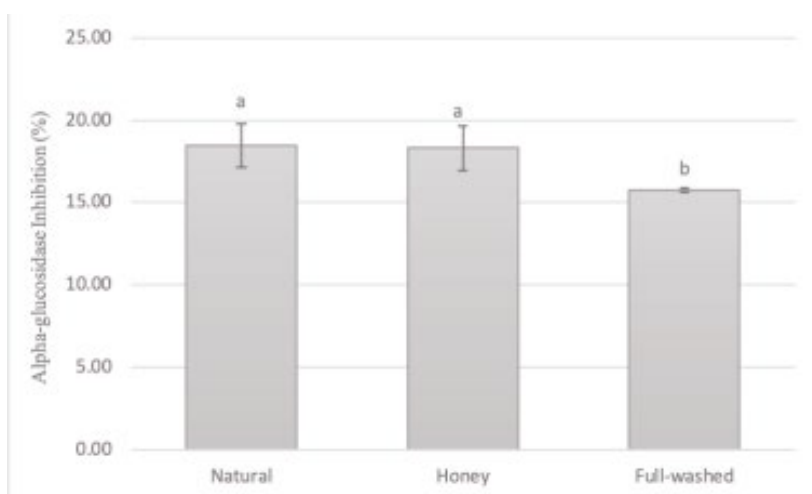


Figure 2: α -glucosidase inhibition activity of tubruk brew from Kalosi-Enrekang Arabica coffee with different post-harvest processing methods. Values are the average values of 3 replications and standard deviations. Values followed by different superscript letters are stated to be significantly different ($p < 0.05$).

3.4 Profile of Tubruk Brewed from Kalosi Enrekang Arabica Coffee

The biplot graph from Principal Component Analysis (PCA) shows the relationship between various active variables (total phenolic content, α -glucosidase inhibition, L^* , a^* , b^* , and hue) and active observations (post-harvest processing methods: full-washed, natural, and honey). The PCA model accounted for 100% of the total variance, with cumulative variability of F1 at 65.37% and F2 at 34.63%. The detailed contributions of each variable are as follows: L contributed 7.413% to F1 and 34.132% to F2, a^* contributed 22.494% to F1 and 5.669% to F2, b^* contributed 7.943% to F1 and 33.133% to F2, Hue contributed 20.532% to F1 and 9.371% to F2, total phenolic content contributed 21.810% to F1 and 6.959% to F2, and α -glucosidase inhibition contributed 19.809% to F1 and 10.737% to F2. The variables that contribute the most to the overall model are a^* , total phenolic content, hue, and α -glucosidase inhibition, as they have high contributions to F1.

Figure 3 shows that brewed coffee from natural processed beans is located in the upper right quadrant (positive F1 and F2) close to the vectors for total phenolic content and α -glucosidase inhibition. This indicates that coffee brewed

from natural processed beans has a high total phenolic content and stronger α -glucosidase inhibition activity. The brew made from full-washed coffee beans is located in the lower left quadrant (negative F1 and positive F2). This position indicates that it is far from the total phenolic content (TPC) and α -glucosidase inhibition vectors, suggesting that the full-washed method produces coffee with lower phenolic content and α -glucosidase inhibitory activity. In contrast, the brew made from honey-processed coffee beans is also located in the lower left quadrant (negative F1 and F2). This indicates that coffee brewed from honey-processed beans contains lower phenolic content than the natural process, but higher than the full-washed method. Furthermore, this also indicates that there is no difference in the α -glucosidase inhibition capacity between brewed coffee from honey-processed and natural.

Figure 3 shows the correlation between total phenolic content and α -glucosidase inhibition. Higher phenolic content tends to be associated with more significant α -glucosidase enzyme inhibition. Based on the results obtained, there are two main clusters: natural and a combination of honey and full-washed. This indicates that brewed coffee from natural processed beans has the highest dissimilarity to honey and full-washed processes.

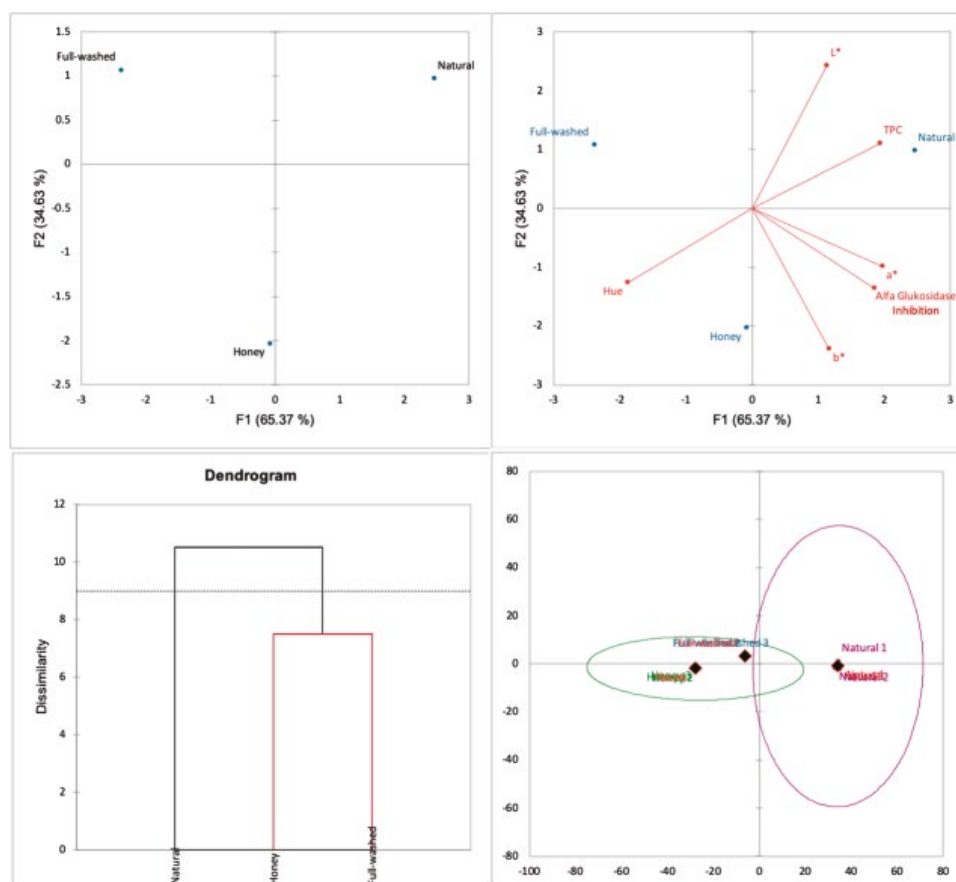


Figure 3: Biplot, dendrogram and discriminant analysis of tubruk brew from Kalosi-Enrekang Arabica coffee with different post-harvest processing methods.

4 DISCUSSION

The color of brewed coffee is not only a matter of consumer preference but is also related to the quality and characteristics of the coffee. The L^* value indicates the brightness of the brew; the lower the L^* value, the darker the coffee (Tsai; Jioe, 2021). Kalschne et al. (2019) and Yeager et al. (2022) stated the color of brewed coffee is correlated with its caffeine and melanoidin content. Melanoidins are formed during the Maillard reaction in roasting, and their concentration increases with roast level (Kurniawan et al., 2017). Besides melanoidin, the color of brewed coffee is also influenced by macromolecular polymer compounds formed from the Maillard reaction, which can result in a brown color (Cwiková et al., 2022). Color, particularly lightness, is an important indicator in determining the roast level of coffee. *In this study, the brewed coffee color was not affected by post-harvest processing, with an L value ranging from 36.66 to 37.25.* According to Fikry et al. (2019), the L^* values for light, medium, medium to dark, and dark roast levels range from 42-51, 37-41, 29-36, and 20-28, respectively. This differs from the findings of Córdoba et al. (2021), who reported that medium roasted coffee has an L^* value of 28-30. The variation in L^* values is influenced by the measurement method and standards applied. The roasting level affects the color of brewed coffee (Yeager et al., 2022).

Phenolic compounds are important in providing health benefits, including antioxidant, anti-inflammatory, and antidiabetic properties (Singh; Yadav, 2022). The phenolic compound with the largest amount in coffee is chlorogenic acid and this compound is influenced by post-harvest processing (Yulianti et al., 2022). In this research, the total phenolic content of tubruk brewed coffee is influenced by post-harvest processing. Várady et al. (2022) similarly stated that post-harvest processing significantly affects the total phenolic content in coffee.

Variations in post-harvest processing steps result in differences the total phenolic content of each brew. The washing process in the full-washed method removes some water-soluble compounds, including phenolic compounds (Duarte; Pereira; Farah, 2010). The natural process retains more bioactive compounds, possibly due to the reduced exposure to water during processing and the skin (exocarp) remains intact. Moreover, the drying process in natural processing takes longer than in other post-harvest processes, leading to abiotic stress and pressure that can affect metabolism and result in the production of compounds such as phenolics (Kulapichitr et al., 2019; Zhang et al., 2019). The high phenolic content in brewed coffee is influenced by the coffee type, post-harvest processing and brewing methods (Herawati et al., 2024).

Arabica coffee brews have the potential to inhibit α -glucosidase. This inhibitory effect is influenced by post-

harvest processing, as different processing methods result in variations in the chemical composition of the coffee brew (Pereira et al., 2019). According to Herawati et al. (2019) dan Ni et al. (2024), phenolic compounds in brewed coffee contribute to antioxidant activity and α -glucosidase enzyme inhibition. In addition to phenolic compounds, caffeic acid, trigonelline, and melanoidin also contribute to α -glucosidase inhibition (Alongi; Anese, 2018). Moreover, caffeine has been reported to possess antidiabetic properties (Jiang; Zhang; Jiang, 2014). No significant differences between brews from natural and honey processing methods may be attributed to the presence of other compounds that contribute to α -glucosidase inhibition. Aswathi et al. (2022) found that honey-processed coffee contains higher levels of trigonelline than full-washed coffee, as well as higher caffeine content (Cortés-Macías et al., 2022). Similarly, Yulianti et al. (2024) reported that Arabica brew prepared using the tubruk technique from green beans processed with the honey method has higher trigonelline and caffeic acid content than those processed using natural or full-washed methods.

The α -glucosidase inhibitory ability of brewed coffee is relatively low compared to acarbose. According to Yang et al. (2021), acarbose is a commonly used antihyperglycemic drug that inhibits α -glucosidase. Based on the findings of this study, approximately 796.5 mL of brewed coffee from natural processed coffee beans is required to achieve the same effect as acarbose. Alongi et al. (2021) reported that three cups of highly concentrated coffee are needed to produce a similar effect as acarbose. However, according to Bhupathiraju et al. (2013), consuming one cup of coffee containing 137 mg of caffeine per day may reduce 4-8% the risk of type 2 diabetes.

The differences between full-washed, natural, and honey processing lie in the cherry peeling, fermentation, and drying duration. These differences lead to variations in the chemical composition and flavor of the brew (Yulianti et al., 2024). Várady et al. (2022) stated that post-harvest processing affects the bioactive components of coffee, one of which is phenolic content. The fermentation process involving soaking and washing of green beans in the full-washed method leads to the loss of water-soluble components, such as chlorogenic acids (Duarte; Pereira; Farah, 2010). The honey process, which retains some pulp during drying, allows certain phenolic compounds to remain, though not as much as in the natural process. According to Yulianti et al. (2022, 2023a, 2023b, 2024), Munyendo et al. (2021), Aswathi et al. (2023) and Aswathi and Murthy (2024), the honey or pulped natural process is a post-harvest method that skips washing and keeps the pulp attached to the coffee beans during drying. According to Bastian et al. (2021) the chemical composition of coffee beans produced from the honey process is between natural and full-washed. From the results of this study, it can

be seen that different post-harvest processing methods affect the total phenolic content and can determine the α glucosidase inhibition of brewed coffee.

5 CONCLUSIONS

Post-harvest processing significantly influences the phenolic content of coffee and its ability to inhibit the α -glucosidase enzyme. Coffee brewed from naturally processed beans exhibited the highest phenolic content and α -glucosidase inhibitory activity compared to those processed using the full-washed method. These findings enhance understanding of how post-harvest processing impacts coffee's phenolic content and bioactivity. However, the study does not directly assess health outcomes, and further investigation is needed to evaluate other brewing techniques.

6 AUTHORS' CONTRIBUTION

Conceptual Idea: Yulianti; Andarwulan, N.; Methodology design: Yulianti; Andarwulan, N.; Adawiyah, D.R.; Hearwati, D.; Indrasti, D.; Data collection: Yulianti; Data analysis and interpretation: Yulianti; Wanita, Y.P., and Writing and editing: Yulianti; Andarwulan, N.; Adawiyah, D.R.; Hearwati, D.; Indrasti, D.; Wanita, Y.P.

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