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ANTIOXIDANT POTENTIAL OF ARABICA COFFEE PROCESSED BY HONEY METHOD

Ayu Puspita Arum*, Susan Barbara Patricia, Dyah Ayu Savitri, and Setiyono
Agricultural Sciences Study Program, Faculty of Agriculture, University of Jember, Jember Regency, East Java, 68121, Indonesia
*Email Correspondence: ayu.puspita@unej.ac.id

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ABSTRACT

This study investigated the impact of different coffee processing methods, the natural method, and fermentation with yellow, red, and black honey methods on the bioactive compounds of Arabica coffee using a Randomized Complete Block Design in five replicate experiments. The antioxidant activity, phenolic, flavonoid, caffeine, total soluble solid content, and pH value were determined. The results showed that the honey processing method could improve bioactive compounds more than the natural method, with higher levels of antioxidants, phenolic, flavonoid, and total soluble solid contents but lower caffeine content and pH value. Arabica coffee-treated yellow honey exhibited the highest Increase in antioxidant activity. These findings suggest that coffee-treated yellow honey could be a functional food.

Keywords: antoxidant, caffeine, fenolic, flavonoid, honey arabica coffee processing

INTRODUCTION

Sumber Wringin Sub-district is a sub-district in Bondowoso Regency, East Java, which is the largest producer of Arabica coffee commodities in Bondowoso which is 1,258 ton/year (Putri, 2023). Regency so Arabica coffee in the area is the primary commodity for the formation of the Bondowoso Regency Agropolitan Area and most of the Arabica coffee in the area is processed naturally which requires a processing time of 50-60 days (Purwatiningsih & Ismanto, 2018). The natural process in arabica coffee processing is the processing of coffee by fermentation and drying the red cherries of coffee in the sun for 40-50 days until the moisture content of the coffee becomes 11-13%. In addition, Arabica coffee that is processed through natural processes produces a fruity and high-complexity of flavor and has a higher price (Asiah et al., 2022). The honey process in Arabica coffee processing is a process by removing the skin of the coffee fruit and leaving a layer of mucilage on the surface of the coffee beans which are then fermented and dried for 10 days in the sun until the moisture content is 11-12% and based on the results of research in the form of organoleptic tests of arabica coffee processed by honey, namely fruity aroma, high body, low acidity and flavor in the form of complex ripe fruit flavors and sweetness (Dalimunthe et al., 2021).

Furthermore, in the honey processing process, which only leaves the coffee mucilage layer during the coffee fermentation and drying process, it is intended that during the drying process the organic acids produced from the fermentation of the mucilage layer can be absorbed by the coffee beans so as to produce a fruity taste typical of fruits and high flavor complexity (Dalimunthe et al., 2021). Coffee mucilage layer contains 85.3% water, 7.2% protein, 0.7% fat, 1.1% ash, 1.5% crude fiber, and 4.3% total sugar (Siridevi et al., 2019). Lactic Acid Bacteria (LAB) have the ability to break down pectin into glucose, giving rise to the sweet taste of coffee and break down glucose into lactic acid, acetic acid, caproic acid, and formic acid, giving rise to the sour taste of coffee during the fermentation process (Wang et al., 2021). Besides that, one of the yeasts such as S. cerevisiae play a role in the fermentation of coffee beans and produces ethanol or alcohol (Elhalis et al., 2023).

In addition, microbial performance during the fermentation process is influenced by the availability of substrates such as sucrose, water, protein, pectin and lipids contained in the mucilage layer of coffee, so the amount of mucilage in coffee beans will affect the flavor of the coffee produced (Dalimunthe et al., 2021). Fermentation time will also affect the resulting coffee flavor. The longer the fermentation time, the more alcohol, aldehyde, and ester compounds that are compounds that form coffee flavors so that the resulting coffee flavors are stronger and more diverse (Ruta & Farcasanu, 2021). The honey process is divided into three types: yellow honey, red honey and black honey. The difference between the three processes is the thickness of the mucilage layer left on the surface of the coffee beans. Yellow honey only has 25-50% mucilage layer on the surface of the coffee beans. Red honey only has 70-85% mucilage layer left on the surface of the coffee beans and black honey has 100% mucilage layer. The difference occurs due to different washing process treatments after the coffee is pulped. Yellow honey is washed twice, red honey only once and black honey without washing (Dalimunthe et al., 2021; Elhalis et al., 2023).

Antioxidants are molecules or compounds that are stable enough to donate their electrons or hydrogens to free radical molecules or compounds and neutralize them, thereby reducing their ability to carry out free radical chain reactions. These antioxidants delay or inhibit cell damage mainly through their free radical scavenging properties. These antioxidants can safely interact with free radicals and stop the chain reaction, and prevent free radicals from damaging vital molecules (Ibroham et al., 2022). Antioxidant compounds found in Arabica coffee are flavonoids and phenolic compounds. (Sellygani Budi Vaelani et al., 2022). Determination of total polyphenol in roasted specialty arabica coffee from Nicaragua which was processed by honey method little bit higher than natural method of 26.3 ± 6.25 μg GAE/mg, 25.3 ± 4.95 μg GAE/mg, and 25.1 ± 4.52 μg GAE/mg, respectively (Várady et al., 2022). Moreover, the similar condition was happened on the impact of honey method and natural method arabica coffee bean in Huila, Colombia to chlorogenic acid content which is one of the type polyphenol compounds of 18.6 ± 0.4 g /kg and 17.9 ± 0.4 g /kg, respectively (Cortés-Macías et al., 2022).

Based on this, it is necessary to examine the effect of different processing honey (yellow, red and black) and natural on arabica coffee processing in Sumber Wringin District, Bondowoso Regency, East Java in the content of antioxidant compounds produced because previous researchers only explained the effect of coffee processing methods on flavor quality, while the potential in terms of health or the content of antioxidant compounds in arabica coffee processed by natural methods with honey methods has not been studied. It is hoped that honey process can be an alternative to a shorter arabica coffee processing with high antioxidant compound content. In this research, the honey process (yellow, red and black) will be modified from the process carried out by (Dalimunthe et al., 2021). This modification is combination the results of interviews from several Arabica coffee processors using the honey method in Jember district, East Java.

METHODS

Design, Location and Time

This research used Arabica coffee fruit from coffee farmers in Sumber Wringi sub-district, Bondowoso Regency, East Java. This study used a completely randomized design with 1 factor consisting of 4 types of treatment, namely yellow honey, red honey, black honey and natural processing methods with 5 replicates for each type of treatment. The parameters used in this study, namely antioxidant activity, caffeine content, total sugar, pH and sugar Brix were analyzed at the Food Analysis Laboratory of Jember State Polytechnic, Jember Regency, East Java in August 2023. Meanwhile, the parameters of total phenolic and total flavonoids content were analyzed at the Chemistry Laboratory, Faculty of Science and Mathematics, Satya Wacana Christian University, Salatiga, Central Java in August 2023.

Material and Tools

The material used in this research was red arabica coffee fruit harvested directly from the coffee plantation of farmers in Sumber Wringin sub-district, Bondowoso Regency, East Java. Meanwhile, the tools used in this study are a pulper machine with a capacity of 150 kg / hour, dimensions 727 X 475 X 1110 mm, power 5.5 HP was made by CV. Vegas Coffee Roaster for peeling coffee skin, para-para for tables to dry coffee in the sun, a huller machine with a capacity of 150 kg / hour power 5-8 HP brand “tehnik Bersama” for removing, skin, husk and dried mucilage and coffee roasting machine with a capacity of 5-10 kg was made by CV. Vegas Coffee Roaster for roasting kopi.

Procedure

Variety of Honey Coffee Processing Treatment

The honey processing on arabica coffee was divided into 3 namely yellow honey, red honey and black honey. At the initial stage, the red arabica coffee fruit was stripped of the coffee fruit skin using a pulper. The second stage, coffee that has been pulped is divided into 3 groups, namely the first group as a black honey process, the arabica coffee would be washed with water once, the second group as a red honey process, the arabica coffee would be washed with water twice and the third group as a yellow honey process, the arabica coffee would be washed with water 3 times. After that, each group would be placed on the surface of the para-para to be dried in the sun. In this drying process there were differences in drying time for yellow honey would be dried for 10 days, red honey would be dried for 20 days and black honey would be dried for 30 days and during the drying process the coffee must be diligently flipped manually every day so that the drying process was perfect. In the third stage, the dried coffee (yellow honey, red honey and black honey) was subjected to a resting process to reduce the temperature of the coffee after drying. The fourth stage was the huller process on dried coffee to remove husk and dried mucilage. In the sixth stage, the hulled coffee was put into an airtight plastic for roasting.

The natural process in arabica coffee was done by directly drying the red arabica coffee fruit on para-para to dry the coffee fruit in the sun for 50 days. During the drying process, the coffee must be turned over every day to ensure a good drying process. Then, the dried coffee was hulled to remove the skin, husk and dried mucilage. And lastly, the hulled coffee was put into an airtight plastic for roasting.

The roasting process for the four type of coffee processing was carried out using the medium roast method at 180°C for 15 minutes. After the roasting process, the coffee beans were aerated on a tray to reduce the temperature to room temperature. Then, the coffee beans were put in airtight plastic and stored for further analysis. This study was conducted using 1 factor with 4 level of treatment especially variety of coffee processing, namely, natural, yellow honey, red honey and black honey on Bondowoso arabica coffee with 5 replication of each treatment and then, coffee roasted would be analyzed with many parameters. The parameters used in this study, namely antioxidant activity, caffeine content, total sugar, pH, sugar Brix, total phenolic content spectrophotometric method (mg gae/g dry sample) and total flavonoids content spectrophotometric method (mg qe/g dry sample).

Analysis of Parameters

Antioxidant Activity

Analysis of Antioxidant activity was conducted with dissolving 100 mg sample to methanol until the volume total of solution was 100 ml and then, 2 ml of sampel was added 2 ml larutan DPPH 0.1 mM and mixing it, then quatificated of the absorbtion using spectrophotometry UV-Vis in wavelength 517 nm with the specification UV-Vis Spectrophotometer was Single-Beam UV-Vis Spectrophotometer type, wavelength Range: 190 nm to 1100 nm, resolution: 1.0 nm and was made by Shimadzu Corporation (Irwinsyah et al., 2019). Determination of Inhibition % was representated as antioxidant activity (% inhibition) which was calculated using equation as follow :

$$\frac{(absorbance\ of\ blank - absorbance\ of\ sample)}{absorbance\ of\ blank}\ X\ 100\%$$

Analysis of Caffeine Content

Analysis of caffeine from ground coffee is done by extraction method, namely as much as 1 g of coffee powder is put into a glass cup and then 150 mL of distilled water is added to it while stirring. The coffee solution was filtered through a funnel using filter paper into an erlenmeyer, then 1.5 g of calcium carbonate ($CaCO_3$) and coffee solution were put into a separatory funnel and extracted 4 times, each with the addition of 25 mL of chloroform. The bottom layer was taken, then the extract (chloroform phase) was evaporated using an evaporator until the chloroform evaporated completely. Then, a total of 250 mg of caffeine was put into a glass cup, dissolved in 250 ml of hot distilled water, obtained a 1000 ppm mother solution. then diluted by taking 9 ml of the mother solution and then adding 1 ml of distilled water to obtain 100 ppm. The caffeine extract solution was put into a 50 mL volumetric flask, diluted with distilled water up to the mark line and homogenized, then determined with a UV-Vis spectrophotometer at a wavelength of 272 nm. The same treatment was done for each sample of coffee powder weighing 1 g (Latunra et al., 2021). Calculation of caffeine content (%) in ground coffee is as follows:

$$= \frac{concentration\ \left(\frac{mg}{l}\right) X\ Volume\ (l) X\ dilution\ factor}{weight\ of\ sample\ \ (mg)}\ X\ 100$$

Analysis of Soluble Solid Content (SSC)

Soluble solid content (SSC) refers to dissolved substances like sugars, salts, and acids in food, and it significantly affects several aspects of food products such as flavor & sweetness: higher SSC enhances sweetness and flavor, especially in juices and fruits; Texture: In products like jams and sauces, higher SSC results in thicker, more viscous textures. Shelf Life: High SSC helps preserve foods by reducing water activity, extending shelf life. Nutritional Value: Higher SSC can increase the concentration of sugars and nutrients, but may also raise calorie content. Appearance: Higher SSC often leads to deeper colors, making products more visually appealing. Processing: Manufacturers adjust SSC to control product quality, texture, and taste. Overall, SSC plays a key role in determining the taste, texture, preservation, and appearance of food products(Luo et al., 2021). The soluble solid content was analysed using refractometer. sugar (degree Brix) is done using a device called a refractometer with the model and company: Mettler Toledo RE40 Digital Refractometer and the specification was digital handheld refractometer type, measurement range: brix: 0-80%, refractive index (nd): 1.3000 - 1.7000, specific gravity: 1.000 - 1.120, accuracy: brix: ±0.1%, refractive index: ±0.0001, resolution: brix: 0.1%, refractive index: 0.0001, temperature compensation: automatic temperature compensation (atc), power supply: 1 x 9v battery and display: lcd digital display. Measurement soluble solids measurement is done by dripping coffee brew on a hand refractometer, then the number of measurements is observed and recorded. Measurement repeated as many as 3x repetition (Nurhayati, 2018).

Dtermination of total sugar was carried out by preparing 25 ml of sample filtrate and putting it into an erlenmeyer, adding 15 ml of distilled water and 5 ml of HCL. Then heated on a water bath at a temperature of 67-70̊ C. Then cooled quickly to a temperature of 20℃. The solution was then neutralized with 45% NaOH and diluted to a volume of 100 ml until the solution contained 2-8 mg/ml reducing sugar. Then the total amount of sugar was determined based on the OD (optical density) of the sample solution and the standard curve of glucose solution (Haryanti & Mustaufik, 2022). Calculation of total sugar content is as follows:

$$\%\,Total\ sugar = \frac{glucose\ (mg) X dilution\ factor}{weight\ of\ sample\ (mg)}\ X\ 100$$

pH Analysis

The pH measurement was calibrated first by inserting the electrode of the pH meter into the buffer at pH 7, then the electrode was rinsed using distilled water and dried with a tissue. The calibrated electrode is then inserted back into the sample solution to be tested, the number that appears on the pH meter screen is waited until constant and then recorded. Then the electrode is rinsed using before pH determination to the next sample and re-calibrate (Dalimunthe et al., 2021). In this study we used pH meter – Orion Star A111 pH Benchtop Meter was made by Thermo Fisher Scientific with the spesificiation as following : measurement range (0.00 - 14.00 pH), accuracy: ±0.01 pH, resolution: 0.01 pH, display type: Digital LCD or LED screen, electrode type: Glass electrode, combination type, temperature compensation: automatic or Manual (0-100°C), power supply: rechargeable battery or DC adapter (9V/12V).

Analysis of Phenolic Content

Measurement of phenolic content using the folin ciocalteu method. The first thing that needs to be done is making a standard solution of gallic acid with 5 concentration variations, namely 10 ppm, 20 ppm, 30 ppm 40 ppm and 50 ppm. Then at each concentration, 1 ml of solution was taken. Then given 0.5 ml folin ciacalteu. then, 7% Na_2CO_3 solution was added as much as 4 ml. After that, it was homogenized using a vortex and allowed to stand for 8 minutes and calculated absorbance with a wavelength of 760 nm. Measurement on the sample by taking 1 ml of concentrated coffee solution sample and poured in a test tube given 0.5 ml of folin ciocalteu. Then added 4 ml of 7% Na_2CO_3 solution. after that it was homogenized using a vortex and allowed to stand for 8 minutes. Calculation of absorbance is done at a wavelength of 760 nm (Rosada et al., 2022). Phenolic content can be calculated using the following equation:

$$TPC\ (\frac{mg}{g} GAE) = \frac{phenolic\ concentration\ from\ gallic\ acid\ standard\ curve\ (ppm)\ x\ extract\ volume\ (ml) x\ dilution\ factors}{weight\ of\ sample\ (g)}$$

Analysis of Flavonoid Content

Measurement of flavonoid content of coffee ground was conducted with the first, Coffee extract (250 µL), distilled water (1 mL) and 75 µL of 5% NaNO2 were mixed together. After 5 min, 10% $AlCl_3$ $6H_2O$ solution (150 µL) was added and incubated for 6 min. We injected 1 N NaOH (500 µL) and the mixture was incubated for 11 min. A blank sample was substituted for the diluted coffee extract with distilled water. The absorbance of the extract sample was measured at 510 nm against the blank sample, using a UV/visible spectrophotometer (U-2900, Hitachi High-Tech Corporation, Tokyo, Japan). The standard solution was prepared using a quercetin solution (0–1 mg/mL) to generate a standard curve (r^2 = 0.999) (Haile & Kang, 2019a). The amount of flavonoids in the coffee was presented as a mg quercetin equivalent/mL of coffee extract. Flavonoid content can be calculated using the following equation:

$$TFC\ (\frac{mg}{g}QE) = \frac{flavonoid\ concentration\ from\ quercetin\ standard\ curve\ (ppm)\ x\ extract\ volume\ (ml) x\ dilution\ factors}{weight\ of\ sample\ (g)}$$

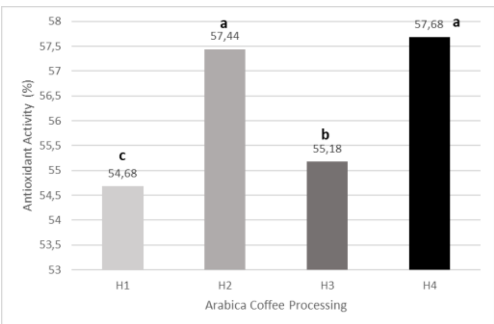
Analysis of Data

The data obtained in this study will be analyzed using the SPSS IBM Statistic 25 program with the type was statistical software and produced by IBM Corporation. The specifications version: SPSS Statistics 25, platform: Windows, macOS, and Linuxwith. And for advanced data analysis tools such as on this study using one way ANOVA (Analysis of Variant) and Duncan Multiple Range Test (DMRT) with a significance level (α) of 0.05.

RESULTS AND DISCUSSION

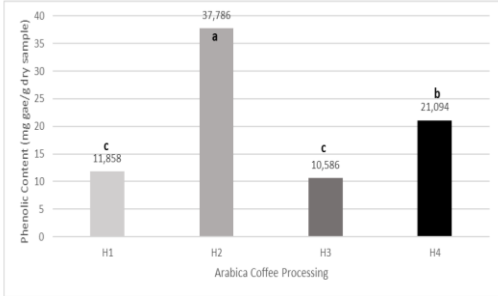
Antioxidant Activity, Phenolic and Flavonoid Content

Antioxidant activity is a compound that can react with free radicals in the human body such as superoxide anions, hydroxyl radicals, hydroperoxyl radicals and others that cause pathological changes in the human body such as premature aging and disease, into stable compounds that cannot react and damage important compounds and organs in living cells such as lipids, proteins, carbohydrates, DNA and cell membranes (Amrullah & Sandi, 2022). Based on the results of the study (Figure 1) showed that Arabica coffee processing with honey method (H2; H3; and H4) was significantly different and provided higher antioxidant activity of 57.44%; 55.18% and 57.68%, respectively compared to processing Arabica coffee with natural method ($p<0.05$). This result was similar to the study of effect coffee processing methods (natural and honey) to specialty arabica coffee from Nicaraguan with the antioxidant capacity of honey method was higher (64.7 ± 9.17 µg TE/mg) than natural method (59.5 ± 13.9 µg TE/mg) (Várady et al., 2022). Furthermore, the research of post harvest treatments (natural and honey method) to arabica coffee bean in Huila, Colombia gave the result of antioxidant capacity of coffee processed by honey method (280 Trolox Equivalent Antioxidant Capacity mmol/kg) was higher than natural method (280 Trolox Equivalent Antioxidant Capacity mmol/kg) (Cortés-Macías et al., 2022). In coffee processing, there will be a fermentation process from various natural microorganisms around the coffee. In the honey processing of Arabica coffee, the fermentation of the mucilage by microorganisms (mainly yeasts, lactic acid bacteria, and acetic acid bacteria) plays a key role in shaping the flavor. After the outer skin is removed, the mucilage remains on the beans, where yeasts break down sugars into alcohol and carbon dioxide. Lactic acid bacteria produce lactic acid, adding creamy notes, while acetic acid bacteria create tangy acidity (Wulandari et al., 2021). This fermentation process produces a variety of organic acids that influence the coffee's flavor, creating a complex, fruity, and sweet profile. After fermentation, the beans are washed and dried, locking in these flavors for a smooth, well-balanced cup with unique sweetness and acidity. and in different coffee processing, the natural microorganisms that ferment the coffee are also different. In the coffee processing with the honey method, natural microorganisms will ferment the mucilage layer of coffee and produce a variety of new compounds including one of which is an antioxidant compound. The most common fermentation microorganisms found in the honey method coffee processing are Saccharomyces cerevisiae and it can not be found in natural method arabica coffee processing (Elhalis et al., 2023; Haile & Kang, 2019b). This is in line with research conducted by (Kwak et al., 2018) that coffee fermentation process using Saccharomyces cerevisiae during coffee processing can increase antioxidant activity (Superoxide dismutase and Oxygen radical absorbance capacity). Antioxidant compounds contained in coffee are phenolic compounds such as chlorogenic acid and hydrocinnamic acid and flavonoid compounds such as catechin, epicatechin and quercetin (Dewajanti, 2019; Saud & Salamatullah, 2021; Sellygani Budi Vaelani et al., 2022).



H1: Natural Processing; H2: Yellow Honey Processing; H3: Red Honey Processing; H4: Black Honey Processing; numbers followed by different letters indicate significantly different ($p<0.05$)

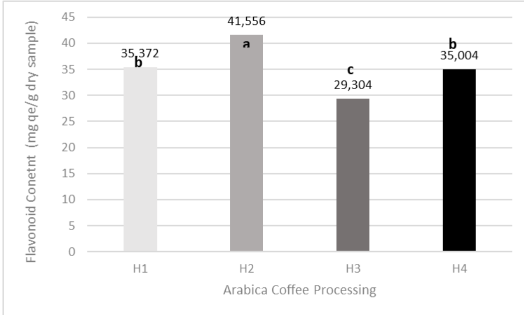
Figure 1. Antioxidant Activity of Many Arabica Coffee Processing



H1: Natural Processing; H2: Yellow Honey Processing; H3: Red Honey Processing; H4: Black Honey Processing; gae : galic acid equivalent; numbers followed by different letters indicate significantly different ($p<0.05$)

Figure 2. Phenolic Content of Many Arabica Coffee Processing

This was also supported by the results of our research on phenolic and flavonoid content in Figure 2 and Figure 3. In the Arabica coffee processing with yellow honey and black honey methods showed significantly different and tended to be higher for phenolic content 37.786 mg gae/g dry sample and 21.094 mg gae/g dry sample respectively compared to the Arabica coffee processing with natural methods 11.858 mg gae/g dry sample ($p <0.05$). it was in accordance with the results of the study of effect coffee processing method by honey method to the total phenolic content (TPC) on the arabica coffee in huila, Colombia was higher (50 ±7 g/kg) than natural method (49 ±8 g/kg) (Cortés-Macías et al., 2022). However, the Arabica coffee processing with the red honey method of phenolic content showed no significant difference 10.586 mg gae/g dry sample with the natural method 11.858 mg gae/g dry sample ($p>0.05$). In addition, flavonoid content in the arabica coffee processing with the yellow honey method showed significantly different and higher 41.556 mg qe/g dry sample compared to the natural method 35.372 mg qe/g dry sample ($p<0.05$). Conversely, in the Arabica coffee processing with the red honey method, flavonoid content showed significantly different and lower 29,304 mg qe/g dry sample compared to the natural method 35,372 mg qe/g dry sample ($p<0.05$). Meanwhile, the black honey method in Arabica coffee processing, the flavonoid content showed no significant difference 35.004 mg qe/g dry sample compared to the natural method 35.372 mg qe/g dry sample ($p>0.05$). However, the interesting thing that we can highlight from Figure 2 and Figure 3 is that coffee processing with the yellow honey method shows the highest phenolic and flavonoid levels compared to other processing methods (natural, red honey and black honey). This is in line with the results of research (Haile & Kang, 2019a) that coffee fermentation with Saccharomyces cereviceae with longer fermentation time will cause a decrease in phenolic and flavonoid levels in coffee. The yellow honey processing is a process that involves fermentation of the mucilage layer in coffee by the dominant natural microorganism is Saccharomyces cereviceae for 10 days. Meanwhile, the fermentation time for red honey is 20 days, black honey is 30 days and natural processing is 50 days. In addition, during prolonged fermentation, a decrease in the content of free phenolic and flavonoid compounds can occur, as these compounds can bind to other molecules present in the food matrix, be degraded by microbial enzymes, and be hydrolyzed by certain microbial strains or other natural microbials (Adebo et al., 2020). The honey process results in a greater increase in soluble solid content during fermentation compared to natural fermentation because it allows for more extensive microbial degradation of the mucilage. This leads to the release of more simple sugars and organic acids into the surrounding liquid, which contributes to a higher soluble solid content, resulting in a sweeter, fuller flavor profile in the coffee. Conversely, the natural fermentation process does not promote as much degradation of the mucilage, leading to a lower increase in soluble solid content (Aswathi et al., 2023).

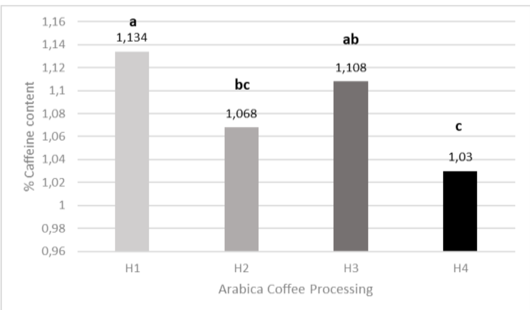


H1: Natural Processing; H2: Yellow Honey Processing; H3: Red Honey Processing; H4: Black Honey Processing; qe: quercetin equivalent; Numbers followed by different letters indicate significantly different ($p<0.05$)

Figure 3. Flavonoid Content of Many Arabica Coffee Processing

Caffeine Content

Caffeine is an alkaloid compound found in coffee that can act as an antioxidant and is also beneficial for preventing eurodegenerative disorders, liver damage, and physical activity (Ősz et al., 2022). Consumption of caffeine-containing beverages at a dose of 250 mg exerts positive effects on the body such as increased arousal, alertness, concentration and well-being (e.g., increased excitement, peace and pleasure), whereas a dose of 500 mg was shown to increase tension, nervousness, anxiety, excitement, irritability, nausea, paresthesias, tremors, sweating, palpitations, restlessness, and possibly dizziness. In addition, high, sub-lethal doses (∼7-10 mg/kg) in normal adults can also cause symptoms such as chills, facial flushing, nausea, headache, palpitations, and tremors (Depaula & Farah, 2019). Based on this, the European Food Safety Authority (EFSA) recommends caffeine consumption of no more than 3 mg/kg per day (Willson, 2018). The results showed that caffeine content in arabica coffee processing with black honey method showed significantly different and the lowest was 1.03% (equal with 1.03 mg/kg) compared to other arabica coffee processing methods (red honey and natural) ($p<0.05$) but, arabica coffee processing with the black honey method was not significantly different from arabica coffee processing with the yellow honey method ($p>0.05$). This was in line with the results of the study effect of coffee processing method to caffeine content in arabica coffee from Huila, Colombia, which the honey method coffee processing gave lower caffeine (12.3 ± 0.3 g/kg) than natural method (13.4 ± 0.5 g/kg) (Cortés-Macías et al., 2022). The black honey method involves the fermentation process of the coffee mucilage layer for 30 days so that the caffeine content in the coffee produced is the lowest. This is in accordance with the results of research from (Purwoko et al., 2023) the longer the fermentation time, the higher the degradation process of caffeine in coffee, the degradation process is through the N-demethylation pathway by the microorganism Saccharomyces cereviceae which is the dominant microorganism fermentation in coffee into paraxanthine compounds. caffeine content of arabica coffee processing with black honey method gave 1.03% or 1.03 mg/kg. it was smaller than recommendation of caffeine consumption per day from the European Food Safety Authority (EFSA), so the coffee is safe to consump.

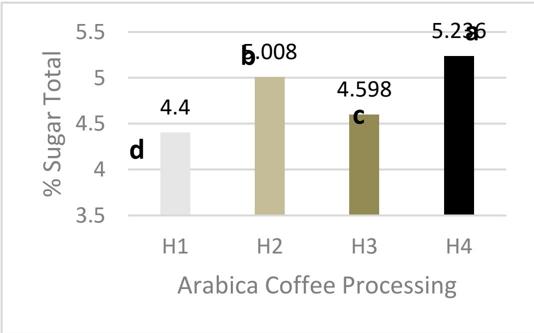


H1: Natural Processing; H2: Yellow Honey Processing; H3: Red Honey Processing; H4: Black Honey Processing; qe: quercetin equivalent; Numbers followed by different letters indicate significantly different ($p<0.05$)

Figure 4. Caffeine Content of Many Arabica Coffee Processing

Sugar Total

Based on Figure 5, it showed that the processing of arabica coffee with the black honey method produces total sugar content (5.236%) which was significantly different and gave the highest results compared to other arabica coffee processing (yellow honey, red honey and natural) ($p<0.05$). This condition is in line with the brix sugar data (graph not shown) on the four arabica coffee processing processes that the arabica coffee processing with the black honey method produced significantly different brix sugar and tended to produce the highest brix sugar value (6.028 brix) compared to the yellow honey, red honey and natural methods which are 5.636 brix; 5.052 brix and 5.04 brix respectively ($p<0.05$). There is a correlation between higher Brix values (indicating higher sugar content) and a greater decrease in pH during fermentation. The black honey method, with the highest sugar content, is likely to produce the greatest amount of acids during fermentation, causing the most significant drop in pH compared to other methods (yellow honey, red honey, and natural). Conversely, methods with lower Brix values will have less sugar to ferment, leading to less acid production and a smaller drop in pH. The increase in sugar in coffee after several days of fermentation is due to mucilage is abundant in sugars, which allows the action of microorganisms, mainly yeasts and bacteria. Microbial growth leads to the production of numerous substances including reduction sugar or non reduction sugar, which can diffuse into the bean and impact the quality of coffee bean (Haile & Kang, 2019b; Zani Agnoletti et al., 2022).



H1: Natural Processing; H2: Yellow Honey Processing; H3: Red Honey Processing; H4: Black Honey Processing; qe: quercetin equivalent; Numbers followed by different letters indicate significantly different (p<0.05)

Figure 5. Sugar Total of Many Arabica Coffee Processing

pH

Based on the results of the study (data not shown) that the arabica coffee processing with the black honey method (4.66) is significantly different and tends to be the lowest compared to other coffee processing methods (yellow honey, red honey and natural) which are 4.78; 5.06; 5 respectively (p<0.05). These results are in line with the results of research from (Mariyam et al., 2022) that the pH of coffee beans decreases during the fermentation process. In addition, all yeast addition treatments can reduce the pH value at the end of fermentation, this is due to the growth and metabolic activity of yeast isolates. Changes in pH to become more acidic are related to the fermentation of sugars, especially glucose and fructose into organic acids. Fermentation can cause the pH of coffee to decline due to the breakdown of sugar or caffeine to be converted into acidic compounds such as lactic acid, acetate, butyric and propionate (Afriliana et al., 2018)

CONCLUSION

The honey processing method could improve bioactive compounds more than the natural method, with higher levels of antioxidants, phenolic, flavonoid, and soluble solid contents but lower caffeine content and pH value. Arabica coffee-treated yellow honey exhibited the highest Increase in antioxidant activity, flavonoid and phenolic content.

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DECLARATION OF INTEREST

The authors have no conflict of interest in preparing the manuscript.

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