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Sensory Analysis, Caffeine, Chlorogenic Acid and Non-Volatile Taste Compounds of Arabica Coffee (Coffea arabica) Fermented with Sugar Addition for Brew Taste

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Abstract: Arabica coffee is the most popular variety of coffee among the people because it has a more complex flavor than other coffee varieties. This study aims to determine sensory properties and non-volatile components in Arabica coffee fermented with sugar addition. The sensory assessment showed that the best cupping score was the samples fermented with the addition of 0.55% fructose with a total score of 85.25 compared to honey (H) and Fullwash (FW) samples. Fermentation with the addition of 0.55% fructose could produce better coffee compared to samples (H) and (FW). Fermentation with the sugar addition of 0.55% could affect non-volatile components such as soluble sugars, organic acids, amino acids, caffeine, and chlorogenic acid. The content of chlorogenic acid and caffeine analysed by HPLC was found relatively stable in green and roasted beans. Amino acids analysed by LCMS showed glutamate was the highest amino acid in all samples and were supposed to have a role in Maillard reaction contributing to coffee flavor. In conclusion, fermentation with the addition of 0.55% sugar in coffee processing could generally enhance the coffee flavor for brew taste with its potential as functional drink.

Keywords: Arabica coffee, sugar fermentation, sensory attributes, non-volatile compounds, coffee flavour

INTRODUCTION

Aroma and taste are the coffee brewed's main character, which formed from a series of post-harvest processing. High-quality coffee flavor is described as a pleasant sensation from the combination of balance between flavor, body, and aroma with no flavor defects such as over-fermented, earthy, and oily (Mori et al., 2003). The aroma and taste of coffee are the combinations of several constituents of volatile chemicals such as acids, aldehydes, ketones, and phenolic compounds with nonvolatile components such as sugars, proteins, amino acids, organic acids, and caffeine (Lee et al., 2015). Volatile and non-volatile compounds of coffee are influenced by coffee varieties, regions, processing methods, levels of roasting, grinding size, and brewing method (Illy and Viani, 2005). Among the processing methods, the fermentation process is the most influential stage in the formation of coffee flavors (Bressani, 2018; Yusianto and Widyotomo, 2013).

The fermentation process is one step in the wet processing of coffee beans. It contributes to forming precursor compounds of flavors such as organic acids, amino acids and reducing sugars. The process not only degrades the mucilage layer, but also occurs a chemical process that is useful in the character and flavor formation of the coffee. (Avvallone et al., 2001; Jackels et al., 2006; Lin, 2010). Uncontrolled fermentation can damage the coffee flavor, due to the growth of microorganisms that naturally exist on the surface of the coffee fruit (FAO, 2004). Previous experiments have been done by Bressani (2018) with adding the yeast starter in the fermentation process of the Arabica coffee (Caturai Amarelo). The addition of a starter is done by directly into the fermentation tank. The differences in the treatment of this starter gave different results. The analysis showed an increase of organic acids in samples treated with the starter addition in the fermentation tank, and it correlated with the sensory value of coffee based on the Specialty Coffee Association of America (SCAA) cupping quality classification.

Wet coffee processing had higher acid content, but the sugar content is lower than the dry processing (Duarte et al., 2010; Knopp et al., 2005). The content of sugars in coffee beans affects the production of non-volatile compounds as precursors of coffee flavor. Several attempts have been made by farmers to improve the quality of coffee beans. One of the efforts carried out by using juice as fermentation believed to increase the content of sugars and organic acids correlated to coffee's sensory value. Low sugar content in the wet-processed coffee may be enhanced by adding sugar during the fermentation process. However, it is also unclear what kind of added sugars can contribute to the coffee sensory.

One of flavor formation is through the Maillard reaction. Wong et al. (2008) concluded that the Maillard reaction is a reaction of specific amino acids and reducing sugars producing the flavor of caramel, fruity, and flowery. Dorfner et al. (2003) and Bytof et al. (2005) stated that sucrose could contribute in the formation of flavor and color in coffee through a caramelization process after the roasting process. Given the importance of the sugar compounds in the formation of coffee flavor, it is necessary to study the addition of sugars during fermentation. The purpose of this research was to study the effect of the addition of glucose, fructose, and sucrose with a different concentration level during the fermentation process to non-volatile taste compounds as precursors of developing coffee flavor. The results of this research are considered useful for developing the wet processing coffee to enhance the quality of the coffee flavor for brew taste.

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MATERIALS AND METHODS

Materials

Arabica coffee Cherries were generated from Matt Husen Andungsari Coffee Plantation, Bondowoso Ijen - East Java Indonesia, located at an altitude of 1,400 meters above the sea level. D-fructose, D-glucose, Sucrose, aquapro (HPLC Grade) and O-phthalaldehyde (OPA) were obtained from Merck (Germany). Standard organic acids (citric, acetic, lactic, malic, succinic, propionic and butyric) were generated from Merck (Germany). Standard amino acids (1-alanine, 1-arginine hydrochloride, 1-aspartic, 1-cysteine, 1-glutamic acid, l-glycine, l-histidine monohydrochloride monohydrate, l-isoleucine, 1-lysine monohydrochloride, 1-leucine, 1-methionine, 1-phenylalanine, 1-proline, 1-serine, 1-threonine, 1-tyrosine, tryptophan and 1-valine) were obtained from Sigma-Aldrich (USA). Caffeine and chlorogenic acid were also obtained from Sigma-Aldrich (USA) and all other chemicals and reagents used were pro analysis and analytical grades.

Sample Preparation

Coffea arabica beans were harvested from selected cherries and then soaked for 12 hours. After that, the coffee beans were pulped using a vis-pulper (Honda GX 160-5.5 HP, Indonesia). Depulped coffee beans (no later than 2 hours) were added with D-fructose, D-glucose and sucrose at the concentration of 0.55%, 1.1% and 1.65%, respectively while honey and fullwash beans were without any addition sugars. A total of 2.5 kg of beans were then put into perforated plastic bags with a thickness of 0.5 mm and a length of 90 cm and fermented for 24 hours. After fermentation, the coffee beans were washed thoroughly, then dried in the sun until the moisture content 12%. The bean skin was then peeled using a huller machine (LM24-2C, China) until a green bean was obtained.

Roasting and Milling of Coffee Beans

Green beans (1 kg) were roasted using a machine (William Edison W600) following the method of Baggenstoss et al. (2008). The roasting process was done at a high temperature for a short time. Roasting temperature was set at 200-210°C and maintained for 18 minutes. Then, the roasted beans were cooled for 5 minutes and stored in the airtight containers. For the milling process, the roasted beans were ground using a grinder (Latina N-600) and sieved using a sieve size of 20 mesh and then stored in a vacuum packaging.

Brewing and Sensory Analysis

A total of 8.25 g of coffee powder was brewed with 150 mL of hot water temp (93 °C) for 4 min. Each unit of experiment was repeated five times, presented in five bowls to test the uniformity. Descriptive analysis was conducted, including in three steps: i) evaluation of coffee aroma by sniffing the dry powder, ii) evaluation of the aroma by sniffing a drink in 3 min after brewing, and iii) 8-10 min after brewing. Sensory analysis was performed by three panelists from Gayo Cupper Team of Nangroe Aceh Darussalam, Indonesia. The assessment used was a system

developed by Specialty Coffee Association of America (SCAA, 2009), by giving a score between 1-10 on each variable tested. Score 1 was the lowest score and 10 was the highest score.

Analysis of Organic Acids

Analysis of organic acids refers to the method (Ribeiro et al., 2017), coffee powder (3 g) was added with 20 mL distilled water at the room temperature. The sample was homogenized for 10 min using a magnetic stirrer and filtered using a whatmann no.3 filter paper. The extract was centrifuged at 10.000 rpm, 4 °C for 10 min. Supernatant was regulated to achieve a pH of 2.11 using a perchloric acid solution (200 µM) before a second centrifugation under the same condition. The second supernatant was filtered using a cellulose acetate filter (0.22 µm) prior to analysis by HPLC system. The organic acids was determined by the HPLC system (ShimadzuRF-10AXL) equipped with C18 column (7.9 mm x 30 cm). The mobile phase used was 100 μM of perchloric acid solution and the flow rate of 0.6 mL/ min. The column temperature was maintained at 50 °C. The detection of organic acids was read by using a wavelength of 210 nm. Identification and quantification were done by comparing to calibration curve of standards compounds.

Sugar Analysis

Analysis of soluble sugars refers to the method (Ribeiro et al., 2017), 10 g of coffee powder was added with 50 mL of distilled water and extracted in the ambient temperature for 10 min using a magnetic stirrer. The extract was centrifuged at 10.000 rpm, 4 °C for 10 min. Supernatant was regulated to achieve a pH of 2.11 using 200 µM perchloric acid solution and then centrifuged again with the same condition. The supernatant was filtered by a cellulose acetate filter 0.45µm prior to analysis by HPLC. HPLC system (Shimadzu SCL-10A) was equipped with Restek columns Finnacle II Amino $5\mu m$ (150 x 4.6 mm) to determine the soluble sugars. 100 µM solution of perchloric acid was used as the mobile phase with a flow rate of 0.6 mL/min. The column temperature was maintained at 40 °C and detected by a Refractive Index Detector (RID). Standard sugar solution was used for confirmation purposes and determining the concentration of the sugar compounds being analysed.

Analysis of Amino Acids

Sample preparation for testing amino acids referred to the Perwita (2016) method. Samples (1 g) and 2 ml of HCl 6 N in glass tubes were homogenized using a vortex. Hydrolysis was done by using an oven at 110 °C for 24 hours, then cooled and neutralized with NaOH 6 N. A mixture of 2.5 mL of 40% lead acetate and 1 mL of 15% oxalic acid was added to each sample before being transferred to a 100 mL flask and added distilled water to the mark. 3 mL of the sample was filtered using a nylon syringe filter Millex 0.45 µm and then dilution was applied for 5 times. Samples were treated for 1 min in OPA solution (5 mL sample added 25 mL OPA). The samples were injected into the HPLC as much as 5 mL. Amino acid analysis refers to the method (Bytof and Peter, 2005) using the HPLC system (CBM 20A). The column used was a column (ODS-2 Thermo

Scientific Hyersil). Buffer A consisting of 2 g Na-acetate (pH 6.5), 0.5 g of Na-EDTA, 90 mL of methanol and 15 ml of THF was dissolved in 1L of distilled water and filtered using a 0.45 μ m milipore paper and stored in dark bottles. Buffer B (95% methanol) was filtered use paper milipore 0.45 μ m. The flow rate at 1 mL/min. The detector used was a fluorescence detector. Identification and quantification of amino acids were done by comparing the retention time, in line with a specific spectrum of authentic amino acid standards (Sigma-Aldrich).

Analysis of Caffeine and Chlorogenic Acid

Analysis of caffeine and chlorogenic acid referred to de Maria et al. (1995) methods. Coffee powder (0.2 g) was dissolved in 60 mL of boiling water, then shaken at room temperature for 15 min at 300 rpm. The mixture was filtered using filter paper (Whatman no. 1) and washed using 30 mL of distilled water. The filtrate was added with 2 mL of saturated lead acetate and the final volume was made with distilled water to 100 mL. Colloid dispersion was then filtered using 4 mm PTFE syringe filter of 0.45 μ m prior to analysis.

Caffeine and chlorogenic acid were analyzed using LCMS with a C18 column (5 μm 4.6 x 150 mm) and mobile phase A (0.3% formic acid in H_2O) and mobile phase B (methanol). The elution gradient was set as follows: 15% B to 28% B in 10 minutes; 28% B to 30% B in 15 minutes; 30% B to 100% B in 3 minutes; 100% B maintained within 2 min and 100% B to 5% B in 5 minutes. The analysis time for 35 min at a flow rate of 0.2 mL/min. The injection volume was 5 mL with detector MS (LCMS-2020), an auto-sampler (SIL-

20A), column oven (CTO-20A), pump (LC-20).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics 21 (IBM, Chicago, USA). The data were summarized as means \pm standard deviation (SD) and all results were in triplicate experiments. One-way analysis of variance (ANOVA) was performed and the statistical significance of differences (p<0.05) was evaluated and followed by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Quality Flavor

Organoleptic assessments with the the SCAA method consist of 11 parameters such as aroma, flavor, aftertaste, acidity, body, balance, sweetness, clean-up, uniformity, overall and total score. Based on the cupping score (Table 1) Samples treated with fructose 0.55% (F-0.55) had the highest score of 85.25, while the Honey (H) 83.00 and Fullwash (FW) 84.50. Sample (F-0.55) had the characteristic aroma of spicy, fruity, tammarine, blackcurrant, grape, grassy, floral, brown dry tea leaf like and molasses. Sample (F-0.55) also had the characteristic tastes of spicy flavor, dry fruit, tammarine, blackcurrant, syrup, like tea, floral, tarty, dry berries, honey, salty, medium to light body and light acidity. Samples with the highest score (F-0.55%) were selected for the next analysis consisting of proximate compotition, organic acids, sugars, amino acids, caffeine and chlorogenic acid that was compared with the samples Honey (H) and Fullwash (FW).

Table 1. Cupping Score of Coffee Fermentation using Different Types of Sugar compared to Honey and Fullwash

	Treatment										
Parameter -	Fructose (F) Concentration				Glucose (G)			Sucrose (S) Concentration			Fullwash
i arameter -				Concentration			(
-	0.55	1.1	1.65	0.55	1.1	1.65	0.55	1.1	1.65	(H)	(FW)
Aroma	8.25 ^b	8.00 ^{ab}	8.00ab	8.00ab	8.00ab	8.25 ^b	8.00ab	7.75a	8.00ab	8.00ab	8.25^{b}
Flavor	8.00^{a}	8.00^{a}	7.75 ^a	7.75a	8.00^{a}	8.00^{a}	7.75a	8.00^{a}	7.75a	7.75^{a}	8.00^{a}
Aftertaste	$7.75^{\rm b}$	7.50^{b}	7.50^{ab}	7.50^{ab}	7.25 ^a	7.25 ^a	7.25 ^a	7.25 ^a	7.25 ^a	7.25^{a}	7.50^{ab}
Acidity	7.75^{b}	7.25a	7.25a	7.25a	7.25a	7.25a	7.25a	7.25a	7.25a	7.25^{a}	7.50^{ab}
Body	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.75^{b}	7.25^{a}	7.75^{b}
Balance	7.75^{a}	7.50a	7.50^{a}	7.75 ^a	7.50^{a}	7.50^{a}	7.50^{a}	7.50^{a}	7.50^{a}	7.75^{a}	7.50^{a}
Uniformity	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
Clean Cup	10.00^{a}	10.00^{a}	10.00a	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
Sweetness	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}	10.00^{a}
Overall	8.00^{a}	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	7.75 ^a	8.00^{a}
Total	85.25 ^d	83.75 ^{bc}	83.50 ^b	83.75 ^{bc}	83.50 ^b	83.75 ^{bc}	83.25ab	0.50h 00.050h	02 05sh	92 00a	9.4.50c
Score	85.25°	83./300	83.50°	83./500	83.50°	83./500	83.25	83.25 ^{ab}	83.25 ^{ab}	83.00^{a}	84.50°

Note: Each value is expressed as an average in SD (n = 3). Numbers followed by the same letter in the same line show no significant difference based on the Duncan test (p < 0.05)

Sample (H) had a characteristic aroma of grassy, dry leaf, scorched rice, spicy, choco, nutty, toasty, creamy, floral and palm sugar with the characteristic taste of grassy, herby, nutty, schorced rice, dry leaf, tea like, toasty, palm sugar, corn like, soy bean, watery, tarty and light acidity. Another sample (FW) had the characteristic aroma of herby, cedar, spicy, roast peanut, sweet potato, grassy, vegetabely, mint, palm sugar and slaged like dry with the characteristic taste

of herby, spicy, nutty, choco, cedar, strawy, vegetable, fruity, mint, palm sugar, dry aged wood like, like green tea and light acidity. Coffee treated with F-0.55 had more various aroma and taste compared with other samples. However, all samples produced cupping score that could be categorized as a specialty coffee because the total value produced >80 (SCAA, 2013).

Coffee fermentation using fructose produced a higher total score than the coffee fermentation using glucose and sucrose. Fructose and glucose are sugars categorized as reducing sugars, while sucrose is classified as a non-reducing sugar. Fructose during the roasting process will act as precursors of flavor (Avvallone et al., 2001; Jackels et al., 2006; Lin, 2010). The fructose will improve aroma and taste such as fruity and floral during the coffee processing (Lee et al., 2016). Therefore, it is understandable if the 0.55% fructose treatment provides a complete flavor response. Glucose is involved in the formation of acid and desired aldehyde compounds in the coffee brew. Fructose and glucose as reducing sugar produce more complex flavor such as acid, sweety, mint, alcohol (fruity, floral, honey, rose), esters (fruity, grape, mint, creamy, floral), furan (fruity, caramel, spicy, almond), phenol (rubbery, spicy, sweet, woody), piroles (nutty, coffee) and pyrazine (cocoa, coffee, roast, nutty, caramel) in the coffee (Lee et al., 2016).

Organic Acids

The profile of organic acids could be seen in the table 2.

The lactic acid is the highest organic acid in all samples (green and roasted beans). The content of lactic acids is dominant in the sample (F-0.55) and (H) with a range of 71.37 ± 0.06 % (db) to 84.32 ± 0.13 % (db). High levels of lactic acid caused by lactic acid bacteria are naturally found in the coffee fermentation (Avallone et al., 2001; Evangelista et al., 2015). Lactic acid bacteria are found in wet processing and semi-dry, but less common in dry processing. It is caused by plastic containers used for fermentation, low oxygen content making it suitable for the growth and metabolism of lactic acid bacteria (Bressani et al., 2018; Silva, 2014). Lactic acid bacteria may play a role in increasing the acidity in coffee. Lactic acid bacteria such as Lactobacillus plantarum can convert energy sources into lactic acids through pyruvic acid cycle to produce pyruvate. In addition, pyruvate can produce many metabolites such as acetate, ethanol, diacetyl, and acetaldehyde. These chemical compounds may play a role in increasing the acidity of the coffee.

Table 2. Organic Acid Compound of Coffee Fermentation using Different Types of Sugar compared to Honey and Fullwash

	Treatment									
Omeonie Aeid	Fructose 0.5	5% (F-0.55)	Hone	ey (H)	Fullwash (FW)					
Organic Acid	Green Bean	Roast Bean	Green Bean	Roast Bean	Green Bean	Roast Bean				
	(%db)	(%db)	(%db)	(%db)	(%db)	(%db)				
Sucsinate	1.86 ± 0.03^{aA}	1.83 ± 0.06^{bB}	0.58 ± 0.04^{aA}	1.53 ± 0.04^{aB}	$1.25 \pm 0.06^{\mathrm{Ba}}$	2.05 ± 0.07^{cB}				
Lactic	74.21 ± 0.03^{b}	71.86 ± 0.13^{c}	84.32 ± 0.13^{c}	71.37 ± 0.06^{b}	65.32 ± 0.03^{a}	60.49 ± 0.15^{a}				
Asetic	$0.52 \pm 0.03^{\circ}$	8.31 ± 0.06^{b}	0.34 ± 0.05^{b}	6.74 ± 0.21^{a}	$0.18\pm0.03^{\rm a}$	6.18 ± 0.49^{a}				
Sitric	6.51 ± 0.06^{b}	2.27 ± 0.09^{a}	11.03 ± 0.04^{c}	3.08 ± 0.03^{b}	4.63 ± 0.09^{a}	2.41 ± 0.19^{a}				
Malic	1.75 ± 0.01^{b}	20.22 ± 0.05^{c}	2.01 ± 0.01^{c}	14.50 ± 0.26^{a}	1.52 ± 0.05^a	17.81 ± 0.16^{b}				
Propionate	0.91 ± 0.11^{b}	1.44 ± 0.01^{b}	0.03 ± 0.01^a	1.25 ± 0.06^{a}	3.10 ± 0.14^{c}	$1.12\pm0.04^{\rm a}$				
Butyrate	nd	nd	nd	nd	nd	nd				

Note: Each value is expressed as an average in SD (n = 3). Numbers followed by the same letter in the same line show no significant difference based on the Duncan test (p < 0.05). (nd = not detected)

Malic acid, succinate and citrate are organic acids naturally present in coffee beans and increases during the fermentation process (Bressani et al., 2018). Based on Table 2, the highest content of malic acid contained in the sample (F-0.55). Malic acid plays a role in green apple flavor. Among all organic acids, succinate and citrate acids are dominant in the TAV (Taste Active Value) correlated to the coffee taste. Increasing acid levels impact to flavor and brewed coffee cupping score. High value organic acids of coffee beans may be generated from the addition of sugars. The organic acid is a result of the metabolic process that remodels sugars into organic acids and energy sources (Avvallone et al., 2001; Jackels et al., 2006; Lin, 2010). In addition, the presence of butyric acid and propionic acid adversely affects the quality of coffee beans (Amorim and Amorim, 1977). However, the content of propionic acid in these samples was found in small quantities while the butyric acid was not detected in these samples. Therefore, the addition of sugar during the fermentation treatment did not affect the coffee quality.

Soluble Sugars

Table 3 shows the content of soluble sugars in coffee beans. Low molecular weight carbohydrates such as sucrose, glucose, and fructose have contributed to the formation

of flavor compounds after roasting (Clarke and Vitzthum, 2008). Among the three types of soluble sugars, sucrose has the highest value compared to fructose and glucose. Sucrose is the sugar in the beans, whereas glucose and fructose are abundantly present in the pulp and muchilage (Kleinwächter, 2010). Sucrose significantly has a higher concentration in the green beans compared to the roast beans. The sucrose content in the green beans is up to 9% of the total weight and reaction of sucrose during the roasting process results in a typical coffee flavor and color. (Murkovic and Karin Derler, 2006). In addition, the soluble sugar has a potential as taste-active components contributed to the sweet taste (Rahmadian et al., 2019).

The decreasing concentration of sugars in green beans could be caused by many factors. The decrease is due to the leaching in the washing process and the use of reduced sugars by the bacteria during fermentation (Wootton, 1974). In addition, processing such as drying and roasting also significantly affects the degradation of sugars (Kleinwächter, 2010). In the fermentation process, glucose and fructose as reduced sugars will be reformed into organic acids and ethanol and energy sources. While sucrose is degraded into volatile compounds such as furan, purine, and pyridine, which give the aroma of caramel

through a degradation process of the Maillard reaction during the process of roasting.

Glucose is one type of soluble sugars that only is found out in the roasted bean (**Table 3**). Glucose gives a sweet taste in the significantly impact the sweet taste of brew coffee in this research due to the low concentration. Soluble

sugars with a lower molecular weight, such as fructose and glucose, were degraded early compared to sucrose. If the lower molecular weight sugars are not available anymore, sucrose will be degraded into glucose and fructose, continuing for the Maillard reaction process producing alkylpirazine compounds and other volatile compounds.

Table 3. Soluble Sugar and Secondary Metabolite Compound of Coffee Fermentation using Different Types of Sugar compared to Honey and Fullwash

_	Treatment									
C-1-11- C	Fructose 0.5	5% (F-0.55)	Hone	y (H)	Fullwash (FW)					
Soluble Sugar -	Green Bean	Roast Bean	Green Bean	Roast Bean	Green Bean	Roast Bean				
	(%db)	(%db)	(%db)	(%db)	(%db)	(%db)				
Fructose	0.10 ± 0.03^a	nd	0.37 ± 0.06^{b}	nd	0.17 ± 0.01^a	nd				
Glucose	4.45 ± 0.03^a	0.47 ± 0.04^{b}	4.92 ± 0.07^{b}	0.11 ± 0.02^a	$5.50 \pm 0.04^{\circ}$	$1.80\pm0.04^{\rm c}$				
Sucrose	9.67 ± 0.05^a	nd	13.15 ± 0.23^{b}	nd	11.37 ± 0.03^{c}	nd				
Caffeine	2.59 ± 0.04^{ab}	$2.12\pm0.04^{\rm a}$	2.63 ± 0.04^{b}	2.05 ± 0.04^{ab}	2.37 ± 0.20^a	2.03 ± 0.04^a				
Chlorogenic Acid	13.21 ± 0.04^{b}	10.51 ± 0.04^a	13.39 ± 0.04^{c}	$10.89 \pm 0.03^{\circ}$	12.70 ± 0.04^{a}	10.60 ± 0.04^{b}				

Note: Each value is expressed as an average in SD (n = 2). Numbers followed by the same letters in the same line show no significant difference based on Duncan's test (p < 0.05). (nd = not detected).

Caffeine and Chlorogenic Acid

The caffeine content in green beans showed differences among treatments. **Table 3** shows that the (FW) caffeine content was lower than (H) and (F-0.55). Caffeine content in samples (F-0.55), (H) and (FW) in green beans were respectively 2.59 ± 0.04 mg/g, 2.63 ± 0.04 mg/g and 2.37 ± 0.20 mg/g. Decreasing caffeine levels in the green bean were due to its natural characteristic soluble in water and organic solvents (Perrone et al., 2008). Therefore, the caffeine content in (FW) sample was decreased. The caffeine content in roasted beans was relatively stable in all types of treatments. TAV value of (F-0.55), (H) and (FW) were respectively 2.65 mg/g, 2.56 mg/g and 2.54 mg/g. It is supposed that caffeine has a relatively stable properties against degradation and in a variety of drying conditions (Dong et al., 2017).

Caffeine is a chemical compound classified as phenolics contributing to the formation of a bitter taste in coffee (Perrone et al., 2008). A high concentration of caffeine in this study has a significant impact on the bitter taste in brew coffee. Caffeine is a derivative xantine known as central nervous system stimulant and is generally associated with improvements in alertness, learning capacity and performance. The characteristic of bitter taste from caffeine is an important determination for the formation of flavor (Farah et al., 2006).

The content of chlorogenic acid in all coffee samples was demonstrated in **Table 3.** The content of chlorogenic acid in green bean samples (F-0.55), (H) and (FW) were respectively 13.21 ± 0.04 mg/g, 13.39 ± 0.04 mg/g and 12.70 ± 0.04 mg/g. The chlorogenic acid content in green beans (H) has a higher value than the green bean samples of (F-0.55) and (FW). This is because of the absence of a washing process in the sample (H). It has been reported the decreased level of chlorogenic acid in the green beans is caused by easily soluble in water and organic solvents (Perrone et al., 2008). Generally, chlorogenic acid content in the green bean samples was higher than the sample

roasted bean (**Table 3**). Decreased levels of chlorogenic acid in the sample roasted beans because of the thermal instability and further processing, especially the roasting process of coffee beans. Chlorogenic acid also has a bitter taste and translucent colour. It has been reported that the increase in the temperature roasting increasingly degrades the chlorogenic acid (Clifford, 1972) up to 93% off for the level of dark (Farah et al., 2006).

The content of chlorogenic acids in (F-0.55), (H) and (FW) were respectively 10.51 mg/g, 10.89 mg/g and 10.60 mg/g which was relatively stable on the all roasted samples. Chlorogenic acid and chlorogenic acid lactones quinat could be formed during the roasting process of coffee (Farah et al., 2005), which contributed to an increase in the bitterness of coffee beverage (Ginz and Engelhardt, 2001). Additionally it has been reported that chlorogenic acid in coffee drinks became one of the highest sources compared to other beverages (Clifford, 2000). Arabica coffee drink cup (200 ml) contains 70-200 mg chlorogenic acid (Clifford, 1999). It is also reported that the chlorogenic acid as sources of natural antioxidants (Chen et al., 2019). Therefore, these coffee beans in this research could be developed as a functional drink with beneficial effect in the body.

Amino Acids

The content of amino acids in all samples were shown in **Table 4**. The heat generated by the coffee beans during roasting for a long time can degrade amino acids in coffee beans (Clark, 1987). Glutamate is the highest amino acid in the green beans and roasted beans which were relatively (F-0.55) 1.87 ± 0.01 mg/g, (H) 2.14 ± 0.04 mg/g and (FW) 2.02 ± 0.01 mg/g while in the roasted beans (F-0.55) 1.34 ± 0.01 mg/g, (H) 1.66 ± 0.05 mg/g and (FW) 1.96 ± 0.05 mg/g. TAV value in green beans of sample (F -0.55), (H) and (FW) were respectively 6.23 mg/g, 7.13 mg/g and 6.73 mg/g while in the roasted bean of sample (F-0.55), (H) and (FW) were respectively 4.47 mg/g, 5.53 mg/g and 6.53 mg/g. Glutamate is an amino acid classified as the umami

taste, but in this study, the high glutamate concentrations did not significantly impact the umami taste in coffee infusion. They only have a small amount of concentration in the total amino acids contained by the coffee beans. In addition, the glutamate and asparagine have been deaminated into glutamic acid and aspartic acid for acid hydrolysis process (Chen et al., 2007).

Table 4. Amino Acid Composition in Fermented Coffee Using 0.55% Fructose

				Treat	ment			
Amino	MW^x	Fructose (),55% (A1)	Honey	y (A10)	Full wa	sh (A11)	Taste
Acid	(g mol ⁻¹)	Green Bean	Roasted Bean	Green Bean	Roast Bean	Green Bean	Roast Bean	Description
		(%db)	(%db)	(%db)	(%db)	(%db)	(%db)	
\mathbf{Asp}^C	133.11	$0.72\pm0.02^{\rm a}$	$0.62\pm0.03^{\rm a}$	0.84 ± 0.03^{b}	$0.77\pm0.02^{\rm b}$	$0.92\pm0.03^{\rm c}$	0.73 ± 0.03^{ab}	1, 3
Thr^B	119.12	0.26 ± 0.02^a	$0.22\pm0.05^{\rm a}$	$0.32\pm0.03^{\rm b}$	$0.26\pm0.02^{\rm a}$	0.35 ± 0.02^{b}	$0.25\pm0.03^{\rm a}$	3, 4
Ser^B	105.09	$0.33\pm0.05^{\rm a}$	0.18 ± 0.01^a	0.41 ± 0.01^{b}	$0.21\pm0.04^{\rm a}$	$0.45\pm0.01^{\rm b}$	0.22 ± 0.04^a	2, 3
Glu^C	147.13	1.87 ± 0.01^a	$1.34\pm0.01^{\rm a}$	2.14 ± 0.04^c	1.66 ± 0.05^{b}	2.02 ± 0.01^{b}	$1.96\pm0.05^{\rm c}$	5
Pro	115.13	0.41 ± 0.06^a	0.33 ± 0.06^a	0.49 ± 0.05^a	0.43 ± 0.02^{b}	0.51 ± 0.06^a	0.46 ± 0.01^{b}	6, 3, 7, 8, 9
Gly^{A}	75.07	0.56 ± 0.01^a	$0.40\pm0.03^{\rm a}$	0.63 ± 0.02^a	0.54 ± 0.04^{b}	0.62 ± 0.08^a	0.56 ± 0.01^{b}	10, 3
Ala^A	89.09	$0.44\pm0.05^{\rm a}$	0.29 ± 0.02^a	0.49 ± 0.05^a	0.38 ± 0.05^{b}	0.45 ± 0.08^a	$0.44\pm0.01^{\rm b}$	1, 3, 6
Cys^F	240.30	0.09 ± 0.01^a	0.01 ± 0.01^a	0.29 ± 0.05^{b}	$0.05\pm0.02^{\rm a}$	0.32 ± 0.02^{b}	$0.04\pm0.03^{\rm a}$	12
Val^A	117.15	$0.48 \pm 0.02^{\rm a}$	0.46 ± 0.01^{ab}	0.54 ± 0.03^{b}	$0.43\pm0.02^{\rm a}$	0.50 ± 0.01^{ab}	0.48 ± 0.02^{b}	10, 9
\mathbf{Met}^F	149.20	$0.11\pm0.03^{\rm a}$	$0.08\pm0.04^{\rm a}$	0.06 ± 0.05^a	$0.04\pm0.04^{\rm a}$	0.11 ± 0.06^a	$0.10\pm0.03^{\rm a}$	13, 15
Ile^A	131.18	0.33 ± 0.06^a	0.26 ± 0.02^a	0.39 ± 0.02^{a}	0.32 ± 0.04^{ab}	0.37 ± 0.02^a	$0.34\pm0.03^{\rm b}$	14, 10
Leu^A	131.18	0.77 ± 0.02^{a}	$0.53\pm0.03^{\rm a}$	0.90 ± 0.04^{c}	0.70 ± 0.02^{b}	0.83 ± 0.04^{b}	$0.82 \pm 0.03^{\text{c}}$	14, 10
Tyr^G	181.19	0.19 ± 0.06^a	$0.13\pm0.02^{\rm a}$	0.27 ± 0.02^a	0.24 ± 0.02^{b}	0.27 ± 0.05^a	0.24 ± 0.03^{b}	16, 19
Phe^G	165.19	0.34 ± 0.01^a	0.32 ± 0.03^a	0.46 ± 0.02^{b}	$0.37\pm0.03^{\rm a}$	0.44 ± 0.01^{b}	$0.43\pm0.03^{\rm b}$	<i>18, 17, 9</i>
His^E	155.18	$0.43\pm0.05^{\rm a}$	0.32 ± 0.04^a	0.43 ± 0.04^a	0.35 ± 0.02^{ab}	0.42 ± 0.05^a	$0.40\pm0.02^{\rm b}$	20, 5
Lys^E	146.19	$0.50\pm0.04^{\rm a}$	0.10 ± 0.03^{a}	0.65 ± 0.06^{b}	0.21 ± 0.04^{b}	$0.74\pm0.03^{\rm c}$	0.21 ± 0.07^{b}	3, 10, 9
\mathbf{Arg}^E	174.20	$0.81\pm0.04^{\rm a}$	nd	0.95 ± 0.06^b	$0.51\pm0.03^{\rm b}$	$1.58\pm0.05^{\rm c}$	0.55 ± 0.03^{b}	9
Try^G	204.23	$0.16\pm0.02^{\rm a}$	0.04 ± 0.01^a	0.11 ± 0.03^a	$0.08\pm0.03^{\rm a}$	0.11 ± 0.07^a	0.19 ± 0.04^{b}	3, 10

Note: (x): Wong. KH et al (2008), (A): simple aliphatic, (B): hydroxy-aliphatic, (C): dicarboxylic (acid), (D): amide, (E): basic amino acids, (F): acid amino with sulfur, (G): aromatic. (1): Fruity (like fresh dates), (2): Fruity (slightly like, fresh dates), (3): Pleasant / sweet, (4): Astringent sweet taste, (5): Sour taste, (6): Flowery, (7): Pandan, (8): Slightly persimmon, (9): Bitter taste, (10): Caramel like, (11): Fruity, (12): Sulphury, (13): Fried potatoes, (14): Burnt, (15): Cracker, (16): Flowery (slightly dried roses), (17): Almonds, (18): Flowery (dried roses), (19): Sweet taste, (20): Slight sweet. Each value is expressed as an average with SD (n = 2). Numbers followed by the same letters in the same line show no significant difference based on Duncan's test (p <0.05). (nd = not detected).

Amino acids classified as the sweet perception consist of five types: glycine, alanine, serine, threonine and lysine. The sweetness threshold of the five amino acids were 25 mg/g, 12 mg/g, 25 mg/g, 35 mg/g and 0.5 mg/g and the lowest threshold value was for lysine. In the coffee samples, the sweet flavour was derived from sugars and

amino acids. However, sugars have a bigger role in the formation of the sweet taste in coffee. In **Table 5** indicated that the value of TAV sugar is higher than the amino acids in the green beans. In the roasted bean sugars much more were involved in the formation of aroma flavor through a series of Maillard and caramelization process.

Tabel 5. Taste Active Value (TAV) of Non-Volatile Compounds Green and Roast Bean Coffee

		-	Taste Active Value (TAV) Green Bean					
m .		Threshold -						
Taste	Components	(mg/g) -	Fructose 0			(A10)	Fullwas	
		(118/8)	Cons (mg/g)	DoT (mg/g)	Cons (<i>mg/g</i>)	DoT (mg/g)	Cons (<i>mg/g</i>)	DoT (mg/g)
Sweet (sugar)	Glucose	18.00a	4.45	0.25	4.92	0.27	5.50	0.31
Sweet (sugar)	Fructose	10.20a	0.10	0.01	0.37	0.04	0.17	0.02
	Sucrose	-	9.67	0.01	13.15	-	11.37	- 0.02
Sour (organic acid)	Suksinate	22.00a	1.86	0.08	0.58	0.03	1.25	0.06
Sour (organic acid)	Laktic	80.00a	74.21	0.93	84.32	1.05	65.32	0.82
	Asetic	1.99ª	0.52	0.26	0.34	0.17	0.18	0.02
	Citric	4.3ª	6.51	1.51	11.03	2.57	4.63	1.08
	Malic	7.3ª	1.75	0.24	2.10	0.29	1.52	0.21
	Propionate	-	0.91	-	0.03	-	3.10	- 0.21
	Butyric	_	nd	nd	nd	nd	nd	nd
Umami (amino acid)	Aspartic	$1.00^{\rm b}$	0.72	0.72	0.84	0.84	0.92	0.92
Cinaini (animo acia)	Glutamic	0.30 ^b	1.87	6.23	2.14	7.13	2.02	6.73
Sweet (amino acid)	Glycine	25.00a	0.56	0.02	0.63	0.03	0.62	0.02
Sweet (minio dela)	Alanine	12.00 ^a	0.44	0.04	0.49	0.04	0.45	0.04
	Serine	25.00a	0.33	0.01	0.41	0.02	0.45	0.02
	Threonine	35.00a	0.26	0.01	0.32	0.01	0.35	0.01
	Lysine	0.50 ^b	0.50	1.00	0.65	1.30	0.74	1.48
Bitter (amino acid)	Tryptophan	-	0.16	-	0.11	-	0.11	-
(Arginine	0.50^{b}	0.81	1.62	0.95	1.90	1.58	3.16
	Histidine	0.20^{b}	0.43	2.15	0.43	2.15	0.42	2.10
	Tyrosine	-	0.19	-	0.27	-	0.27	-
	Methionine	5.00a	0.11	0.02	0.06	0.01	0.11	0.02
	Valine	0.40^{b}	0.48	1.20	0.54	1.35	0.50	1.25
	Phenylalanine	0.90^{b}	0.34	0.38	0.46	0.51	0.44	0.49
	Isoleucine	0.90^{b}	0.33	0.37	0.39	0.43	0.37	0.41
	Leucine	1.90 ^b	0.77	0.41	0.90	0.47	0.83	0.44
Sour (amino acid)	Aspargine	-	-	-	-	-	_	-
Bitter (phenolic)	Caffeine	0.80^{a}	2.59	3.24	2.63	3.29	2.37	2.96
~ /	Chlorogenic	-	13.21	-	13.39	-	12.70	-

Continue

		Threshold – (mg/g) –	Taste Active Value (TAV) Roasted Bean							
Taste	Components		Fructose 0).55% (A1)	Honey (A10)		Fullwash (A11)			
			Cons	DoT	Cons	DoT	Cons	DoT		
			(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)	(mg/g)		
Sweet (sugar)	Glucose	18.00a	0.47	0.03	0.11	0.01	1.80	1.10		
	Fructose	10.20^{a}	nd	nd	nd	nd	nd	nd		
	Sucrose	-	-	-	-	-	-	-		
Sour (organic acid)	Suksinate	22.00^{a}	1.83	0.08	1.53	0.07	2.05	0.09		
	Laktic	80.00^{a}	71.86	0.90	71.37	0.89	60.49	0.76		
	Asetic	1.99 ^a	8.31	4.18	6.74	3.39	6.18	3.11		
	Citric	4.30^{a}	2.27	0.53	3.08	0.72	2.41	0.56		
	Malic	7.30^{a}	20.22	2.77	14.50	1.99	17.81	2.44		
	Propionate	-	1.44	-	1.25	-	1.12	-		
	Butyric	-	nd	nd	nd	nd	nd	nd		
Umami (amino acid)	Aspartic	1.00^{b}	0.62	0.62	0.77	0.77	0.73	0.73		
•	Glutamic	0.30^{b}	1.34	4.47	1.66	5.53	1.96	6.53		
Sweet (amino acid)	Glycine	25.00a	0.40	0.02	0.54	0.02	0.56	0.02		
· · · · · · · · · · · · · · · · · · ·	Alanine	12.00^{a}	0.29	0.02	0.38	0.03	0.44	0.04		
	Serine	25.00a	0.18	0.01	0.21	0.01	0.22	0.01		
	Threonine	35.00^{a}	0.22	0.01	0.26	0.01	0.25	0.01		
	Lysine	0.50^{b}	0.10	0.20	0.21	0.42	0.21	0.42		
Bitter (amino acid)	Tryptophan	-	0.04	-	0.08	-	0.19	-		
,	Arginine	0.50^{b}	nd	nd	0.51	1.02	0.55	1.10		
	Histidine	0.20^{b}	0.32	1.60	0.21	1.05	0.21	1.05		
	Tyrosine	-	0.13	-	0.24	-	0.24	-		
	Methionine	5.00^{a}	0.08	0.02	0.04	0.01	0.10	0.02		
	Valine	0.40^{b}	0.46	1.15	0.43	1.08	0.48	1.20		
	Phenylalanine	0.90^{b}	0.32	0.36	0.37	0.41	0.43	0.48		
	Isoleucine	0.90^{b}	0.26	0.29	0.32	0.36	0.34	0.38		
	Leucine	1.90 ^b	0.53	0.28	0.7	0.37	0.82	0.43		
Sour (amino acid)	Aspargine	_	0.62	-	0.77	-	0.73	-		
Bitter (phenolic)	Caffeine	0.80^{a}	2.12	2.65	2.05	2.56	2.03	2.54		
- /	Chlorogenic	_	10.51	-	10.89	-	10.60	-		

Note: GB: Green Bean; RB: Roasted Bean; (a): Hufnagel and Hofmann, 2008; (b): Chen and Zhang (2007)

Nine amino acids were classified as having a bitter taste consisting of arginine, histidine, methionine, valine, phenylalanine, isoleucine, leucine, tyrosine and tryptophan with a taste threshold value of respectively 0.50 mg g, 0.20 mg/g, 5.00 mg/g, 0.40 mg/g, 0.90 mg/g, 0.90 mg/g and 1.90 mg/g but tyrosine and tryptophan were undiscovered in the literature. Three of them are aromatic amino acids consisting of phenylalanine, tyrosine and tryptophan (Wong et al., 2008).

According to Wong et al. (2008), temperature, substrate ratio, pH, and time can affect flavor notes formed during the Maillard reaction. Some amino acids, such as aspartic acid and glutamic acid have naturally low pH values (respectively pH 3.0 and 3.2), while others are in the pH range of 5.3 to 5.8. Various detectable aroma of arginine, aspartic acid and cysteine in pH 5.2 implies conformity to the formation of flavor. Serine, proline, and phenylalanine provide significant fruity taste and floral aroma. Alanine produces the smell of flowers and also aspartic acid produces a pleasant smell (pleasant odor). The aroma is detected from isoleucine, leucine, lysine and threonine like caramel and become more distinct in a longer heating. No odor was detected from arginine, glutamic acid and histidine, even after a change of color. Detection of odor depends on the concentration and threshold of major odor impact compounds as well as the sensitivity of the human nose. In addition, it was reported that Maillard reaction between amino acids and reducing sugars produced smells like popcorn roast (Hunter et al., 1969). In this point of view, Maillard reaction may affect the coffee flavor during the processing. Generally, all of the amino acid contents were similar in all samples.

CONCLUSION

Based on these results, fructose is a type of sugar suitable as a fermentation substrate used in the coffee processing by 0.55%. Fermentation using 0.55% fructose gave positive effect on the profile of nonvolatile compounds such as organic acids, amino acids, caffeine, and chlorogenic acid content of which is correlated to the increase steeping coffee cupping score until it reaches a value >84 (85.25), where it is classified as an excellence coffee. The content of caffeine and chlorogenic acid was relatively stable in all samples and contributed to the coffee flavour. The chlorogenic acid also has the potential as an antioxidant. Therefore the coffee processing with sugar addition may affect the coffee flavour in general for the brewed taste and beneficial effects in body. Furthermore, to understand the effect of sugar addition to the flavor coffee, study of key aroma should be conducted and to understand the beneficial effects of the coffee beans, the study of antioxidant activities should be examined.

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