The influence of coffee cherry fermentation on the properties of *Cascara arabica* from Subang, West Java

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Abstract. Cascara is an infusion beverage of dried coffee cherry pulp prepared from a by-product of coffee production. Cherry of arabica coffee was fermented with spontaneous fermentation, *Lactobacillus plantarum* and yeast (*Saccharomyces cerevisiae*) at room temperature for 24 hrs. The properties of cascara including total phenolic, flavonoid, and caffeine contents, antioxidant activity, colour, and sensory acceptability were evaluated. Results showed that cascara from *L. plantarum* fermentation (Y) exhibited the highest polyphenols and caffeine contents of 46.78 ppm and 52.8 ppm, respectively. Cascara from spontaneous fermentation (X) resulted the highest flavonoids content and antioxidant activity of 0.28 ppm and 89.52%, respectively. The result of sensory evaluation showed that cascara from yeast fermented cherry obtained the highest overall acceptance score of 4 out of 6.

1. Introduction
Cascara also is known as cherry tea coffee is defined as dried coffee cherry pulp which is a by-product of coffee bean production [1]. Cascara beverage is usually prepared by infusing dried coffee cherry porridge with hot water to extract active compounds that provide health benefits. Polyphenol compounds such as chlorogenic acid, protocatechuic acid, gallic acid, and are present in cascara and result in high antioxidant activity [2]. Mullen, *et al.* (2013), reported that cascara from China, India, and Mexico contain active compound that are chlorogenic acids (CGA), caffeine, and polyphenolic content [3]. The utilization of cascara as a derivative product from coffee production can increase the economic value of coffee cherry pulp and its nutritional benefits [4].

The processing of coffee production was developed continuously to improve the coffee bean quality (flavor and unique taste) and fulfill the costumer or market demand. Coffee cherry fermentation was used to reduce the caffeine content and obtained the best flavor quality [5–7]. Caffeine is the major compound of whole coffee cherry, coffee bean and coffee husk [3]. Caffeine consumption can enhance mood, exercise performance and reduce the symptoms of Parkinson’s disease and tremors. It has been reported that high caffeine consumption also stimulates the central nervous system, increases the blood circulation and respiration [8]. Coffee cherry fermentation is intended to reduce the caffeine content in coffee beverage.

Luwak coffee is a very popular fermented coffee because of the good flavor, taste, aroma and lower acidity and caffeine [5]. Luwak (*Paradoxurus hermaphroditus*) eats coffee cherry in the coffee plantation, then it defecates the remaining seeds as feces. Coffee cherry was fermented by the digestion...
system in luwak body. Usman et al (2015) reported that the predominant bacteria in the luwak feces was lactic acid bacteria. Hatiningsih et al (2018) reported that lactic acid bacteria and yeast were the main microbes in the spontaneous fermentation for Green Coffee (Coffea arabica) [9]. In vitro fermentation method using Lactobacillus bacteria and yeast has been developed to improve the quality of coffee. Fermentation of green coffee resulted green coffee bean with higher acid content, lower caffeine and polyphenol while the antioxidant activity was stable [9]. Lactic acid bacteria act in solubilization digests the complexed pectin producing more soluble pectin, while Saccharomyces cerevisiae hydrolyses cellulose to become glucose, reduced crude fiber and tannin effecting the increase of amino acids [10]. Coffee cherry fermentation influences the physicochemical characteristic of coffee bean, moreover it also affects the properties particularly the chemical content such as phenolic, alkaloid compounds and others soluble components of cherry pulp as the raw material of cascara. The aim of this study was to investigate the effect of in-vitro fermentation condition on cascara infused water includes colour, total phenolic, flavonoid, antioxidant activity and sensory acceptability.

2. Materials and methods

2.1. Materials
Arabica coffee cherry was obtained from coffee plantation of BUMDES Mukti Harja Subang-West Java. The chemicals for analysis were sodium carbonate (Na2CO3), Folin-Ciocalteu reagent, gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol absolute, quercetin, aluminum chloride (AlCl3), sodium acetate (CH3COONa), methanol, caffeine, and distilled water. Microbes used in the fermentation process were Lactobacillus plantarum (isolated from coffee cherry and identified based 16S rRNA) and commercially available yeast of Saccharomyces cerevisiae (fermipan) which were in the dried powder form.

2.2. Preparation of fermented cascara
Arabica coffee cherry was fermented in 3 fermentation methods (Table 1) including spontaneous fermentation as the control (X), fermentation by L. plantarum starter (Y), and S. cerevisiae starter (Z) in room temperature for 24 h. The medium for fermentation was prepared by dissolving 2g of starter powder in 2 l of distilled water and mixing well. The fermentation process was done by soaking 1 kg of arabica coffee cherry to the fermentation liquid in triplicate. Fermented coffee cherry was pulped using coffee pulper to separate the pulp and coffee bean. The coffee pulp was dried using oven dryer in temperature 50°C for 12 h. The dried of coffee pulp was ground and packed in the tea bag which was 3 g per pack, the secondary packaging was aluminum foil (10x10cm). Cascara samples for analysis were prepared by pouring 150 ml of the boiled water into a tea bag of cascara and infusing for 15 minutes and then removing the cascara. The cascara infusion water was the sample for analysis steps.

Table 1. Arabica Coffee Cherry fermentation methods.

| Sample | Fermentation Methods                  |
|--------|---------------------------------------|
| X      | spontaneous fermentation               |
| Y      | Lactobacillus plantarum starter       |
| Z      | Saccharomyces cerevisiae starter      |

2.3. Colour analysis
The colour of cascara arabica infused water was measured by colourimeter (3NH-Taiwan). The sample was placed in a cuvette then colour reading was carried out. The obtained value was 3 colour coordinates namely, L* (represents lightness index), a* (represents the tons from red to green colour) and b* (represents the tons from yellow to blue colour) [11].
2.4. Total phenolic content
Total phenolic content was determined according to the method of the Indonesian National Standard 3753: 2014 for black tea bag with some modifications [12]. Gallic acid was used as the standard which the concentrations were of 10, 20, 30, 40, and 50 ppm. An amount of 1 ml of standard solution or sample was added by 5.0 ml of Folin-ciocalteu 10% and allowed for 3-8 minutes. Four ml of 7.5% Na₂CO₃ solution was added and mixed homogenously. The mixture then placed for 50 minutes at room temperature. All steps done in the dark bottles. Distilled water was used as a blank solution. The samples solution was treated as the standards solution. The next step was measuring the absorbance of blank, standards and samples solutions using the Shimadzu 1900 UV-Vis spectrophotometer at 765 nm. The total phenolic content was calculated based on the standard curve of gallic acid (y=0.0147x + 0.0074; R²=0.99).

2.5. Flavonoid content
Determination of flavonoid content of samples used the aluminium chloride colourimetric method [13] with slightly modification. Quercetin was used as the standard calibration curve with serial concentration is 0.2; 0.4; 0.6; 0.8 and 1 ppm, ethanol was as a blank. An amount of 1.5ml of standard, samples and blank were added by 1.5 ml of 1.2% AlCl₃ solution and 1.5 ml of CH₃COONa. Then all dark bottles cover tightly and mix using vortex, then were placed in a dark room for 30 minutes. The absorbance was measured by Spectrophotometer UV-Vis Shimadzu 1900 in wavelength of 415 nm. (y= 0.7843x-0.0055; R²= 0.9958).

2.6. Caffeine content
Determination of caffeine in the cascara infused water was use HPLC Infinity II 1260-AGILENT according to the method of Shrestha et al [14]. A C-18 column was used for HPLC analysis (ODS 5 μm, internal diameter 4.6 nm and length 150 mm) with isocratic mobile phase methanol : water (40:60) at flow rate of 1 ml/min and column temperature at 40°C. The peak of caffeine was observed at 2.56 minutes using UV detector set at 275 nm. The concentration of caffeine standard was 2, 4, 6, 8, and 10 ppm. Distilled water was used for blank. Caffeine content of samples was determined based on caffeine standard curve (y= 25.156x+4.3998; R²=1).

2.7. Antioxidant Activity
2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used for antioxidant activity measurement [15]. Quercetin was used as positive control in range concentrations were 5, 10, 15, 20, 25 and 30 ppm. DPPH solution was made in 0.3mM and ethanol was the solvent. An amount of 2 ml standard and samples were placed in dark bottles, then added by 2 ml of DPPH 0.3 mM and 1 mL of ethanol. The mixed solution was homogenized and incubated in the dark room for 30 minutes. The absorbance of standard and samples were measured using Spectrophotometer UV-Vis Shimadzu 1900 at 517 nm. Antioxidant capacity reported as percent radical scavenging activity (% RSA) and calculated based on equation 1 [16].

\[
(\%RS\text{A}) = \frac{A_b - A_s}{A_b} \times 100 \% \quad (\text{Eq. 1})
\]

Where:
- \(A_b\) : absorbance of blank
- \(A_s\) : absorbance of sample

2.8. Sensory analysis
Sensory test of the cascara infused water was assessed by hedonic test with 30 panelists, in which the samples consisted 3 cascara infused water (X, Y, Z). The panelist evaluated the samples through hedonic parameters including colour, aroma, taste, after taste and overall acceptability with the hedonic scale from 1-6 in which 1 represents “extremely dislike,” 6 indicates “extremely like.”. The data was
calculated the average value of every parameter for quantification the cascara acceptability. The mean value of each sensory parameter was reported.

2.9. Statistical analysis
Statistical analysis for all parameters determination including colour, total phenolic, flavonoid, caffeine content, and antioxidant activity was conducted by Analysis of variance (ANOVA) and post-hoc (P<0.05) test was performed to find out the difference between the variables. Microsoft Excel version 2016 program was employed for Statistical analysis.

3. Results and discussion

3.1. Colour Analysis
Colour of the cascara infused water reported in Table 1, expressed in L, a and b. L* was indicate the lightness, a* represent the difference in the colours red (+) and green (-), and b* was the indicator coordinates yellow (+) and blue (-) [17]. Cascara infused water were resulted from L. plantarum and S. cerevisiae fermentation methods had lower value of lightness, redness and yellowness than spontaneous fermentation, although insignificantly different (p<0.05). Yellowish colour of cascara infused water can be produced by epicatechin compound, and the redness was resulted by rutin, beta carotene and anthocyanin [18,19]. Colour of unfermented cascara infused water (3:100) had value of L was 32.27, a* was 7 and b* 17 [20]. The sample X showed higher lightness, redness and yellowness than sample Y and Z. Organic compounds such as phenolic, flavonoid and alkaloid can dissolve in the water during fermentation thereby reducing the lightness, redness and yellowness. Microbes digest the matrix of coffee cherry which consists of macromolecules such as complexed carbohydrate, lipid and protein due to may increase solubility of flavonoid, alkaloid and phenolic [10].

| Table 2. Colour of fermented Cascara. |
|---|---|---|
| L | a | b |
| X | 28.33±0.29<sub>n</sub> | 2.62±0.14<sub>m</sub> | 3.94±0.19<sub>n</sub> |
| Y | 28.15±0.44<sub>n</sub> | 2.58±0.22<sub>m</sub> | 3.69±0.27<sub>n</sub> |
| Z | 28.18±0.4<sub>n</sub> | 2.57±0.17<sub>m</sub> | 3.53±0.25<sub>n</sub> |

3.2. Total phenolic content
The major component of polyphenols in the whole coffee fruit were flavan-3-ols and flavonols, further the arabica coffee contain greater diversity of polyphenol compound than robusta [3]. The result showed that yeast fermentation (Z) produced cascara with the lowest value of total phenolic (Figure 1), meanwhile the L. plantarum fermentation displayed no effect on total phenolic compound. S. cerevisiae produced extracellular enzyme including amylase which can hydrolyze the α(1,6)- bonding of amylopectin and change sugar become alcohol [21]. In the coffee cherry, phenolic compounds conjugated with amino acids and glycosides [3,22], so it was possible the hydrolysis reaction can increase the solubility of glycosides and phenolic compound in the water. Lactobacillus act to convert carbohydrates of substrate into lactic acid. Carbohydrate in coffee cherry is a complex carbohydrate such as cellulose and pectin, so fermentation by L. plantarum failed to decrease the content of phenolic compounds in cascara.
3.3. Flavonoid content

Colourimetric method for flavonoid determination based on the formation of complexed between AlCl₃ and keto groups of C-4 and hydroxyl groups of C-3 or C-5 in the flavon and flavonols [23]. Quercetin is a flavonol compound used as standard in flavonoid determination. Figure 2 shows that the highest total flavonoid content was obtained of sample X that was 0.278 ppm. Fermentation by L. plantarum and S. cerevisiae decreased the total flavonoid content even though it was not significantly. L. plantarum and S. cerevisiae cause amount of the flavonoid release into water or fermentation medium.

3.4. Caffeine content

The amount of caffeine is influenced by many factors that are the processing method, type of coffee cherry, plantation [2,3,9]. Whole arabica coffee cherry contain 1.3-5.2 mg/g of caffeine, coffee bean 1.3-5.4 mg/g, coffee husk are between 1-1.3 mg/g [24]. The concentration of caffeine in the arabica coffee cherry is lower than robusta coffee [3]. Caffeine is alkaloid compound contained mostly in the coffee, tea and cocoa. Caffeine consumption recommended in moderate level to get mood booster effect and health benefits [24].

Figure 1. Total Phenolic Content of Fermented Cascara.

Figure 2. Flavonoid content of fermented cascara.
Determination of caffeine using HPLC detected the peak of caffeine in the retention time was 2.5 minute. The obtained caffeine concentration in the cascara infused water showed in Figure 3. The result express that spontaneous fermentation produced cascara with the lowest caffeine significantly (p<0.05). In the spontaneous fermentation may consist many kind of microbes but the dominants were Lactic acid bacteria and yeast which could produce pectinolytic and proteolytic enzymes [9]. Furthermore, the microbes have abilities to hydrolyze and degrade the caffeine content in the cherry coffee during the fermentation. Caffeine can be derived become chlorogenate acid and continuing degradation to form smaller compound that can release or soluble in the water [7].

![Figure 3. Caffeine content of fermented cascara.](image)

### 3.5. Antioxidant activity
Coffee cherry was reported as a potential source of antioxidant because it was confirmed containing rich of the active compound such as phenolic, flavonoid, alkaloid and organic acids. Antioxidant activity indicate the capability to inhibit the oxidation process or radical reaction [16]. Sources of antioxidant compound are required to improve the immunity and maintain the healthy especially in this pandemic Covid-19 [25]. Mullen et al [3], reported that the antioxidant activity of coffee cherry related to the phenolic content but it did not related to flavonoid content.

Fermentation of coffee cherry in 3 fermentation methods resulted cascara which were stable in its antioxidant activity. Spontaneous fermentation produced higher antioxidant activity that was 89.52% followed by L. plantarum (87.63%) and S. cerevisiae (84.76%) (Figure 4).

![Figure 4. Antioxidant activity of fermented cascara using DPPH method.](image)
3.6. Sensory analysis

Figure 5 shows that sample Z possessed the highest value of overall obtained acceptability with the score was 4 from 6. While the values for samples X and Y were 3. These results indicated that the fermentation process by S. cerevisiae can improve the taste quality of cascara beverage better than with L. plantarum and spontaneous fermentation. This was in line with research by Neto el al [26] which was fermented coffee cherry by yeast produced cascara with better overall acceptance than spontaneous fermentation. Cascara fermentation by L. plantarum was not showed significantly different from spontaneous fermentation in the overall acceptance. The results of sensory test of fermented coffee beverages using yeast get score 91 points [27], while fermented coffee beverage L. plantarum showed 88.3 points, based on the results of the Specialty Coffee Association of America's Cupping (https://sca.coffee/research/coffee-standards). This was due to the fermentation process using S. cerevisiae in coffee cherries, the mucilage of coffee cherry was acted as a source of carbon and nitrogen which could produce ethanol, lactic acid, and other metabolites that produce better taste, aroma, flavor and acceptance. The fermentation process can produce volatile compounds that are diffused in the beans and cascara that have an impact on the final quality of the product. Yeast, in this case S. cerevisiae can produce compounds, such as esters, alcohols, aldehydes, ketones, and terpenoids, that could affect the product's aroma through carbon metabolism so that a distinctive aroma is produced and differentiates it from other drinks.

![Figure 5. Result of organoleptic analysis.](image)

4. Conclusions

Fermentation processing method of arabica coffee cherry influenced the total phenolic, flavonoid, caffeine content and sensory acceptability. Lactobacillus plantarum fermentation (Y) showed the highest polyphenols and caffeine contents of 46.78 ppm and 52.8 ppm, respectively. Cascara from spontaneous fermentation (X) resulted the highest flavonoids content and antioxidant activity of 0.28 ppm and 89.52%, respectively. The result of sensory evaluation showed that cascara from yeast fermented cherry obtained the highest overall acceptance score of 4 out of 6. Fermentation method of coffee cherry suggest to be continuously developed to optimize the fermentation condition to improve the coffee bean and cascara quality.

Conflict of interest

The authors have no conflict of interest to state regarding this manuscript.
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