Heliyon 6 (2020) e04462

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Fermentation of coffee pulp using indigenous lactic acid bacteria with simultaneous aeration to produce cascara with a high antioxidant activity

Lina Oktaviani [a], Dea Indriani Astuti [a], Mia Rosmiati [a], Muhammad Yusuf Abduh [a,b,*]

[a] School of Life Sciences and Technology, Institut Teknologi Bandung, Jalan Ganesha 10, 40132 Bandung, Indonesia
[b] Center of Excellence for Nutraceuticals, Bioscience and Biotechnology Research Center, Institut Teknologi Bandung, Jalan Ganesha 10, 40132 Bandung, Indonesia

ARTICLE INFO

Keywords:
Food science
Food technology
Aeration
Antioxidant activity
Cascara
Coffee pulp
Lactic acid bacteria

ABSTRACT

Coffee pulp which is a by-product of coffee production contains considerable amounts of phenolic compounds that can be valorised to produce cascara as an antioxidant beverage. The fermentation and drying conditions of the coffee pulp have a great influence on the bioactive compounds in the cascara. This study aimed to investigate the effect of natural fermentation with simultaneous aeration on the phenolic content and antioxidant activity of cascara. A systematic study was carried out using a response surface methodology with a face-centered central composite design to determine the effect of fermentation time (0–8 h) and temperature (27–37 °C) on the number of bacteria in the coffee pulp after natural fermentation with simultaneous aeration (an air flowrate of 4 m/s) as well as phenolic content and antioxidant activity of cascara. The experimental dataset was modelled with an empirical model using multi-variable non-linear regression. A good agreement between model and experimental data was obtained. At the optimum conditions (4.2 h, 31.8 °C), the phenolic content was 6.72% whereas the antioxidant activity was 27.6%. Indigenous lactic acid bacteria were also isolated from the coffee pulp and determined as *Leuconostoc pseudomesenteroides*. The isolated bacteria can be used as a starter for controlled fermentation of coffee pulp as it increased the antioxidant activity up to 15% higher than the antioxidant activity of cascara obtained at the optimum conditions for natural fermentation with simultaneous aeration and 30% higher from the fresh coffee pulp.

1. Introduction

Coffee is a popular beverage brewed from roasted coffee beans that have been produced in more than 70 countries (Alves et al., 2017). In 2017–2018, the global production of coffee was around 9.5 million tons and increased to approximately 10.2 million tons in 2018–2019 (Adam et al., 2020). A global increase in coffee production also results in a huge number of residues. The residues contain caffeine, polyphenol and tannin that may cause severe contamination and serious environmental problems if not treated properly (Murthy and Naidu, 2012; Oliveira and Franca, 2015; Alves et al., 2017).

According to Bakker (2013), 15 million tons of residues are generated annually from the global coffee production from which 9.4 million tons comes from the coffee pulp. Coffee pulp is still underexploited because it contains protein (9–11%), lipid (2–17%), cellulose (13–27%), tannin (4.5%), pectin matter (6.5%), reducing sugar (12.4%) and non-nitrogen extract (57–63%) (Pleissner et al., 2016; Woiciechowski et al., 2000). Hence, coffee pulp may be further valorized for producing bioproducts such as compost and vermi-composting (Murthy and Naidu, 2012; Pandey et al., 2000), animal feed (Murthy and Naidu, 2012; Oktaviani et al., 2019), bioenergy (Bonilla Hermosa et al., 2014; Pandey et al., 2000), sugars (Murthy and Naidu, 2012; Pandey et al., 2000), flour (Velez and Lopez, 2015), aroma compounds (Bonilla Hermosa et al., 2014) and cascara (Heeger et al., 2017).

Cascara which is also known as a coffee cherry tea is an infusion of dried coffee cherry pulp. The global demand of cascara is increasing with a market price of US$13 - US$17 per kg (Ciummo, 2014) and it has been the subject of several studies in recent years due to the composition of phenolic compounds in the coffee pulp (Heeger et al., 2017). Chlorogenic acid is the predominant phenolic compound in addition to flavonols and anthocyanidins (Ramirez-Coronel et al., 2004; Rodríguez-Durán et al., 2014) which highlights the potential of coffee pulp as a source of antioxidant and phenolic compounds. Heeger et al. (2017) has investigated the production of cascara by aqueous extraction of dried coffee pulp from different origins. The extracted samples contain total polyphenols in the range of 4.9 and 9.2 mg gallic acid equivalents (GAE)/g dry matter with

* Corresponding author.
E-mail address: yusuf@sith.itb.ac.id (M.Y. Abduh).

https://doi.org/10.1016/j.heliyon.2020.e04462
Received 28 March 2020; Received in revised form 8 June 2020; Accepted 10 July 2020

an antioxidant activity between 51 and 92 μmol Trolox equivalents (TE)/g dry matter.

Other studies have investigated the effect of fermentation of coffee pulp with different types of microbes on the phenolic and antioxidant content of the extracted samples (Arellano-González et al., 2011; López et al., 2013; Rios et al., 2014; Kurniawati et al., 2016; García et al., 2015; Santos da Silveira et al., 2019). The studies concluded that fermentation of coffee pulp with microbes highly influenced the phenolic and antioxidant content of the extracted samples. For instance, natural fermentation of coffee pulp using indigenous microbes from molasses for 60 days decreased the antioxidant activity from 215 to 206 μmol trolox g^{-1} dry matter (Rios et al., 2014). In another study, solid state fermentation of coffee pulp with *Aspergillus tamari* for 5 days increased the percentage of phenolic content from 12.2% to 13.7% (Arellano-González et al., 2011).

The antioxidant activity in cascara is highly influenced by indigenous bacteria in the coffee pulp, one of them is heterofermentative lactic acid bacteria (LAB). The bacteria increased the antioxidant activity of coffee pulp by converting phenolic compounds to other compounds that have a higher antioxidant activity (López et al., 2013). López et al. (2013) has investigated the effect of fermentation of fresh coffee pulp with 5 different types of LAB at 37 °C under anaerobic condition for 48 h. The antioxidant activity of the fermented coffee pulp increased from 72% up to 81.8% depending on the types of LAB. The total phenolic content of the coffee pulp increased from 1% to 1.2% when fermented with *Lactobacillus casei* but decreased with other types of LAB. As such highlights that different types of bacteria have different effects on the total phenolic content of the coffee pulp.

Although several studies on the fermentation of coffee pulp have been reported, systematic studies that optimize the conditions for natural fermentation with simultaneous aeration using indigenous microbes isolated from the coffee pulp particularly heterofermentative LAB are still very scarce. Hence, this study attempts to optimize the conditions of natural fermentation particularly temperature and incubation time on the number of LAB, antioxidant activity, phenolic content of the fermented coffee pulp. The indigenous bacteria on the coffee pulp were also isolated and determined for future application as a starter culture for controlled fermentation of coffee pulp to produce a high antioxidant beverage.

2. Material and methods

2.1. Materials

Coffee pulp was obtained from a local coffee plantation in West Java, Indonesia. Ethanol, methanol, de Man, Rogosa, and Sharpe (MRS) medium, bacto agar, Na_2CO_3, Folin-ciocalteau, and NaCl were obtained from School of Life Sciences and Technology, Institut Teknologi Bandung. 2,2-diphenyl-1-picrylhydrazyl reagents, chlorogenic acid standards, and caffeic acid standards were obtained from Sigma Aldrich.

2.2. Isolation of lactic acid bacteria

Two g of fresh coffee pulp was mixed with 20 ml of sterile MRS broth media (pH 5.8) in an Erlenmeyer flask. The mixture was mixed slowly followed by incubation at 37 °C for 48 h. After that, different concentration of bacterial culture was inoculated on the MRS media using a spread plate technique. Incubation was carried out at 37 °C for 3–5 days (Leong et al., 2014).

2.3. Selection and identification of lactic acid bacteria

Screening of isolates involved the ability of bacteria to produce lactic acid and CO_2 as well as gram tests. For determination of acid-producing bacteria, 1% $CaCO_3$ was added to MRS-agar plates. The isolates were spread directly onto the surface of MRS-agar plates followed by incubation at 30 °C for 3–5 days. The isolates were spread directly onto $CaCO_3$-agar medium. Acid-producing bacteria were identified by a clear zone around each colony (Leong et al., 2014). For a gram staining test, the isolates were sequentially stained with crystal violet, iodine, and then destained with alcohol and counter-stained with safranin. Gram-positive bacteria were identified when the colour turned blue-purple whereas gram-negative bacteria were identified when the colour turned red (Cappuccino and Sherman, 2014). The ability of bacteria to produce CO_2 was determined tested using Durham tubes. Five ml of sterile MRS both medium was poured into a Durham tube inoculated with 1 ml of LAB inoculum for 24 h. The gas released through the top of the Durham tube indicates production of CO_2 (Cappuccino and Sherman, 2014). The isolated bacteria were identified with 16s RNA sequencing followed by further analysis with a phylogenetic tree based on the maximum likelihood method using a MEGA software (Hall, 2013).

2.4. Natural fermentation of coffee pulp

Preliminary experiments on natural fermentation of coffee pulp with and without simultaneous aeration were carried out prior to systematic studies of natural fermentation with simultaneous aeration. For natural fermentation of coffee pulp without simultaneous aeration, 500 g of coffee pulp was placed inside a closed container for 24 h. After that, the sample was dried inside an oven at 32 °C for 24 h followed by extraction and further analysis. For natural fermentation of coffee pulp with simultaneous aeration, 500 g of coffee pulp was placed on a perforated plat inside an oven set at 32 °C and an air flowrate of 4 m/s for 24 h followed by extraction and further analysis. For the systematic studies of natural fermentation with simultaneous aeration, 500 g of fresh coffee pulp were placed on a perforated plate inside an oven at different temperature and incubation time as shown in Table 1. The air flowrate inside the oven was 4 m/s whereas relative humidity in the oven lies in the range of 66–68%.

2.5. Design of experiments, statistical analysis, and optimization

Non-linear multi-variable regression was used to model the experimental data using a Design Expert software. The data were modelled using the following equation.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1^2 + \beta_4 x_2^2 + \beta_5 x_1 x_2 + e \quad (1)$$

where y is a dependent variable (phenolic content, antioxidant activity, and number of LAB), x_1 and x_2 are independent variables (temperature and fermentation time) that were varied systematically using a face-centered central composite design. β_i are regression coefficients of the model and e is a model error. The regression equations were obtained by elimination of non-significant coefficients. A coefficient was considered statistically relevant when the p-value was less than 0.05. The optimum conditions for the coffee pulp fermentation were determined using the numerical optimization function provided in the software (Abduh et al., 2016).

2.6. Fermentation of coffee pulp using heterofermentative lactic acid bacteria inoculum

Isolates of *Leuconostoc sp.* obtained from the isolation step were used as starters for the fermentation of coffee pulp. *Leuconostoc sp.* in the agar medium was inoculated into 100 ml of MRS broth at 37 °C for 24 h. The inoculum was then cultivated on MRS medium mixed with coffee pulp at 37 °C for 24 h (Kurniawati et al., 2016). After that, the coffee pulp was fermented with 10^7 cfu/ml inoculum of *Leuconostoc sp.* using the optimum conditions obtained from the regression equations. The concentration of the inoculum was varied 0–15% (v/w) where v is the volume of the inoculum (ml) and w is the weight of the fresh coffee pulp (g). The number of *Leuconostoc sp.* was calculated during the fermentation using a

Table 1. Factors and level for the natural fermentation with simultaneous aeration of coffee pulp.

Factors	Level		
	-1	0	+1
Temperature (°C)	27	32	37
Incubation Time (h)	0	4	8

spread plate method and the antioxidant activity of the fermented coffee pulp was determined.

2.7. Extraction of coffee pulp

Ten g of coffee pulp was mixed with 100 ml of methanol. Phenolic compounds in the mixture were extracted using a maceration technique for 24 h at room temperature (27 °C) followed by a filtration using Whatman No. 1 filter paper (Geremu et al., 2016). The filtered extract was analysed to determine the concentration of total phenolic content and antioxidant activity.

2.8. Determination of antioxidant activity

Three ml sample of varying concentration (100–1000 ppm) was mixed with 2 ml of 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) in a test tube. The mixture was mixed well and incubated at room temperature (27 °C) in a dark condition for 30 min. The absorbance of the solution was measured using a spectrophotometer at a wavelength of 517 nm (Blois, 1958). In this study, the antioxidant activity is expressed in DPPH scavenging activity (at 100 ppm) which can be calculated using the following equation:

$$Antioxidant\ activity\ (\%) = DPPH\ scavenging\ activity\ (\%) = \frac{Ac - As}{Ac}\ x\ 100 \quad (2)$$

Where A_c is the absorbance of the control (DPPH solution) and A_s is the absorbance of the test solution (Geremu et al., 2016).

2.9. Determination of total phenolic content

Total phenolic content of the sample was determined using a Folin–Ciocalteu method. Gallic acid was used as a standard and the total phenolic content was expressed as mg/g gallic acid equivalents (GAE) based on a calibration curve using gallic acid. Gallic acid (0.5 g) was weighed into a 10 ml volumetric flask before dissolved in 10 ml of absolute methanol and the solution was made up to 100 ml with methanol. In order to prepare a standard curve, the standard solution was diluted into 0–100 mg/l of gallic acid. Then, 0.5 ml of each sample mixed with 2.5 ml of a tenfold diluted Folin–Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with an aluminium foil and allowed to stand for 30 min at room temperature (27 °C). The absorbance of the sample was measured at 765 nm using a spectrophotometer (Geremu et al., 2016).

3. Results and discussions

3.1. Isolation and identification of heterofermentative lactic acid bacteria

In this study, there were 6 colonies with different morphology that were isolated from the coffee pulp. The isolated bacteria were grown on MRS agar medium with a total number of 10^6 cfu/ml up to 10^7 cfu/ml which resembles the previous results obtained by Zhang et al. (2019). MRS agar medium can be used to predict the number of LAB because it acts as a selective medium for LAB at low pH values (Corry et al., 2011). A total of 4 acid-producing bacterial colonies were found from the 6 isolated bacteria. These isolates showed a clear zone around the colonies in the MRS-$CaCO_3$ agar medium which indicates the presence of lactic acid bacteria as demonstrated by Onda et al. (2002).

Based on the gram staining assay, the 4 acid-producing bacterial colonies showed a violet color which indicates that the bacteria belong to gram-positive bacteria which agrees with the results obtained by Cappuccino and Sherman (2014). From the 4 acid-producing bacterial colonies, 1 colony produced CO_2 gas when tested with a Durham tube procedure. The CO_2-producing bacteria indicates that the bacteria belong to heterofermentative LAB because they produce ethanol, acetic acid, and CO_2 during glucose metabolism while homofermentative LAB only produce lactic acid (Ankolekar, 2013). The isolated bacteria were then analyzed using 16s RNA sequencing and phylogenetic tree to determine the species of the bacteria. The phylogenetic tree shown in Figure 1 was constructed based on the maximum likelihood method using a MEGA with 500 iterations. The MEGA 10.0.5 software was used for all analyses.

The phylogenetic tree showed its closeness to *Leuconostoc pseudomesenteroides*. *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides* are common types of LAB found in fresh coffee cherries. After the depulping process of coffee cherries to produce coffee beans and coffee pulp, the presence of *Leuconostoc sp.* increases in the mucilage area of coffee and becomes the dominant bacteria in the early stages of the coffee fermentation (De Bruyn et al., 2017). A previous study by Zhang et al. (2019) also demonstrated that 3 species of homofermentative LAB and 6 species of heterofermentative LAB were found in fresh coffee cherry. Sulmiyati et al. (2018) also reported a similar size, shape, margin, elevation, and color between different genus of LAB.

3.2. Preliminary experiments for natural fermentation with simultaneous aeration on the antioxidant activity of cascara

The effect of simultaneous aeration on natural fermentation of coffee pulp towards the antioxidant activity of cascara is shown in Table 2. From the table, it can be observed that the highest antioxidant activity (8.17 %) was obtained from the cascara produced by natural fermentation of coffee pulp with simultaneous aeration. This value is 43% higher as compared to the cascara produced by natural fermentation of coffee pulp without simultaneous aeration. According to Nakagawa and Miyazaki (2017), oxygen from aeration may trigger production of reactive oxygen species by bacterial cell. Consequently, cell produces enzymes to neutralize the reactive oxygen species by increasing production of components that act as antioxidants. This mechanism is supported by previous studies that observed an increase in antioxidant activity after an aerobic fermentation using *Lactobacillus sp*. which has a positive correlation to the production of a catalase-like enzyme which protect the cell from oxidative damage (Ianniello et al., 2015; Maresca et al., 2018).

These values are also higher than the antioxidant activity of fresh coffee pulp. As such indicates that natural fermentation of coffee pulp influences the antioxidant activity of cascara. This is because coffee pulp contains enough numbers of bacteria for natural fermentation to occur. De Bruyn et al. (2017) has observed an increased in antioxidant activity of coffee mucilage after natural or spontaneous fermentation with heterofermentative LAB particularly *Leuconostoc mesentoroides*. As such because heterofermentative LAB able to modify the structure and conformation of phenolic compounds to increase the antioxidant activity (Hur et al., 2014).

3.3. Effect of natural fermentation with simultaneous aeration on number of lactic acid bacteria, phenolic content and antioxidant activity of cascara

The effects of natural fermentation conditions with simultaneous aerations particularly temperature and incubation time on number of LAB, phenolic content and antioxidant activity of cascara is shown in Table 3 and Figure 2. All response surfaces shown in Figure 2 show a similar pattern; an increasing trend before a plateau was reached at certain temperature and incubation time for all dependent variables.

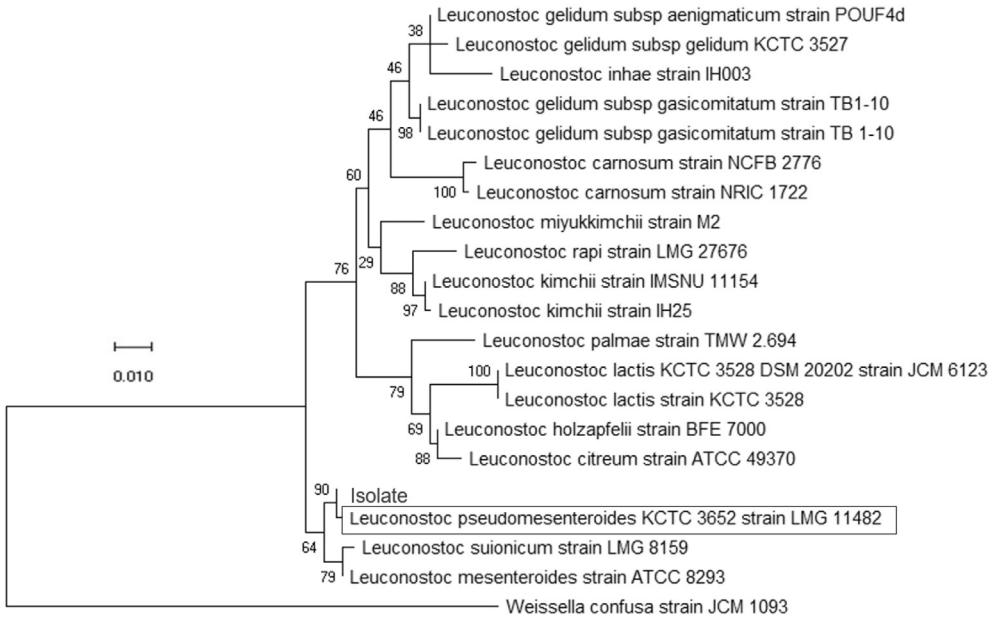

Figure 1. Phylogenetic tree of 16s rRNA gene of heterofermentative lactic acid bacteria isolate.

Table 2. Antioxidant activity of coffee pulp and cascara produced from natural fermentation of coffee pulp with and without simultaneous aeration.

Sample	Antioxidant Activity	
	IC50 (ppm)	Percentage (% at 100 ppm)
Fresh coffee pulp	1194.47 ± 10	5.16 ± 0.44
Cascara produced from natural fermentation without simultaneous aeration	989.47 ± 17	5.72 ± 0.81
Cascara produced from natural fermentation with simultaneous aesration	584.39 ± 29	8. 17 ± 0.5

From the figure, it can be observed that the phenolic content and the antioxidant activity reached a plateau after natural fermentation with simultaneous aeration was carried out for 4 h. The phenolic content increased from 3.2% to 6.4% and the antioxidant activity increased from 12.2% (IC50 of 396.08 ppm) to 30.7% (IC50 of 185.29 ppm). According to Geremu et al. (2016), the phenolic content in coffee pulp lies in the range of 0.7%–1.8% whereas the antioxidant activity varies from 153 ppm to 400 ppm. The differences may be influenced by the origin of coffee pulp used in the study.

López et al. (2013) has investigated the effect of fermentation of fresh coffee pulp with 5 different types of LAB at 37 °C under anaerobic condition for 48 h. The antioxidant activity of the fermented coffee pulp increased up to 10% after the addition of a homofermentative LAB inoculum (*Lactobacillus casei*) for 48 h. In this study, a slightly better results were obtained as the antioxidant activity increased up to 14.8% within 4 h when natural fermentation with simultaneous aeration was carried out in an oven maintained at 32 °C with an air flowrate of 4 m/s and relative humidity of 66–68%.

Table 3. Experimental conditions and the response of phenolic content, antioxidant activity, and number of lactic acid bacteria.

Run	Independent Variables		Response		
	Incubation time (h)	Temperature (°C)	Phenolic content (%)	Antioxidant activity (%)	Number of lactic acid bacteria (log cfu/ml)
1	4	27	6.39	27.95	4.01
2	4	32	6.78	28.84	4.39
3	4	37	5.79	23.65	3.47
4	4	32	6,78	28.84	4.39
5	8	32	3.77	20.55	4.30
6	0	32	3.19	12.16	3.46
7	4	32	6.78	28.84	4.39
8	0	37	3.19	12.16	3.46
9	8	27	3.91	12.57	3.60
10	4	32	6.78	28.84	4.39
11	4	32	6.78	28.84	4.39
12	8	37	3.75	22.14	3.19
13	0	27	3.19	12.16	3.46
14	4	32	6.78	28.84	4.39

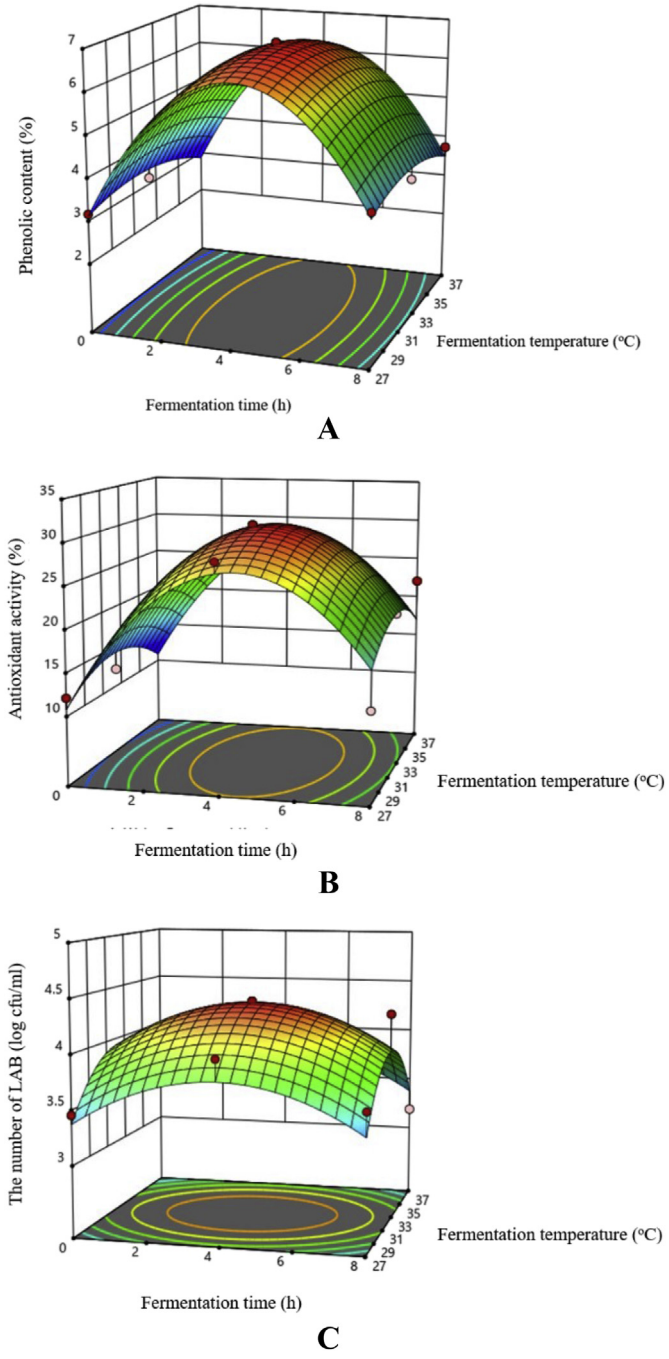

Figure 2. Response surface showing the interaction between fermentation time and temperature on (A) phenolic content, (B) antioxidant activity, and (C) the number of lactic acid bacteria.

Based on Figure 2 (A) and Figure 2 (B), there are positive correlations between the phenolic content and antioxidant activity. After natural fermentation with simultaneous aeration was carried out for 4 h, the phenolic content and antioxidant activity increased up to twice than the initial value. Hur et al. (2014) reported that the phenolic content can affect antioxidant activity due to the presence of a hydroxyl group (-OH). As a result, the antioxidant activity will increase with an increase of the phenolic content. A change in the structure of phenolic compound due to enzymatic activity also can increase the antioxidant activity. For instance, quercetin and catechin molecules have the same number of hydroxyl groups but quercetin has a double bond on its benzene ring structure which makes its antioxidant activity greater than the catechin (Hur et al., 2014).

The increase of phenolic content may be caused by the activity of heterofermentative LAB as demonstrated in Figure 2 (C). The number of heterofermentative LAB and phenolic content were increased within 4 h of natural fermentation with simultaneous aeration and decreased after 8 h. The increase in phenolic content may occurs due to the metabolism of heterofermentative LAB such as hydrolysis and deglycosylation (Zhao and Shah, 2016). In the hydrolysis reaction, LAB can convert complex phenolic compounds into simpler phenolic compounds. Particularly, feruloyl-esterase which converts methyl ferulate to ferulic acid. The structure of ferulic acid has more hydroxyl groups that can increase the measurement of phenolic content and increases the antioxidants activity (Fessard et al., 2017).

The increase of phenolic content may also be caused by the activity of endogenous enzymes on the surface of coffee pulp such as pectinase. This enzyme can be produced naturally from plant cells or microorganisms (Silva et al., 2013). Pectinase can decrease pectin in the cell wall. The reduction of pectin can improve the extraction process of phenolic compounds (Verma et al., 2018). According to a study conducted by Yazdi et al. (2019), pectinase enzymes in pistachio pulp increased the concentration of phenolic content after 2–4 h of enzymatic reaction. As such resembles the results obtained in this study that recorded an increase of phenolic content after natural fermentation with simultaneous aeration for 4 h.

After 4 h of natural fermentation with simultaneous aeration, the phenolic content shows a decreasing profile, as well as antioxidant activity and number of heterofermentative LAB (Figure 2). The decrease can be caused by a feedback inhibition from the phenolic content produced during the fermentation process. For instance, the presence of protocatechuic acid, caffeic acid, and chlorogenic acid that that act as a growth inhibitor may cause heterofermentative LAB to release reductase enzymes that decrease hydroxyl groups (Filannino et al., 2014). As a result, phenolic content and antioxidant activity decreased due to the inhibition of heterofermentative LAB.

3.4. Regression model for natural fermentation of coffee pulp with simultaneous aeration

Regression model coefficients for predicting number of LAB after natural fermentation with simultaneous aeration as well phenolic content and antioxidant activity of cascara are shown in Table 4. The analysis of variance (ANOVA) for the phenolic content of cascara is shown in Table 5. The model describes the experimental data very well with a low p-value and a high R^2 value. The ANOVA for other repsonse parameters also have similar values. Fermentation time also had a significant effect ($p < 0.05$) on all responses (data not shown). Optimum fermentation conditions were determined based on highest bacterial growth, phenolic content, and antioxidant activity which occurred when the natural fermentation with simultaneous aeration was carried out for 4.2 h at 31.8 °C.

The regression model was validated, and the experimental data was compared with estimated values from the model as shown in Table 6 and the parity plot for one of the response variables particularly phenolic content is shown in Figure 3. Data from the model and experiment is not significantly different ($p > 0.05$). Hence, it can be concluded the model was valid and can be used to predict the phenolic content, antioxidant activity, and number of heterofermentative LAB during a natural fermentation with simultaneous aeration. The optimum conditions obtained in this section were used for subsequent fermentation and simultaneous aeration of coffee pulp with heterofermentative LAB inoculum to produce cascara with a high antioxidant activity.

3.5. Fermentation of coffee pulp with simultaneous aeration and addition of heterofermentative lactic acid bacteria inoculum

Fermentation of fresh coffee pulp with simultaneous aeration was carried at the optimum conditions obtained in the previous section (4.2 h

Table 4. Regression model coefficients for predicting number of bacteria after fermentation as well phenolic content antioxidant activity of cascara.

Variable	Coefficient		
	Number of bacteria	Phenolic content	Antioxidant activity
Constant	4.38	6.74	28.76
X_1	-	0.31	3.13
X_2	-	-	-
X_1^2	-0.43	-3.01	-11.19
X_2^2	-0.57	-0.4	-2.46

X_1: incubation time (h), X_2: temperature (°C).

Table 5. Analysis of variance for the phenolic content of cascara.

	Sum Square	Degree of Freedom	F-value	p-value	R^2 *value*	
Model	32.36	3	261,19	<0.0001	R^2	0.99
Error	0.37	9			$R^2_{adjusted}$	0.98

Table 6. Phenolic content, antioxidant activity and number of lactic acid bateria of cascara obtained from experiment and model.

Response variable	Experiment	Model	Reference
Phenolic content (%)	6.72 ± 0.8	6.75	0.9–1.81[1]
Antioxidant activity (%)	27.6 ± 4.4	30.7	15–70[2]
Number of lactic acid bacteria (log cfu/ml)	4.17 ± 0.2	4.37	-

[1] López et al. (2013).
[2] Geremu et al. (2016).

at 31.8 °C). Inoculum of heterofermentative LAB (*Leuconostoc pseudomesenteroides*) isolated from the previous section was added at different concentrations (5–15% volume/weight). The initial and final number of heterofermentative LAB in the sample is shown in Table 7 whereas the antioxidant activity of the produced cascara is illustrated in Figure 4. Based on the data from Table 7, the heterofermentative LAB able to grow well in the coffee pulp medium, reaching approximately 10^8 log cfu/ml after fermentation with simultaneous aeration for 4.2 h.

The antioxidant activity of cascara increased when concentration of the inoculum was increased from 0 to 10% with an antioxidant activity of 42.6%. This value is 15% higher than the antioxidant activity of cascara obtained at the optimum conditions for natural fermentation and 30% higher from the fresh coffee pulp. The results obtained in this study are slightly higher than the 10% increased of antioxidant activity after coffee pulp was fermented with *Lactobacillus casei* as reported by Lopez et al. (2013). The differences may be due to different types of bacteria used and different fermentation conditions. LAB used in the previous study was a homofermentative LAB that has a limited metabolic pathway in comparison to the heterofermentative LAB used in this study (Fugelsang

Table 7. Initial and final number of heterofermentative lactic acid bacteria in fermented coffee pulp (4.2 h at 31.8 °C).

Concentration of inoculum (% volume/weight)	Number of lactic acid bacteria (log cfu/ml)	
	Initial	Final
5	5.73 ± 0,04	8.13 ± 0,02
10	5.99 ± 0,20	8.18 ± 0,11
15	6.16 ± 0,30	8.20 ± 0,01

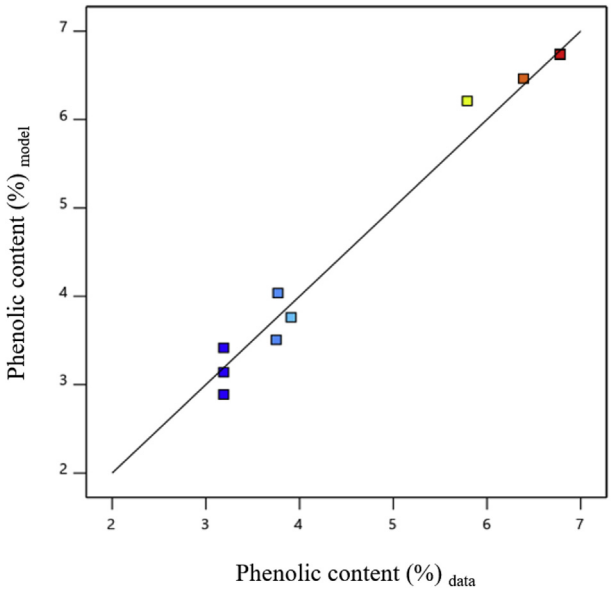

Figure 3. Parity plot for the regression model of phenolic content of cascara.

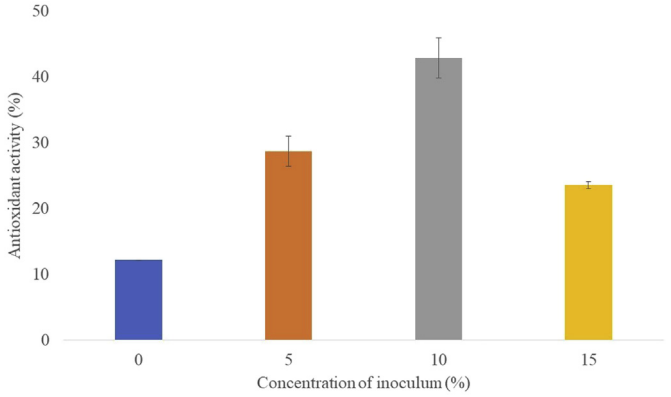

Figure 4. Antioxidant activity of cascara produced from fermentation of coffee pulp with simultaneous aeration and addition of lactic acid bacteria inoculum.

and Edwards, 2007). Homofermentative LAB also do not have enzymes to convert or modify phenolic compounds which corresponds to the antioxidant activity (Ankolekar, 2013). In addition, long fermentation time (48 h) in the previous research may have caused the conversion of phenolic compounds into inactive compounds (Fessard et al., 2017).

4. Conclusion

A systematic study using a response surface methodology with a face-centered central composite design has been carried out to determine the effects of fermentation time and temperature on the number of bacteria in the coffee pulp after natural fermentation with simultaneous aeration (an air flowrate of 4 m/s) as well as phenolic content and antioxidant activity of cascara. The experimental dataset has been modelled using a multi-variable non-linear regression which results in a good agreement between the model and experimental data. At the optimum conditions (4.2 h, 31.8 °C), the phenolic content is 6.72% whereas the antioxidant activity is 27.6%. Indigenous heterofermentative lactic acid bacteria particularly *Leuconostoc pseudomesenteroides* have been isolated from the coffee pulp and used as a starter for controlled fermentation of coffee pulp. The antioxidant activity of the cascara is 15% higher than those obtained at the optimum conditions for natural fermentation with simultaneous aeration and 30% higher than the fresh coffee pulp. Hence, fermentation of coffee pulp using indigenous lactic acid bacteria with a simultaneous aeration would be a good strategy for producing cascara with a high antioxidant activity.

Declarations

Author contribution statement

Lina Oktaviani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Dea Indriani Astuti: Analyzed and interpreted the data.

Mia Rosmiati: Contributed reagents, materials, analysis tools or data.

Muhammad Yusuf Abduh: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by Ministry of Research, Technology and Higher Education of the Republic of Indonesia (002/SP2H/PTNBH/DRPM/2019).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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