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ORIGINAL ARTICLE

Impact of controlled fermentation on the volatile aroma of roasted cocoa

Impacto da fermentação controlada no aroma volátil do cacau torrado

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Abstract

The study of controlled methods of cocoa fermentation on a small scale is important to assess the maintenance of heat generated in the last days of fermentation. The research aimed to study the impact of spontaneous fermentation in controlled fermentation systems on the quality and acceptability of fermented cocoa beans. A 2×3 complete factorial design used different controlled fermentation systems (jacket system, solar heater and wooden box) and pulp reduction as variables. Samples were analyzed for fermentation index and volatile aroma composition profile using Headspace-Solid Phase Microextraction (HS-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). The profile of volatile compounds is evaluated for the studied variables using a multivariate Principal Components Analysis (PCA). The results showed increasing fermentation times in the jacket system seeing that it raised the fermentation rate and accelerated it to five days of fermentation combined with pulp reduction. The PCA analysis showed differences in the chemical composition of volatile compounds that were mainly associated with the reduction of the pulping process than the type of controlled system in four days of fermentation.

Keywords: Acceleration; Jacket system; Fermenter machinery; Pulp reduction; Solar heater; Spontaneous fermentation.

Resumo

O estudo de métodos controlados de fermentação de cacau em pequena escala é importante para se avaliar a manutenção do calor gerado nos últimos dias de fermentação. A pesquisa teve como objetivo estudar o impacto da fermentação espontânea em sistemas de fermentação controlada na qualidade e aceitabilidade da amêndoa de cacau fermentada. Um planejamento fatorial completo 2×3 utilizou como variáveis diferentes sistemas de fermentação controlada (encamisado, aquecedor solar e caixa de madeira) e redução de polpa. As amostras foram analisadas quanto ao índice de fermentação e ao perfil da composição do aroma volátil, usando *Headspace-Solid Phase* (HS-SPME) e cromatografia gasosa acoplada à espectrometria de massa (GC-MS). O perfil dos compostos voláteis foi avaliado para as variáveis estudadas usando uma análise multivariada de componentes principais (PCA).

Os resultados mostraram que o aumento do tempo de fermentação no sistema encamisado elevou a taxa de fermentação e acelerou para cinco dias de fermentação combinado com a redução de polpa. A análise de PCA mostrou diferença na composição química dos compostos voláteis que foram mais associados à redução do processo de despolpamento do que ao tipo de sistema controlado, em quatro dias de fermentação.

Palavras-chave: Aceleração; Sistema encamisado; Máquinas de fermentação; Redução de polpa; Aquecedor solar; Fermentação espontânea.

1 Introduction

The fermentation of cocoa beans was an important step in the development of the aroma during roasting; however, immature and unfermented beans generate a weak chocolate aroma and flavor, whilst the undesirable odor of excessive fermentation is similar to that of putrid ham (Gutiérrez, 2017; Rohan, 1964). In a spontaneous process, microorganism succession breaks down the sugars from pulp and produces alcohol and acid after being placed in large wooden boxes (Schwan & Wheals, 2004). The microorganism metabolite (alcohol and acids) moved into bean cotyledon, and the heat generated in the process converted biochemical reaction inside the bean made seed death. It also decreases bitterness and astringency, flavor precursors formed such as free amino acid, peptides, and sugars (Rodriguez-Campos et al., 2011; Thompson et al., 2012). Schwan & Wheals (2004) could highlight the control regarding the amount of pulp at the start of fermentation to minimize acidity, thus using superior starting culture to perfect microorganism succession and accurate timely production of acids diffusing in beans, and enhancing the fermenter utilized are all required for an optimum process.

In recent years, there has been an increased interest in exploring methods for controlled cocoa fermentation. Fermentation using a submerged artificial fermentation system based on Biehl & Passern (1982) was built to bring up new perspectives on cocoa fermentation under controlled environmental conditions (Voigt et al., 1994a). The effect of acetic acid on beans during fermentation increases the amount of free amino acid content compared to traditional methods (Kadow et al., 2015; Pasau, 2013). The activity of natural endogenous enzymes of cocoa bean such as aspartic endoprotease, aminopeptidase and carboxypeptidase were breakdown protein into peptide and free amino acid (Hansen et al., 1998; Kongor et al., 2016; Voigt et al., 1994b). The protein breakdown results were similar to well-fermented bean in SDS-Page analysis in 24 hours (John et al., 2016). Critical fermentation process depends on sufficient heat and acid in the fermentation system to make endoprotease enzyme activated (Ho et al., 2018; Rottiers et al., 2019).

After two days of fermentation, the temperature begins to rise, and perfect fermentation conditions are achieved when the temperature reached 45 °C. As reported by Hernández-Hernández et al. (2016), on the third day of fermentation, the fermentation temperature in a box with a capacity of 300-1000 kg was consistent at 49 °C, however the highest quality fermented cocoa beans were found in a box with a capacity of 300 kg. The cocoa bean fermenter has a maximum capacity of 2000 kg of beans, although the depth should not exceed 50 cm to avoid inadequate aeration (Schwan & Wheals, 2004). In small capacity fermenters (8-10 kg), maintaining sufficient heat during the last several days of spontaneous fermentation is a challenge. An alternative method of tackling these problems is to use addition heater source but it should be economical and applicable. Solar heater and jacket systems, which are utilized following box fermentation, could provide heat and intended to produce consistently high-quality goods in a regulated environment.

Cocoa bean feature developed from several components such as volatile aroma, nutritional composition, antioxidant-polyphenolic content, and fermentative form. The volatile aroma affects cocoa bean acceptability (Krähmer et al., 2015). The high acidity of the cocoa beans is caused by genetic factors of planting material cultivated in Malaysia and Indonesia, which is characterized by a higher amount of pulp (Lima et al., 2011). Postharvest pod storage, mechanical pulping, enzymatic de-pulping, and spreading bean are all pulp preconditioned in order to reduce nib acidification (Meyer et al., 1989; Schwan & Wheals, 2004). Modifying pulp quantity also accelerates fermentation from six to four days based on study by Afoakwa et al. (2012) using pod storing for 14 days. Mechanical pulping using a washing machine approach also accelerates fermentation if the amount of cocoa pulp removed is less than 20% (Schwan & Wheals, 2004). The research aims to

determine the impact of controlled cocoa fermentation, which combined with spontaneous fermentation and pulp preconditioned to accelerate fermentation and volatile aroma of the roasted cocoa bean.

2 Materials and methods

2.1 Samples preparation

The cocoa pods were obtained from the Indonesian Industrial and Beverage Crops Research Institute (IIBCRI), Parungkuda- Sukabumi, West Java. The Forastero varieties, Scavina 6, was used in the experimental fermentation. The collected pods were placed in a holding place with ambient air temperature for five days then broken down with an unwashed machete. The Beans plus their pulps were scooped out manually, collected, and weighed in 8 kg per sample. For each pulp preconditioned variable, cocoa pulps were reduced by mechanical pulping. The bean immediately transferred to the fermentation site at IIBCRI. The mechanical pulping, wooden box, jacket system and solar heater are shown in Figure 1.

Figure 1. (A) Mechanical pulping, (B) wooden box, (C) jacket system and (D) solar heater.

2.2 Fermentation procedure

The fermentation was conducted in a 0.03 m^3 wooden box that capable to hold 20 kg of cocoa beans. The wooden boxes are solid on four sides, and it will be covered by a gunnysack at the top of the box. The bottom of the boxes, however, have holes between joints of woods to allow drainage of the liquid generated during fermentation. All fermentation was done in triplicate and rotated every day. After three days of fermentation, the cocoa beans were transferred to a heat-controlled incubator. There are two types of heat-controlled incubator, namely jacket system (F2) and solar heater (F3). In Table 1, the sequence fermentation design experiment is shown.

Table 1. Sequence Fermentation Design Experiment.

Incubator Type	Sequence Fermentation	Pulp Type	
		Normal Pulp (A)	Reduced Pulp (B)
Wooden Box (F1)	Spontaneous fermentation for five days. Solid on four sides. Holes between joints of woods to allow drainage in bottom.	F1A	F1B
Jacket System (F2)	Spontaneous fermentation used wooden box for three days. Two days in incubator with additional heat from water heated at 45 ºC, the cocoa beans putted into a plastic container then dipped in it.	F2A	F2B
Solar Heater (F3)	Spontaneous fermentation used wooden box for three days. Two days in incubator with add heat from solar radiation during the daylight and the heating blower at night.	F3A	F3B

Spontaneous fermentation was processed at ambient temperature for five days. In the plenary experiment, the temperature inside the bean mass was decreased to 30 °C at the 5 days of fermentation. After fermentation,

it was rinsed and then dried for 3 to5 days in a solar drying facility. Every 24 hours after turning the beans, 100 g of each sample was randomly collected, placed in plastic bags, and transferred to a refrigerator.

2.3 Determination of fermentation temperature

The temperature data logger is a set of tools that functions as a continuous temperature recorder that detects changes in heat inside mass beans. The process of changing the temperature changes will be converted into electrical signals and send them to the microprocessor in the data logger into data (in the form of a log). These changes were recorded every half hour during the fermentation process.

2.4 Determination of fermentation index

Fermentation Index (FI) detects color changes in bean cotyledon due to decreasing anthocyanin content during fermentation. The fermentation index was determined using the modified method of (Gourieva & Tserevitinov, 1979) that analyzed determination of cocoa bean pigment extraction using methanol-HCL and ratio absorbance at 460/530 nm of 0.5 gram of cocoa powder that was placed into a dark bottle then added 50 ml methanol-hydrochloric acid mixture (97:3 v/v) and shook. Then the samples were kept in refrigerated condition overnight for 16 to 18 hours. The samples were filtered using the Whatman paper no 4 before being tested for absorbance using a spectrophotometer. The absorbance ratios at 460 nm and 530 nm were calculated to determine the degree of fermentation.

2.5 Determination of bean cut test

The fermentation degree of cocoa bean was also determined with bean cut test. One hundred bean were cut lengthwise through the center. Both sides were visually inspected in broad daylight and classified as slaty (unfermented), purple (insufficiently fermented), purple-brownish (lightly fermented), or brown (well fermented). Following that, the beans were inspected for defects (moldy and germinated). The cut test score was determined using Hii et al. (2011) equation. Cut test score = (10 X (% brown+%brownish)) + (5 x %purple) + (0 x %slaty).

2.6 Determination of volatile aroma composition

The volatile aroma compounds were analyzed in flavor laboratories of Indonesian Center for Rice Research Sukamandi-Subang, West Java. Thus, 3 kg cocoa beans were roasted at 100 °C and 110 °C for 15 minutes then it deshelled. Cooled roasted beans were grounded to obtain cocoa liquor. In addition, 5 g cocoa liquor and 0.6 uL 0.001% internal standard (2,4,6-Trimethyl pyridine) were mixed in a 22 ml capped glass vial. This study was used 2 cm SPME fiber made from DVB/CAR/PDMS. The extraction was held for 45 minutes at a temperature of 60 °C.

The extraction results were analyzed using the Gas Chromatography (GC) system consisting of an Agilent 7890A equipped with a Mass Spectrometer (MS) Detector (MS Agilent 5975 C Triple Axis, USA). The GC operation method was setup based on several experiments that recommended the condition of splitless injection at 250°C. The column used was DB-Wax with an inner diameter of 250 µm, a length of 30 m, and a thickness of 0.25 um. The MS detector temperature was at 250 °C. The initial temperature was 44 °C which then raised to 170°C at a speed of 3 °C/min and increased again to 240 °C for 15 minutes (rate 8 °C/min) programed by GC-MS. The helium used as a carrier gas had a speed of 0.8 mL/minute.

The Linear Retention Index (LRI) for each peak was calculated based on standard n-Alkane retention time data (C9-C33) which were injected with the same conditions as the sample. Identification of volatile database components using identification NIST 14 is compared with the calculation of the LRI value. The Linear Retention Index calculation uses an Equation 1:

$$LRI = \left\{ \left(\frac{(t_x - t_n)}{(t_{n+1} - t_n)} \right) + n \right\} \times 100 \quad (1)$$

LRI = component x linear retention index value

t_x = component retention time x (minutes)

t_n = standard alkane retention time, with n carbon atoms appearing before the x component (minutes)

t_{n+1} = standard alkane retention time, with n + 1 carbon atoms that appear after the x component (minutes)

n = number of standard alkane carbon atoms that appear before the x component

2.7 Principal component analysis of GC–MS peak areas

Utilizing XLstat version 20, the Principal Component Analysis (PCA) was carried out on the peak areas of selected aroma compounds. The data were mean-centered by deducting the mean estimation of every variable and scaled to get the unit difference for every factor (automatic scale).

3 Results and discussion

3.1 Fermentation temperature

Monitoring changes in temperature, pH, and oxygen availability during fermentation can ascertain the transform of microorganisms succession (Kongor et al., 2016).

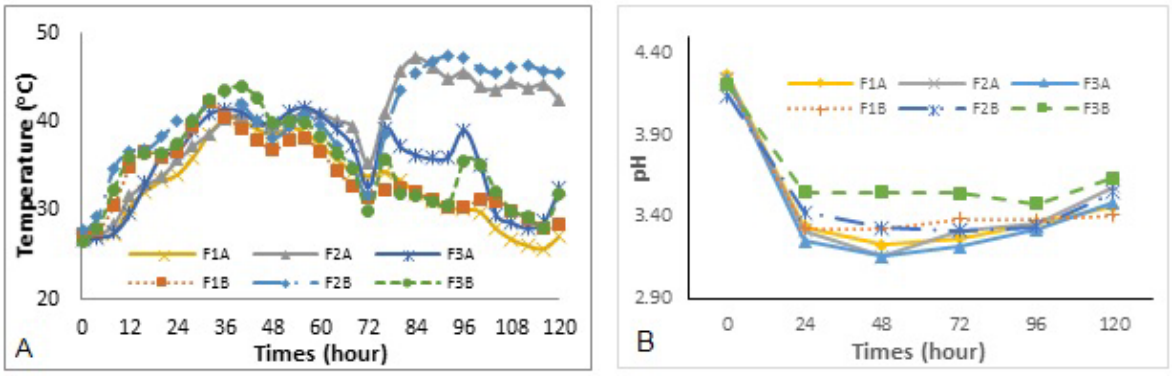

Figure 2. Temperature changes during fermentation, each dot is related to 4 hours (A); pH pulp (B).

In Figure 2, the highest mean temperature of the very small capacity (8 kg) of bean in the box reached 43.9 °C for 40 hours. The reduced pulp treatment reached a higher temperature than natural pulp in 12 hours then reached a similar temperature in 36 hours but fall rapidly at 72 hours. A good condition for yeast growth was 36-48 hours of fermentation and remained stable until temperature reach 45 °C. It was reported that yeast – pectinolytic strains was not growth in temperature above 45 °C but adapted at 40 °C (Samagaci et al., 2014).

Keeping heat sufficient for the last few days of fermentation (72-120 hours) was failed on the solar heater incubator because the temperature difference between the environment and inside heater was 23 °C at night. An additional heating blower attached to the solar heater cannot maintain the temperature. An average incubation temperature inside beans mass in the jacket system was 45 °C, so that the expected results were similar to the fermentation capacity of 300 to 500 kg. After a 72-hour fermentation period, the temperature was maintained above 40 °C in the 500 kg box fermentation, resulting in a percentage of fermented beans of over 87.03 percent (Peláez et al., 2016). As the temperature rises during fermentation, proteins decompose into flavor precursors

such as amino acids (Chetschik et al., 2018). In Figure 2, pulp pH decreased significantly in 24 hours, and reached the lowest value after 48 hours, the pH media increased to 3.5 after 5 days of fermentation. All treatments created a similar pH value at the final fermentation. Thus, pH media in the jacket system was not increased significantly in final fermentation because the discharge pulp was not flown out from the system.

3.2 Fermentation index in cocoa beans during fermentation

The level of fermentation depends on the color changes of cocoa beans to brown as measures by the Fermentation Index (FI). The cocoa beans turn brown due to the dispersion of polyphenols during fermentation, followed by oxidation and reduction with a mixture of chemicals from outside the seeds (Hernández-Hernández et al., 2016). According to Sulaiman et al. (2014), fermented beans have a fermentation index of greater than 1, over-fermented beans have a fermentation index of 1.6, and unfermented beans have a fermentation index of less than 1.0.

The beans treated with jacket system reached an FI value of more than one (1) after five days of fermentation. Another treatment that used solar heater and wooden box fermentation was under fermentation (max 0.90) with a fermentation duration of 5 days. An increase in the value of the Fermentation Index and a decrease in the level of epicatechin (polyphenols) has a relationship with the quality of the fermentation product (Jinap et al., 2003). Polyphenols will be degraded during fermentation, resulting in a substantially lower polyphenol content than fresh beans (Jalil & Ismail, 2008). As a result of the decrease in bean polyphenol concentration, the overall bitterness of the roasted product is reduced (John et al., 2019). Under-fermented beans will cause a loss of flavor in the final product after further processing of cocoa. Figure 3 shows the fermentation index for each treatment.

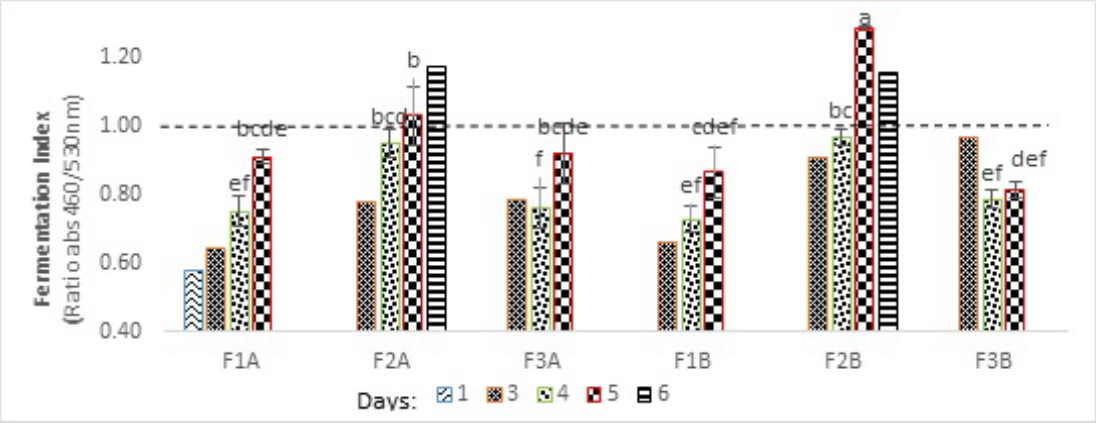

Figure 3. Fermentation Index in 1-6 days of fermentation, vertical bars represented standard deviation of three separate determination followed by different superscripts are significantly different (Duncan, $p < 0.05$).

3.3 Bean cut test

The cut test was used to determine the fermentation quality of completely fermented dried beans. In Table 2, there were significant differences in brown - the cut bean test in five days of fermentation using jacket system and pulp reduction (F2B-5) and four days of fermentation using wooden box – original pulp (F1A-4). The results indicated a significant difference in fermentation duration between four and five days except using solar heater equipment. According to Guehi et al. (2010), a quantity of dry fermented cocoa beans is considered to be of high grade if it comprises at least 60% brown cocoa beans. In Table 2, if the percentages of brown and 2/3 of purple-brown are calculated together, the percentage of brown exceeds 60%. According to the Federation of Cocoa Commerce (FCC), cut beans with 5% slaty or defect beans might be classified as properly fermented (Rottiers et al., 2019). In Table 2, the overall proportion of slaty-colored

cocoa beans was still greater than 5% after five days of cocoa fermentation. In general, the percentage of slaty cocoa beans reduces as fermentation duration increases. A successful fermentation level can be accomplished by using a jacket system for up to six days of fermentation.

Table 2. Bean cut test (N=3).

Code	Bean color (%)				Defect (Moldy-germinated, %)	Cut test score
	Brown	Purple-brown	Purple	Slaty		
F1A-1	22.0	12.0	61.0	5.0	0.0	645.0
F1A-3	26.0	15.0	37.0	20.0	2.0	595.0
F1A-4	36.3 ± 8.2^{a}	16.8 ± 4.0	34 ± 4.9^{b}	9.8 ± 5.1^{b}	3.3 ± 1.1^{c}	700 ± 44.3
F1A-5	47.8 ± 1.8^{bc}	18.3 ± 2.3	27.3 ± 3.1^{ab}	6.8 ± 2.4^{ab}	0.0^{a}	796.3 ± 25.8
F2A-3	21.0	16.0	50.0	13.0	0.0	620.0
F2A-4	41 ± 5.2^{ab}	17.2 ± 2.9	35 ± 5.4^{b}	6.6 ± 0.4^{ab}	0.2 ± 0.4^{a}	757 ± 42
F2A-5	47 ± 7.3^{bc}	19.8 ± 1.6	26.4 ± 2.1^{ab}	6 ± 1.2^{ab}	0.8 ± 1.2^{a}	800 ± 53.9
F2A-6	64.0	21.0	15.0	0.0	0.0	925.0
F3A-3	21.0	11.0	46.0	22.0	0.0	550.0
F3A-4	42.3 ± 17.1^{ab}	17.3 ± 7.2	31.5 ± 13.8^{ab}	8.3 ± 4.8^{ab}	0.8 ± 1.2^{a}	752.5 ± 28.4
F3A-5	46 ± 18.7^{ab}	18.5 ± 7.5	30.5 ± 12.6^{ab}	3.8 ± 1.8^{a}	1.3 ± 1.3^{ab}	797.5 ± 22.5
F1B-3	34.0	23.0	31.0	12.0	0.0	725.0
F1B-4	42.8 ± 17.3^{ab}	20 ± 8.3	30 ± 12.3^{ab}	6.8 ± 4.2^{ab}	0.5 ± 0.8^{a}	777.5 ± 30.1
F1B-5	47.5 ± 19.9^{bc}	19.5 ± 7.9	27.3 ± 11.5^{ab}	5.3 ± 2.6^{ab}	0.5 ± 0.8^{a}	806.3 ± 41.7
F2B-3	27.0	13.0	38.0	22.0	0.0	590.0
F2B-4	42 ± 17.1^{ab}	18.8 ± 7.9	32.8 ± 13.4^{b}	6.5 ± 3.5^{ab}	0.0^{a}	771.3 ± 21.3
F2B-5	50.8 ± 18.7^{c}	18.8 ± 7.9	24 ± 9.6^{a}	6 ± 4.9^{ab}	0.3 ± 0.5^{a}	816.7 ± 68.2
F2B-6	64.0	15.0	20.0	0.0	1.0	890.0
F3B-3	34.0	20.0	31.0	15.0	0.0	695.0
F3B-4	39.3 ± 16.3^{ab}	16.8 ± 7.2	33.8 ± 14.0^{b}	7.3 ± 3.2^{ab}	3 ± 2.6^{bc}	728.8 ± 49.2
F3B-5	43.8 ± 18.8^{ab}	20 ± 8.1	29.8 ± 12.7^{ab}	5.3 ± 3.2^{ab}	1.3 ± 1.5^{ab}	786.3 ± 61.0

The last digit in the code is the total number of days of fermentation. A different superscripts are significantly different in each column (Duncan, $p < 0.05$).

3.4 Volatile aroma composition and Concentration in PCA

Using headspace GC–MS, 222 volatile compounds were identified in all samples, wherein 26, 24, 32, 19, 11 compounds were detected in pyrazine, ketones, alcohol, ester, and aldehydes, respectively in each sample. Only 39 volatiles compounds were calculated in PCA for identified compound related to treatment. The distribution of different classes of compound are acids (26.71%), pyrazines (21.67%), aldehydes (12.61%), alcohol (12.67%), ketones (5.97%), ester (2.46%), terpenes (3.46%) and other compounds (pyrans, fatty acids, furans, benzene and pyrroles). Pyrazines (22.79%) and aldehydes (15.62%) were increased after roasting, and new compound was made (del Rosario Brunetto et al., 2020).

To visualize the impact of the variable fermentation treatment and pulp reduction treatment on the volatile aroma profile, a two-part PCA model was determined based on the GC–MS concentration data. With a clarified variation of 97.63%, the score plot in Figure 4 shows the separation of samples due to differences in pulp treatment along PC2 within 4 days of fermentation. Thus, 39 of volatile compounds were identified to separate F2A-4, F1A-4, and F3A-4 on the positive y-axis, while other roasted samples clustered along the negative axis. Some of the volatile compounds that are on the positive y-axis were acetic acid (A2), 2-butenal, 2-phenyl (B9), 2,5-dimethyl -1,5-hexadien-3-ol (C8), 2,3-butanediol, [R- (R *, R *)] – (C13), 2,3-butanediol (C15), ethyl isocaproate (D5), 2-pentanone (E1); 2-Heptanone (E2); 3-Methyl-2-cyclohexenone (E14); Pyrazine, tetramethyl- (F11); Trichloromethane (M1). The types of volatile compounds that were close to the treatment without pulp preconditioned for four days of fermentation were associated with the ketones and alcohol groups (Table 3). It may be caused by the formation of acetic acid and alcohol during fermentation correlated to pulp quantity. The reduction in pulp volume will suppress the anaerobic stage during the beginning of fermentation (Meyer et al., 1989). As a result, the fermentation degree might be marginally limited so that reducing acidification of the beans (low amounts of acids). In Figure 4B, it could be noted all the volatile compounds that cause the separation of re-analyzed samples using PCA to find the relationship associated with the treatment of fermentation.

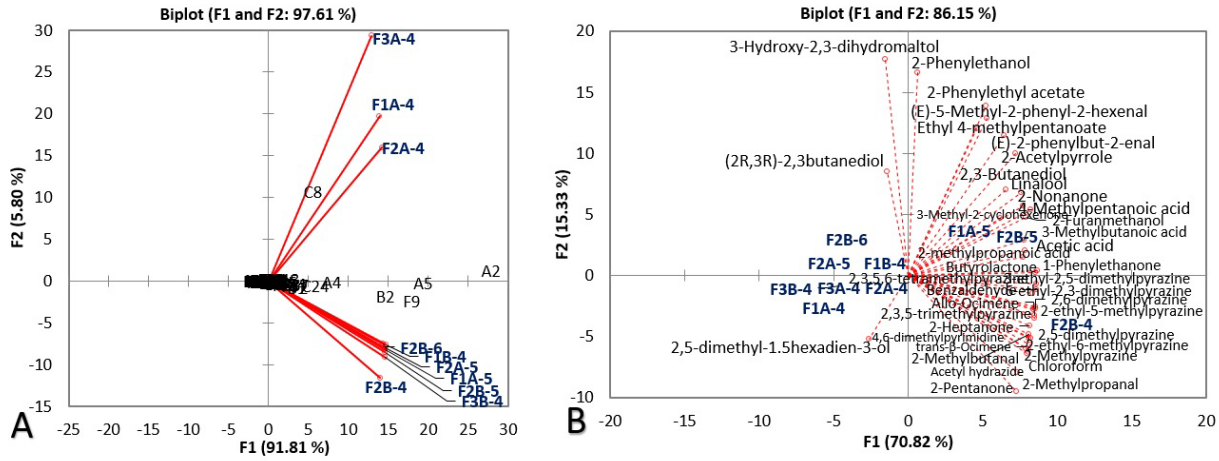

Figure 4. Separation of samples due to differences in pulp treatment along PC2 in 4 days of fermentation (A); volatile compounds identified related to the separation- PC1 positive (B).

In cocoa, pyrazines are a significant group of volatiles known for their distinctive earthy, nutty, roasted, and chocolate flavor notes. In Figure 4B, F2B-4 (pulp precondintioned and jacket system in four days) had the most abundant pyrazine than other treatment. The Strecker degradation in the Maillard reaction generates the majority of pyrazines that used precursors (free amino acids and reducing sugars) for their formations. According to Santander Muñoz et al. (2020), most peptides, free amino acids and reducing sugars (glucose and fructose) after 3 to 4 days during spontaneous fermentation increased to maximum levels and reduced in 5 to 6 days. The shorter fermentation (4 days) was sufficient to produce quality cocoa beans if the fermentation temperature of 45 ºC reached for several days. The decrease concentration of pyrazine formed also occurred in the fermentation treatment for 5 to 6 days (F2B-5 and F2B-6) in jacket system (Table 3). The formation of peptides and amino acids depends on both the structure of the protein and aspartic protease enzyme that had performance optimal at pH 3.5 and a temperature of 40 ºC to 45 ºC (Santander Muñoz et al., 2020; Voigt & Biehl, 1995).

Table 3. Main volatiles compounds identified to separation - PC1 positive in PCA. Means represent averages of concentration (ng/g) volatile compounds in 2 replicates.

Code	Compounds	RT (min)	LRI	LRI*	Concentration (ng/g)									
					F1B-4	F1A-4	F2A-4	F2B-4	F2B-6	F3A-4	F2B-5	F3B-4	F1A-5	F2A-5
	The positive y-axis													
A2	Acetic acid	17.03	1439	1430[a]	72.4	46.8	100.6	115.6	51.5	69.9	107.0	40.5	105.5	59.3
B9	(E)-2-phenyl-2-butenal	34.84	1916	1898[b]	2.9	1.8	3.0	4.1	3.7	2.6	5.8	3.1	5.6	3.5
C8	2,5-dimethyl-1,5-hexadien-3-ol	13.58	1351	1351[c]	0.0	35.3	58.5	1.5	0.0	68.5	1.9	0.6	1.4	0.6
C13	(2R,3R)-2,3-butanediol	21.31	1548	1547[c]	12.0	4.5	10.6	3.5	14.7	9.5	8.2	3.2	6.9	3.7
C15	2,3-Butanediol	22.74	1584	1557[d]	8.6	4.5	10.7	9.9	7.8	8.0	14.0	4.2	9.8	4.8
D5	Ethyl 4-methylpentanoate	27.15	1700	1193[c]	2.1	1.2	1.8	2.8	3.1	2.6	3.5	2.6	4.1	2.6
E1	2-Pentanone	3.18	859	964[a]	5.1	4.3	5.9	14.5	2.0	5.2	7.0	1.9	4.8	3.9
E2	2-Heptanone	7.60	1172	1180[e]	3.2	2.2	3.0	11.5	2.0	2.8	8.8	1.6	4.0	3.0
E14	3-Methyl-2-cyclohexenone	22.61	1581	1605[b]	3.6	2.3	4.3	4.4	3.0	4.6	4.8	1.9	4.7	2.8
F11	2,3,5,6-tetramethylpyrazine	18.46	1475	1482[b]	3.7	3.4	3.0	7.4	4.1	7.3	5.0	2.2	7.1	4.6
M1	Chloroform	3.65	868	1010[c]	3.0	2.5	3.4	9.7	1.1	2.0	7.0	1.9	3.0	2.5
	The negative y-axis													
A1	Acetyl hydrazide	2.52	847	863[g]	6.8	3.4	5.6	12.0	3.9	4.7	7.8	3.7	6.4	4.1
A4	2-methylpropanoic acid	22.08	1567	1568[a]	24.6	11.8	36.7	40.6	17.1	17.3	39.3	12.5	30.1	15.7
A5	3-methylbutanoic acid	25.96	1668	1676[a]	50.1	29.9	80.5	89.1	31.2	38.8	107.1	30.1	70.1	37.3
A7	4-Methylpentanoic acid	30.86	1802	1809[b]	3.1	1.6	3.1	4.9	2.7	2.3	5.6	2.0	4.5	2.1
B1	2-methylpropanal	2.12	839	817[a]	11.1	5.5	11.6	44.3	5.6	7.4	12.6	7.8	19.3	9.4
B2	2-methylbutanal	2.67	849	864[f]	37.8	28.1	35.9	119.3	20.3	23.2	70.7	24.7	48.1	34.2
B3	Benzaldehyde	19.91	1512	1508[a]	11.1	5.5	11.6	44.3	5.6	7.4	12.6	7.8	19.3	9.4
B10	(E)-5-Methyl-2-phenyl-2-hexenal	39.62	2059	2078[b]	4.4	2.7	4.5	5.7	6.4	3.7	10.2	5.6	8.4	5.9
C17	2-Furanmethanol	25.70	1662	1666[c]	4.0	2.3	4.6	5.7	2.7	3.4	7.3	2.7	5.1	3.0
C24	2-Phenylethanol	34.46	1905	1891[a]	20.7	11.1	14.0	8.1	20.7	11.9	21.0	9.6	25.8	20.8
D9	2-Phenylethyl acetate	31.01	1806	1810[a]	4.0	2.9	4.2	4.5	5.5	2.8	8.7	3.3	7.8	5.0
E11	2-Nonanone	14.98	1387	1389[a]	3.5	1.9	3.8	6.5	2.0	3.3	9.4	3.0	6.3	3.2
E15	1-Phenylethanone	24.80	1638	1642[a]	3.2	2.1	3.9	7.9	2.7	3.2	6.2	2.7	6.1	3.3
F1	2-Methylpyrazine	10.37	1264	1273[b]	6.7	3.3	5.7	22.9	3.3	4.0	12.9	3.9	9.7	5.4
F2	2,5-dimethylpyrazine	12.46	1322	1346[d]	12.7	5.9	12.2	37.5	7.7	8.0	24.2	7.6	18.6	8.7
F3	2,6-dimethylpyrazine	12.68	1327	1334[b]	9.2	4.6	9.3	25.8	4.6	6.5	18.9	5.5	13.6	6.9
F5	2-ethyl-6-methylpyrazine	14.89	1385	1393[b]	2.6	1.3	2.1	11.3	1.7	1.5	5.1	1.8	4.3	1.7
F6	2-ethyl-5-methylpyrazine	15.12	1391	1393[b]	8.6	4.6	9.1	27.4	5.9	5.9	18.7	6.3	15.3	6.2
F7	2,3,5-trimethylpyrazine	15.73	1406	1408[a]	13.2	6.9	11.1	32.9	9.8	11.1	21.4	7.1	19.7	7.5
F9	3-ethyl-2,5-dimethylpyrazine	17.35	1447	1453[b]	42.7	23.0	49.6	111.7	35.1	33.9	80.9	31.8	78.4	37.4
F10	6-ethyl-2,3-dimethylpyrazine	17.91	1461	1470[b]	3.5	1.8	2.8	8.9	3.0	2.8	5.8	2.3	6.0	2.4
G2	Trans-β-Ocimene	9.28	1233	1250[c]	2.5	1.6	3.0	8.6	1.7	1.7	6.1	1.8	2.7	2.1
G4	Linalool	21.50	1552	1559[b]	9.9	5.6	12.0	17.1	8.2	9.3	19.0	9.7	21.3	8.1
H4	3-Hydroxy-2,3-dihydromaltol	44.96	2249	2239[e]	6.7	4.0	5.2	2.0	6.3	4.6	6.7	4.8	7.3	6.0
L6	2-Acetylpyrrole	36.38	1961	1968[b]	6.1	3.5	6.5	8.4	5.9	4.8	11.7	5.1	10.8	5.9
M7	4,6-dimethylpyrimidine	12.85	1332	1363[e]	3.2	1.7	3.1	10.8	1.7	1.9	5.8	1.6	4.3	2.2
M9	Allo-Ocimene	14.30	1396	1394[e]	2.6	1.6	3.6	8.7	1.7	1.9	5.9	1.9	4.6	2.1
M15	Butyrolactone	23.88	1614	1618[a]	6.4	4.1	6.7	13.0	4.8	5.3	11.1	4.1	9.7	5.1

Retention time (RT). Compounds identified by probability based matching of mass spectra and Linear retention index (LRI). Source: LRI* (literature); [a](Assi-Clair et al., 2019), [b](Barbosa-Pereira et al., 2019), [c](Linstrom & Mallard, 2014), [d](Crafack et al., 2014), [e](National Center for Biotechnology Information, 2020), [f](El-Sayed, 2014), [g](Royal Society of Chemistry, 2020).In Figure 4, the concentration of 2,5-Dimethyl -1,5-hexadien-3-ol was significantly higher in F1A-4, F2A-4, and F3A-4. This might be explained by the activity of Lactic Acid Bacteria (LAB) through malolactic fermentation process (conversion of malic acid to lactic acid) under acidic conditions, low temperatures and alcohol (VDM Vinicius De Melo Pereira et al., 2020).

The volatile compounds which had specially robust chocolate character were 2-Methylbutanal, 2-Methylpropanal and 3-Methylbutanal (Counet et al., 2002). On the other hand, 2-Methylbutanal and 2-Methylpropanal were abundant in jacket system treatment in 4 days and decreased in 6 days, but the concentrations increased with fermentation time in spontaneous fermentation (F1A-4 and F1A-5). Agreeing with Counet et al. (2002), 2-Methylbutanal was a degradation product from the hydrophobic amino acids isoleucine/valine and leucine during Stecker degradation and provide a fruity flavor.

Another key chocolate aroma was 5-methyl-2-phenyl-2-hexenal that develops from phenyl acetaldehyde, acetaldehyde, and 2-methylpropanal through aldol condensation (del Rosario Brunetto et al., 2020; Owusu et al., 2012) which is deep bitter (Afoakwa et al., 2008). The 5-methyl-2-phenyl-2-hexenal concentration of the F2B method also increased in days of fermentation. In Figure 4B, F1A-5 and F2B-5 are in the same quadrant as 5-methyl-2-phenyl-2-hexenal. The concentration of this compound was also higher compared to other methods. The volatile compounds in the F1A-5 and F2B-5 quadrants are Phenyl ethyl alcohol, 2-phenyl acetate, and 3-Methylbutanoic acid. In addition, 2-phenyl ethyl alcohol that was identified as fruity (Rodriguez-Campos et al., 2011). However, 2-Phenylethyl acetate might be formed through yeast metabolism (Perotti et al., 2020).

Thus, 3-Methylbutanoic acid found higher concentration in incubator heater addition (Jacket system and solar heater) than traditional fermentation used box fermentation. Overproduction of 3-Methylbutanoic and 2-methylbutanoic acids is caused by a shift in metabolic processes in the LAB under stress-acid conditions accompanied by a decrease in the availability of sugars and the production of primary carbohydrate metabolites (Serrazanetti et al., 2011).

4 Conclusion

A total of 223 compounds in cocoa liquors were identified, of which 26, 27, 30, 14, 12 compounds were detected in pyrazines, ketones, alcohols, esters, and aldehydes, respectively in each sample. The difference in the chemical composition of the volatile compounds was associated mainly with the reduction of the pulping process than the type of controlled heat incubator in four days of fermentation. Increasing the fermentation times in the jacket system will increase the fermentation index and it is an opportunity to use this technology in a very small capacity of bean fermentation. The recommended alternative technology is five days of fermentation using a jacket system and pulp reduction that has a fermentation index of more than one.

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