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Microorganism population, theobromine, antioxidant, and FTIR analysis of Samarinda cocoa bean fermented with *Saccharomyces cerevisiae* and *Acetobacter aceti*

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Abstract

This research aimed to observe *S. cerevisiae* and *A. aceti* induced fermentation of cocoa bean from Samarinda, Indonesia, in comparison to commercial cocoa bean in terms of microbial population, pH, total acids, total phenols, theobromine, antioxidant capacity, and FTIR profile. Cocoa beans were fermented with a boxed fermentation method resembling commercial plantation for four days at ambient box temperature (35-40°C). Four fermentation samples were produced which were spontaneous, 2% *Saccharomyces cerevisiae, Acetobacter aceti*, or mixed culture (*S. cerevisiae* and *A. aceti*) induced fermentations. Total Plate Count (TPC) and Total Yeast-Mold (TYM), pH, total phenol, theobromine, antioxidant activity, and FTIR analyses were performed according to the established method. There was no significant difference in the microbe population in all fermented cocoa. Mixed culture fermented cocoa had a slightly lower final pH. *S. cerevisiae* fermented cocoa produced the highest total phenol compared to the same compound content in other fermented cocoa. The mixed culture fermented cocoa had better theobromine content 162.3±22.6 ppm, antioxidant capacity 424.9±3.3 ppm, and the closest theobromine and caffeine identification zones to commercial cocoa samples. The use of mixed culture of *S. cerevisiae* and *A. aceti* is suggested as the better inoculum to ferment cocoa bean at local farms.

1. Introduction

The cocoa bean is a popular research subject considering its strong antioxidant capacity and indulged taste. Various techniques of fermentation of cocoa bean in commercial plantation allow standardized but distinct products. The local cocoa bean farmers in Indonesia commonly did not conduct fermentation but only drying. This problem results in lower quality of the cocoa bean. In combination with less compliant to good agricultural practice, the cocoa bean from local Indonesian farmers is not internationally competitive (Rahmadi and Fleet, 2008).

Researchers introduced and implemented the use of culture cocktail to ferment the cocoa bean in large plantations (Cempaka *et al*., 2014; De Vuyst and Weckx, 2016; Meersman *et al*., 2016). It is essential to use a straightforward but widely available culture source to introduce simple but successful fermentation of cocoa beans to local farmers (Meersman *et al*., 2015). Therefore, baker's yeast (*Saccharomyces cerevisiae*) and acetic acid (*cuka*) starter (*Acetobacter aceti*), locally available in Samarinda, Indonesia, were selected as strong microbial cocktail candidates.

Of all parameters observed, this research employs a Fourier Transformed Infra-Red (FTIR) analysis that produces a unique profile. Therefore, it is proposed to be a fast but reliable method to detect high quality of cocoa bean fermentation. The FTIR has an identification regiment at wavelengths of 600 to 1500 cm^{-1} (Altemimi *et al.,* 2017). The identification regiment can approximate identical covalent binds when comparing measurement results from two different samples (Koch *et al.,* 2013). Therefore, a similar quality of fermented cocoa bean can be identified.

This research aimed to observe *S. cerevisiae* and *A. aceti* induced fermentation of cocoa bean from Samarinda, Indonesia, in comparison to commercial cocoa bean in terms of microbial population, pH, total

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acids, total phenols, theobromine, antioxidant capacity, and FTIR profile.

2. Materials and methods

2.1 Materials

Method of fermentation was learned from PT Perkebunan Nusantara (Nusantara Plantation XIII in Jember, Indonesia in January-February 2018. The techniques obtained from commercial plantation were then employed to local farmers in Samarinda, Indonesia, in April-December 2018. Instant *Saccharomyces cerevisiae* (Fermipan, Indonesia) and *Acetobacter aceti* (Segiri Market, Samarinda) and were used to induce fermentation on Samarinda cocoa beans for four days, with a boxed fermentation method resembling commercial plantation. The starter inoculum was used at a concentration of 2% (v/w). Therefore, it was either a 2% concentration for single culture inoculum or 1% each for in the mixed culture inoculum *of S. cerevisiae* and *A. aceti*. The observed parameters were measured on day-4 of the fermentation period, while the FTIR measurements were conducted on the initial day and final day of fermentation. For FTIR analysis, dried commercial samples were obtained from PT Perkebunan Nusantara (Nusantara Plantation XIII) in Jember, Indonesia. Samples were spontaneously fermented for three days on-site in January-February 2018. The dried cocoa bean from commercial samples were stored in 4±3 °C before further analysis.

2.2 Total plate count and total yeast-mold

Total Plate Count (TPC) and Total Yeast-Mold (TYM) was performed as described by Fardiaz (1993). Nutrient Agar (NA) (Accumedia, USA), De Mann Rogosa Sharpe Agar (Himedia, India), and Dichloran Rose-bengal Chloramphenicol Agar (DRBCA) (Oxoid, USA) was used for TPC, Lactic acid bacteria (LAB) count, and TYM, respectively. The sample weighed about 25 g in 0.86% Nacl solution. As many as 0.1 mL that had been aseptically poured into a petri dish andSprate plate. For TPC and LAB observations, the samples were incubated in the incubator in a reversed position at 37°C for 24 hours. For TYM, the samples were incubated at (27±3°C) for 72-96 hours. Calculations according to SPC (Standard Plate Count) number of colonies range from 25 to 250 CFU / mL, taking into account the dilution factor. The non-LAB population calculation was performed by subtracting the log of the total bacterial population obtained from the NA medium with the log of the LAB population of the MRSA medium.

2.3 pH

The degree of acidity was measured using the Sudarmadji *et al.* (2007) method. About 50 mL of sample product was placed into a small jar, and the pH was measured in duplicate for each product. Before analysis, the pH meter was checked and calibrated by comparing the values with buffer pH 4 and 9.

2.4 Total phenol

The total phenol analysis was carried out based on The previous methods by (Mu'nisa *et al*., 2012; Nurhayati *et al*., 2012). Cocoa bean powder was weighed at 0.3 g, and then dissolved to 10 mL in absolute ethanol (SmartLab, Indonesia): aqua distillate (1: 1). The extract solution was taken as much as 0.2 mL. To the solution, 15.8 mL aqua distillate was added, followed by the addition of 1 mL Folin-Ciocalteu reagent (Sigma-Aldrich, USA) 50% (v/v) in ethanol. The mixture was left to stand for 8±2 mins, then 3 mL of Na_2CO_3 5% (w/v) (Sigma-Aldrich, USA) was added. The solution was left to stand for 2 hrs in The dark at 28±2°C room temperature (28±2°C), then the absorbance was measured at 725 nm (Eppendorf BioSpectrometer®, Germany). Quantification of total phenol was carried out using the Gallic acid (Sigma-Aldrich, USA) standard curve, which had been prepared in the same way. Total phenol was expressed in mg equivalent Gallic acid per kg dry weight.

2.5 Total theobromine

The total measurement of theobromine (TB) by UV spectrophotometry was done according to the method by Li *et al*. (1991). lead II solution was made by mixing 15 g of Pb (II) acetate (Merck, USA) with 150 mL of water in a 250 mL flask, and then the solution was heated while stirring until the color of the solution turned white. The control used was 0.1 g TB (Sigma-Aldrich, USA). Cocoa bean powder was weight at 0.1 g and transferred into a 150 mL glass, and 96 mL of distilled water was added. The solution was heated (70 ± 10°C) for 5 mins while stirring. About 4 mL of lead II solution was added and mixed. Distilled water was added until the volume of the solution became 100 mL. To the mixture, 1 g of $NaHCO_3$ (Sigma-Aldrich, USA) was added. Then, the precipitate was filtered with filter paper three times until the solution became transparent. The filtrate (50 mL) was removed, and about 5.5 mL of 1 M NaOH (Sigma-Aldrich, USA) was added to produce pH 10.5. Then, the filtrate was extracted with chloroform (Fulltime, China) in five repetitions, each of which was added 25 , 20 , 15 , 15 and 10 mL of chloroform. In each repetition, the mixture was shaken for 1 min and stand for about 5 mins. Chloroform extract was combined into a volumetric flask and added with chloroform so that it reached a volume of 100 mL. About 10 mL of extract in

chloroform was added with 40 mL of aqua distillate and 0.55 mL of 10% HCl. The determination of theobromine spectrophotometry was carried out by transferring 1 mL of chloroform extract into the cuvette and measured the absorbance at a wavelength of 302 nm. The results obtained were then plotted in the standard curve of TB (Sigma-Aldrich, USA).

2.6 Antioxidant activity

Total antioxidants were performed by a spectrophotometric method using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA) reduction principle (Farhan *et al.,* 2012). A total of 1 mL of diluted extract in ethanol (Kimia Farma, Indonesia) was added to 1 mL DPPH (prepared at 0.15 mM in ethanol), and at the same time, a control consisting of 1 mL of DPPH with 1 mL of ethanol was prepared. The reaction mixture was vortexed and then incubated in the dark at room temperature for 30 mins. The absorbance was measured at 519±2 nm (Genesys 20, Thermo-Fischer, USA). Vitamin C (Sigma Aldrich, USA) was used as a positive control, and ethanol was used as the subtract. The ability to inhibit DPPH reduction of the extract was calculated by comparing the absorbance of the reduced control of sample absorbance divided by the absorbance of the control. The total antioxidant value was then plotted in a linear regression equation: [antioxidant potential] = a [ingredient in ppm] + b to obtain its IC_{50} value.

2.7 FTIR Analysis

The samples were in the form of powder. All samples then analyzed to obtain infrared (IR) spectrogram with Shimadzu FTIR-8400S (Michelson interferometer, single-ray optical system, a globular infrared ceramic source with an S/N ratio of 20000: 1, Happ-Genzel Apodization, and 10x readings at the resolution of 4.0).

3. Results and discussion

3.1 Microbial population, pH, and total acids

Microbial populations, pH, and total titrated acid from fermented cocoa using starter cultures were compared with the same parameters of spontaneously fermented cocoa (Table 1). On the 4th day of fermentation, spontaneously fermented cocoa had a TPC value of 8.11±0.83 log CFU/mL, a total LAB of 5.87±0.91 log CFU/mL, and a total YM of 4.22±0.65 log CFU/mL. In general, there was no significant difference in total microbes between spontaneously fermented cocoa and fermented cocoa using a starter. Based on previous research (Da Veiga-Moreira *et al.,* 2013), the difference between fermented cocoa with a starter and without a starter in terms of population size generally did not experience a difference ($p>0.05$), except for the use of a mixed culture starter. In this case, the Total Yeast-Mold (TYM), fermentation by mixed culture starter inoculation was higher when compared to the total YM in spontaneous fermentation. Mixing of mixed culture starters was thought to cause a decrease in the activity of microorganisms that inhibit YM growth, which was indicated by the final pH of the product being significantly different. Nutritional use competition is thought to be the cause that the final pH of fermented cocoa with various inoculums becomes different (Lee *et al.,* 2019).

The cocoa fermentation process involves a microbial complex that has a successful role in shutting down the viability of the cocoa germ, reducing bitter taste by reducing polyphenols and flavonoids, and reducing the final pH (Da Veiga-Moreira *et al.,* 2013). In terms of the use of a starter, the benefit is a more consistent end result with an emphasis on certain fermentation stages. The intended outcomes include stabilizing the process of turning pulp into alcohol, forming the aroma of vinegar and lactic acid, or reducing the pH rapidly to inhibit the growth of mycotoxin-producing molds (De Melo-Pereira *et al.,* 2012).

Two parameters commonly used to monitor the ongoing process of cocoa fermentation are pH and temperature of fermentation (Lefeber, Gobert and Vrancken *et al*., 2011). In this study, the fermentation temperature was not reported, but somewhat dependent on the pH. Total acid can be used to confirm changes in pH during the cocoa fermentation process. In addition to vinegar, succinic acid is the dominant source of pH reduction in cocoa fermentation, with the help or without the help of a starter (De Melo-Pereira *et al.,* 2012). In general, the final pH and total acid observed at the end of

Table 1. Microbial populations, pH, and total acids of fermented Samarinda cocoa bean

Type of Cocoa Bean Fermentation	TPC (log CFU/mL)	LAB (log CFU/mL)	YM (log CFU/mL)	pH	Total acids (%)
Spontaneous	8.11±0.83	5.87±0.91	4.22±0.65	4.33±0.03	0.09±0.01
S. cerevisiae induced	7.97±0.85	6.12±0.68	5.14±0.77	4.30±0.28	0.11±0.01
A. aceti induced	7.89±0.91	6.02±0.88	5.22±0.83	4.38±0.00	0.10±0.02
Mixed culture induced	7.96±0.81	5.58±0.85	5.94±0.81*	4.98±0.00*	0.08±0.01

* indicates significant different ($p<0.05$)

the fermentation process for four days did not experience a significant difference ($p>0.05$), except for mixed culture fermentation. The pH value of cocoa fermentation products with the help of mixed culture inoculum was significantly different ($p<0.05$) compared to the final pH on spontaneous fermentation of cocoa.

In many cocoa fermentation operations in the world, spontaneous fermentation is the first choice with a note that the stages of fruit shell opening, removal of fruit pulp, and laying on a fermentation container are done hygienically (Papalexandratou *et al*., 2011). This means that the use of a starter, in general, does not significantly affect the etiology of the decrease in pH and the increase in total acid during the cocoa fermentation process. Except, inoculum conditions do not quickly enough converting sugar to alcohol and alcohol to vinegar (Lefeber, Janssens and Moens *et al*., 2011).

3.2 Total phenol, theobromine, and antioxidant capacity

Table 2 explains the changes in total phenols, theobromine, and antioxidant capacity of cocoa fermentation using several starters compared to spontaneously fermented cocoa. The antioxidant capacity of cocoa fermented by these four methods was significantly different ($p<0.05$). However, when viewed from its antioxidant source, cocoa fermented with *S. cerevisiae* inoculum had the highest total phenol compared with the same compound content in other fermented cocoa. The total phenols in cocoa fermented with *A. aceti* inoculum and mixed culture were lower when compared to the other two fermentation methods. It is notable that the phenolic content was more affected by the presence of the *Acetobacter* inoculum than the *S. cerevisiae* inoculum (Lee *et al*., 2019). In another study, spontaneous fermentation produced a better phenolic profile and total antioxidant compared to fermentation with the help of *S. cerevisiae* starter, because of continuous fermentation that involved many microbes (Batista *et al*., 2016).

The total theobromine in spontaneously fermented and mixed culture cocoa did not differ significantly ($p>0.05$). The lowest total theobromine was obtained in fermented cocoa using S. cerevisiae inoculum. This indicates the theobromine degradation process occurs more at the alcoholic fermentation stage (Sandhya *et al.,* 2016). One suspected cause is the presence of fungi that can degrade theobromine as occurs in cocoa husk fermentation (Adamafio *et al.,* 2012).

There was generally no strong correlation between the total polyphenols and antioxidant capacity (Table 2). Pearson correlation test (data not shown) showed no relationship between the total phenolic component in the fermented cocoa from various starters and their antioxidant capacity. Then, there was an indication that a group of methylxanthine compounds such as theobromine and caffeine could be considered as sources of antioxidants. However, the radical scavenging ability of methylxanthine compounds has not been proven in major reports (Sroka *et al.,* 2015; Petrucci *et al.,* 2018). Although Table 2 indicates that there was a possible relationship between total theobromine and antioxidant capacity, previous studies had focused only on the antioxidant capacity of cocoa sourced from polyphenol contents (Cádiz-Gurrea *et al*., 2014; Todorovic *et al.,* 2015). Species of polyphenols determines the antioxidant activity strength of cocoa (Carrillo *et al.,* 2014), so the quantification of each polyphenol species from fermented cocoa with various starters needs to be conducted in the future.

3.3 FTIR analysis of fully fermented cocoa bean

Figure 1 illustrates the FTIR spectrum of cocoa beans that had been fermented spontaneously or with the help of *A. aceti* and *S. cerevisiae* inoculums. For comparison, the results of this fermentation were compared with cocoa beans obtained from a commercial factory owned by PTPN XIII. In general, all fermented cocoa beans had almost the same FTIR spectrum in the range of wavenumbers from 400 to 4000 cm^{-1}. Based on Figure 1, fermentation caused a change in% T of the FTIR spectrum, especially in the wavenumber between 3600 and 3800 cm^{-1} (O-H stretching), 1600-1700 cm^{-1} (C = O), and 400-950 cm^{-1}. In addition to carbonyl and O-H, the functional group markers of 120 hours fermented cocoa butter shell consist of C-H aldehyde, C-H, CH_2, CH_3, C-O (ester), and C-O-C (Utami *et al.*, 2016).

The lactic acid bacteria fermentation has a zone of identification or marker of proteins, carbohydrates, nucleic acids, and bacterial DNA detected in the wavenumber between 900 and 1300 cm^{-1} (Vodnar *et al.,*

Table 2. Total phenol, theobromine, and antioxidant capacity of fermented Samarinda cocoa bean

Types of fermentation	Total phenol (ppm GAE)	Antioxidant Capacity IC_{50} against DPPH (ppm)	Total theobromine (ppm)
Spontaneous	3, 027.6±110.5[a]	461.6±2.0[a]	162.3±22.6[a]
S. cerevisiae induced	3, 183.8±47.3[b]	521.7±3.8[b]	53.3±13.8[b]
A. aceti induced	2, 173.9±102.6[c]	892.8±2.3[c]	93.2±2.5[c]
Mixed culture induced	2, 134.8±31.6[c]	424.9±3.3[d]	154.4±1.3[a]

Values with the same superscript indicate no significant difference ($p>0.05$).

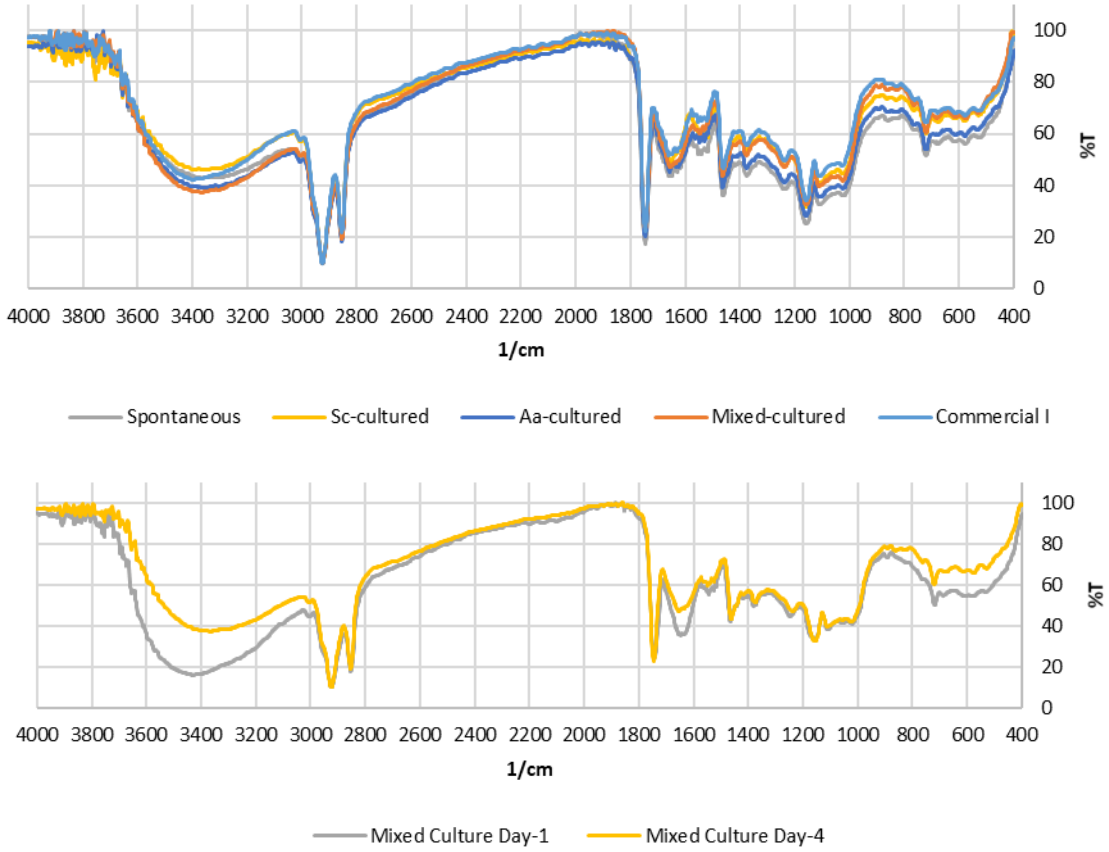

Figure 1. (a) Full range FTIR analysis of fermented Samarinda cocoa bean compared to commercial I (b) Full range FTIR analysis of partly and fully fermented Samarinda cocoa bean.

2010). In other studies, fermented cocoa with various inoculums has a slightly different FTIR profile, mainly on antioxidant components (Batista *et al.,* 2016). Fermentation causes changes in C=O stretching vibrations detected in the wavenumber range 1300-1900 cm^{-1}. Changes in moisture and water activity (O-H stretching) as a result of bacterial fermentation were detected in the wavenumber range 3100-3700 cm^{-1}, as seen in variations of cellulose products from bacterial fermentation (Halib *et al.,* 2012). The FTIR spectra of fermented products has unique characteristics, depending on the dominant microbes present at the time of the fermentation (Shalin *et al.,* 2014).

3.4 FTIR analysis of fully fermented cocoa bean against theobromine identification regiment

One effort to see changes due to fermentation is to compare the FTIR spectrum of fermented cocoa with theobromine standard, especially in the wavenumber FTIR identification zone from 600 to 1500 cm^{-1} (Figure 2 and Table 3). the FTIR spectrum of the theobromine standard was measured with the same tool to avoid too much peak shifting. The shift is around 5% due to differences in the complexity of biological tissue, methods, tools, and binding media (Movasaghi *et al.,* 2008). FTIR spectrum reading for theobromine standard produced peak identification on wave numbers 1485, 1456, 1425, 1412, 1366, 1335, 1294, 1225, 1173, 1138, 1070, 939, 889, 783, 750, 731, 683 and 615 cm^{-1}. After comparing with the database and composition of theobromine deduction, the FTIR peaks occurred because of vibrations from the covalent bonds of C=O, N -H, C=N, and C-N-C (Figure 2). The calculations in Table 3 attempted to quantify how much %A difference from each fermented cocoa with a different inoculum when compared to theobromine standards. Spontaneously fermented cocoa had the highest absorbance difference in theobromine FTIR identification peaks.

Meanwhile, cocoa fermented by mixed culture (*A. aceti* and *S. cerevisiae*) and had the lowest absorbance difference in theobromine FTIR identification peaks. This difference was caused by differences in the inoculum used as induction of the fermentation process as previously described (Vodner *et al.,* 2010; Halib *et al.,* 2012; Shalin *et al.,* 2014). Based on the FTIR spectrum in the theobromine identification zone, fermented cocoa with a mixture of *A. aceti* and S. *cerevisiae* had the closest quality to commercial cocoa samples.

3.5 FTIR analysis of fully fermented cocoa bean against caffeine identification regiment

Not only does cocoa contain theobromine, but it also contains significant amounts of caffeine (Batista *et al.,*

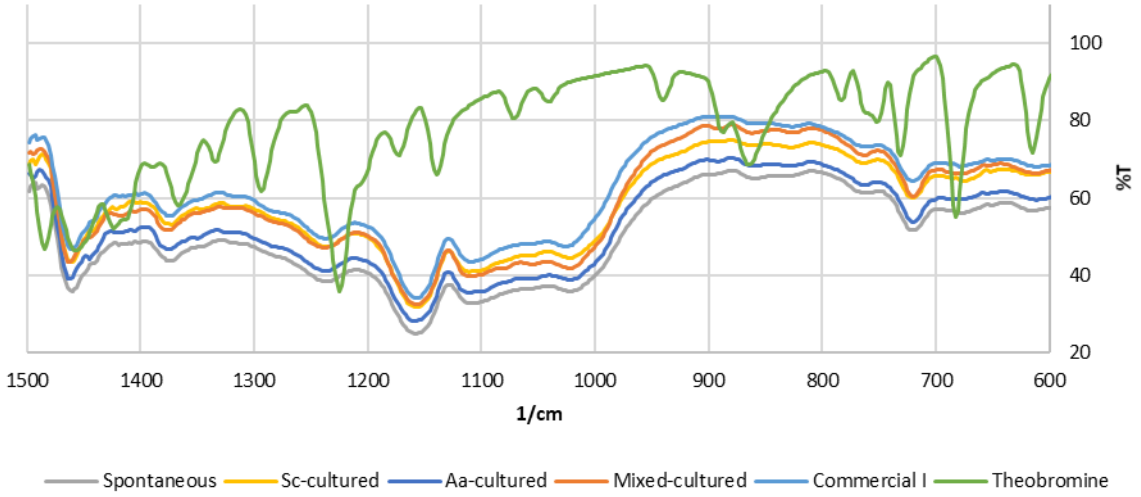

Figure 2. Theobromine IR identification regiment of fermented Samarinda cocoa bean compared to commercial I

Table 3. Peak wavelengths of theobromine IR identification regiment of fermented Samarinda cocoa bean

Wave Number (1/cm)	%A vs. Theobromine				
	Spontaneous	*S. cerevisiae* -induced	*A. aceti* -induced	Mixed culture-induced	Commercial I
1485	-16.2	-24.12	-19.25	-25.16	-28.82
1456	9.56	1.48	4.6	0.5	-1.2
1425	4.37	-5.18	1	-3.68	-8.07
1412	6.49	-3.98	3.16	-1.33	-5.9
1366	12.91	3.24	9.79	4.35	1
1335	20.55	11.21	17.76	12.04	8.2
1294	15.3	5.52	12.95	6.53	3.66
1225	-3.87	-13.26	-6.9	-13.11	-15.66
1173	41.89	34.11	38.75	33.7	30.93
1138	33.19	25.47	30.14	24.98	21.83
1070	44.46	35.97	41.62	37.6	32.86
939	23.69	15.02	19.5	11.58	8.02
889	10.7	2.55	7.59	-0.74	-3.7
783	20.55	13.11	18.57	10.38	8.7
750	18.1	10.06	15.85	7.7	6.22
731	15.48	6.98	12.42	5.04	3.29
683	-1.52	-10.36	-4.64	-11.22	-13.6
615	14.92	5.78	12.03	5.27	3.51

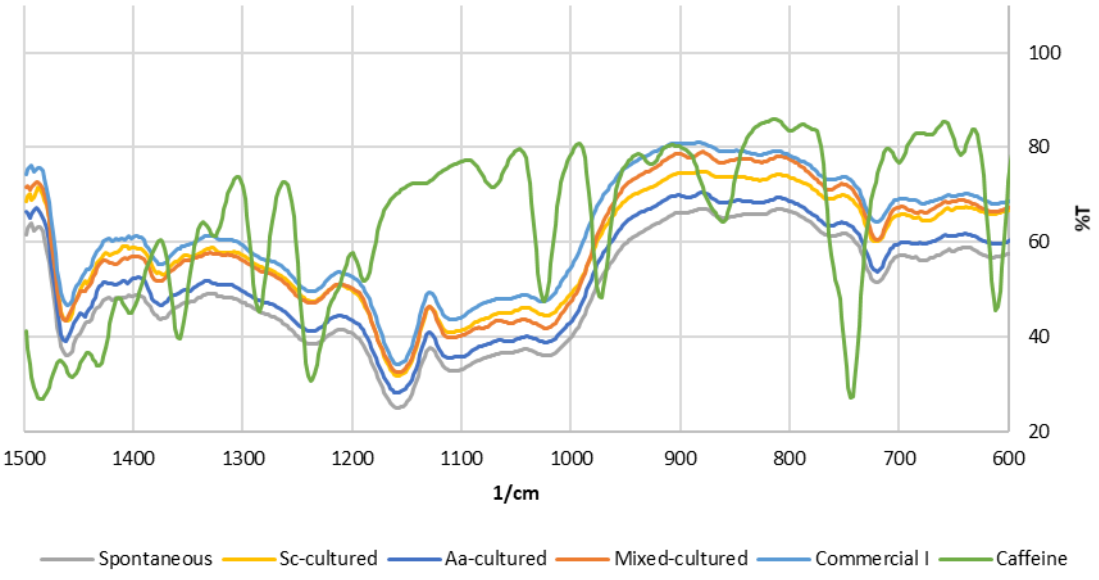

Figure 3. Caffeine IR identification regiment of fermented Samarinda cocoa bean compared to commercial I

Table 4. Peak wavelengths of caffeine IR identification regiment of fermented Samarinda cocoa bean

Wave Number (1/cm)	%A vs. Caffeine				
	Spontaneous	*S. cerevisiae* -induced	*A. aceti* -induced	Mixed culture-induced	Commercial I
1483	-14.90	-22.44	-17.69	-23.73	-27.59
1454	8.43	0.25	3.98	-0.10	-2.44
1431	10.76	1.18	6.49	1.76	-1.29
1402	17.83	7.44	14.23	9.47	5.47
1358	17.00	7.27	14.11	8.17	4.93
1325	28.44	19.20	26.35	19.98	16.38
1285	26.85	17.29	24.56	18.21	15.53
1236	17.09	8.06	14.29	8.25	5.98
1188	38.98	30.15	35.86	29.48	26.84
1072	44.61	36.20	41.82	37.90	33.01
1026	53.57	44.99	50.63	47.60	42.18
972	40.74	31.40	36.32	29.81	24.06
928	29.11	20.81	25.18	17.11	13.64
862	3.86	-4.59	0.81	-7.73	-10.16
800	26.11	18.93	24.00	15.19	14.31
743	29.04	20.96	26.64	18.72	17.10
698	38.72	30.12	35.99	28.49	26.75
642	34.70	26.11	31.79	24.58	23.37
611	18.77	9.61	16.10	9.23	7.51

2016). Changes that occur in fermented cocoa to caffeine standards need to be observed, especially in the FTIR identification zone in the range of wavenumbers from 600 to 1500 cm^{-1} (Figure 3 and Table 4). Caffeine identification peaks were found on wavenumber of 1483, 1454, 1431, 1402, 1358, 1325, 1285, 1236, 1188, 1072, 1026, 972, 928, 862, 800, 743, 698, 642, and 611 cm^{-1} (Table 4). Based on the structure of caffeine, the identification peaks can be assumed to be originated from vibrations of covalent bonds consisting of =C=N- (conjugated, cyclic), =N-N=O, $-CH_3$, $=CH_2$, C-N=O, -OH, =C-O-C-, $-CH=CH_2$, three neighboring aromatic C-H, and ethylene (Rahmadi *et al*., 2019).

As is the case with the theobromine functional groups, the absorbance value of the FTIR spectrum in the caffeine identification zone was compared with the absorbance of the FTIR spectra in fermented and commercial cocoa (Table 4). Industrial cocoa had the most absorbance difference in the value of caffeine when compared to fermented cocoa used in this study. The use of *A. aceti* and *S. cerevisiae* mixed culture in the fermentation process produces cocoa with the difference of caffeine absorbance, which was the closest to commercial cocoa. However, quantification of caffeine levels in fermented cocoa with the help of an induction inoculum needs to be further confirmed.

4. Conclusion

There was no difference in the total population of bacteria, LAB, and yeast and mold in all fermented cocoa samples. Mixed culture fermented cocoa has a slightly lower final pH compared to other cocoa samples. Spontaneously fermented cocoa and mixed culture have the highest levels of theobromine compared to other fermentation methods. Fermented cocoa with *S. cerevisiae* inoculum has the highest total phenol compared to the same compound content in other fermented cocoa. Better antioxidant capacity was obtained from fermented cocoa with a mixed culture. Based on FTIR analysis in the theobromine and caffeine identification zones, fermented cocoa with a mixture of *A. aceti* and *S. cerevisiae* had the closest quality to commercial cocoa samples.

Conflict of Interest

The authors declare no conflict of interest.

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