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Effects of *Candida* sp. and *Blastobotrys* sp. Starter on Fermentation of Cocoa (*Theobroma cacao* L.) Beans and Its Antibacterial Activity

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The effects of *Candida* sp. and *Blastobotrys* sp. starters on fermentation of cocoa beans were studied to determine the potential antibacterial activity of its extracts against foodborne pathogens. The fermentations of cacao beans using *Candida* sp. and *Blastobotrys* sp. were conducted for seven days. The observed parameters including pH and temperature monitoring during fermentation, detected active compounds, and *in-vitro* antibacterial activity against several foodborne pathogens at on sampling day 0, 3 and 7. Spontaneous fermentation (without starter culture added) was used as control. The pH during fermentation increased from pH 3.00 to 7.97, pH 3.00 to 7.68, and pH 3.00 to 7.54 for spontaneous, *Candida* sp. and *Blastobotrys* sp. fermentation respectively. The temperatures of fermentation ranged from 28°C to 33°C, 28°C to 32°C, and 28°C to 32°C for fermentation by spontaneous, *Candida* sp. and *Blastobotrys* sp., respectively. Gas Chromatography Mass Spectrometry (GC-MS) analyses showed several active compounds including caffeine, theobromine, gamma-tocopherol, stigmasterol and beta-sitosterols in all three fermentations. Caffeine content was the highest (74.59%) in control fermentation in earlier process. Theobromine content was higher for control fermentation compared to other *Candida* sp. and *Blastobotrys* sp. fermentation. Generally, gamma-tocopherol, stigmasterol and beta-sitosterols contents declined in the middle of the fermentation period but increased again towards the end. Fermented cocoa beans extract exhibited antibacterial activity against *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella enteritica,* and *Staphylococcus aureus*. However, the extracts did not show any antibacterial activity against *Listeria monocytogenes* and *Pseudomonas aeruginosa.* In summary, the addition of starter cultures namely *Candida* sp. and *Blastobotrys* sp. in fermentation of cocoa beans able to trigger off the active compounds and show potent antibacterial activity against several foodborne pathogens.

Keywords: *Blastobotrys* sp.; *Candida* sp.; *Theobroma cacao* L; cocoa beans; fermentation; foodborne pathogens.

In food industry, safety, quality, and taste are among the substantial criteria that were concerned most to produce excellent characteristic of food products apart from promising health-promoting foods. Unfortunately, foodborne pathogens are one of the potent roots of food contaminations and spoilage that cause countless foodborne outbreaks. In 2005, 1.8 million people reported died from diarrheal diseases largely due to consumption of contaminated food and drinking water (Newell *et al*., 2010). Nowadays numerous

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researches have proved that natural products especially plant sources are plenteous with preservative properties that could reduce the pathogens growth at the same time prolong the shelf life of the products (Calatayud *et al*., 2013).

Cocoa (*Theobroma cacao* L.) in Greek means 'Food for God' (Knight, 1999) which was first bred in 250-900 AD by the Mayans and Aztec in Mesoamerican regions (Knight, 1999). It is regarded as one of the precious food for preserving healthy life and longevity as well as other uses ranging from medicines to currency (Dillinger *et al*., 2000). Approximately 10% of the dry weight of the gross bean enriched with polyphenols contents making them one of the major contributors of antioxidants to the American diet after vegetables and fruits (Rusconi and Conti, 2010). Fermentation of the cocoa beans is a crucial step to discard the mucilaginous pulp and develop the flavor precursors of the chocolate (Fowler, 2009; Thompson *et al*., 2013). The microbial ecology in the fermentation system works wondrous and intricate at the same time. Recent research has found that some strains present in the microbial ecology of the fermentation could inhibit the growth of pathogens namely *L. monocytogenes*, *E. coli* and *Salmonella* sp. (Adimpong *et al*., 2010; Saltini *et al*., 2013). Fermentation of cocoa beans was reported to enhance the development of flavor, aroma and color precursors (Saltini *et al*., 2013). It was also reported to improve the rate and potential yield of cocoa sweating and shorten the fermentation duration (Dzogbefia *et al*., 1999). Yeast, lactic acid bacteria, and acetic acid bacteria are among the proclaimed microflora involved in the fermentation of the cocoa beans (Gálvez *et al*., 2007; Schwan and Wheals, 2010; Lima *et al*., 2011). Microflora such as *Kloeckera apiculata* and *Saccharomyces cerevisiae* var. *chevalieri* were reported to produce volatile compounds such as isopropyl acetate, ethyl acetate, 1-propanol, isoamyl alcohol, 2,3-butanediol, diethyl succinate and 2-phenylethanol during fermentation (Rodriquez-Campos *et al*., 2012). Hence, in this study the antibacterial activity of the fermented cocoa beans extracts using starter *Candida* sp. and *Blastobotrys* sp. starter cultures with regards to fermentation time was analyzed against several foodborne pathogens. Therefore, it is imperative to know the potential of the fermented cocoa beans extracts in impede growth of foodborne pathogens in order to exploit the resourcefulness of the natural products as food preservative.

MATERIALS AND METHODS

Preparation of starter culture

Candida sp. and *Blastobotrys* sp. were acquired from Barry Callebout Services Asia Pacific Sdn. Bhd., Pahang, Malaysia. The cultures were cultivated in yeast peptone dextrose broth media for 24 hours at 30°C prior to fermentation process (Barry Callebout). The cultures were then transferred into molasses-yeast media and finally diluted in sterilized distilled water of ratio 1:9.

Sample preparation (cocoa beans)

Cocoa pods (*Theobroma cacao* L.) were purchased from cocoa plantation, Lembaga Koko Malaysia, Jengka, Pahang, Malaysia. Fresh cocoa pods were opened to obtain the beans for further fermentation process. Briefly, cocoa beans were piled into basket with drainage-holes to allow air and for fluids to flow out. Banana leaves were laid over the base of the baskets. Three separate heaps of cocoa beans were contrived for three different treatments namely spontaneous fermentation (control), fermentation with starter culture *Candida* sp. and lastly fermentation with starter culture *Blastobotrys* sp. The heaps were covered with banana leaves to content the heat in the system along with the observation of pH and temperature in the center of the heap. Samplings of cocoa beans were done at day 0, 3, and 7. Afterwards, the beans were oven dried at 50°C for 48 hours. Then the dried fermented cocoa beans were manually dehusked and grinded for further extraction process. Extractions of fermented cocoa beans were based on Rukayadi *et al*. (2008) with slight modification. 100 g of cocoa beans were soaked in 400 ml methanol (R&M Marketing, Essex, UK) for four days with occasionally shake. The solutions were filtered and evaporated to obtain crude extracts. The extracts were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Saint Louis, MO, USA) for further analysis.

Bacterial strains

Bacterial strains namely *Bacillus cereus* ATCC 21772, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Klebsilla pneumoniae* ATCC 13773, *Listeria monocytogenes*

ATCC 15313, *Proteus mirabilis* ATCC 21100, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enterica* ATCC 13311, and *Staphylococcus aureus* ATCC 29737 were obtained from the American Type Culture Collection (Rockville, MD). Bacterial strains were growth in Mueller-Hilton broth (MHB) (Difco Becton Dickinson, Sparks, MD) at 37°C overnight prior to antibacterial-activity analysis.

***In-vitro* susceptibility test**

Each extract was tested for antibacterial-activity using the disc-diffusion method based on Clinical and Laboratory Standard Institute (CLSI, 2012). The pathogenic microorganism is grown on Mueller-Hinton agar (MHA) in the presence of 10ìl of 10% extract impregnated onto disk of filter paper. Evidence of clear zone after 24 hours incubation surrounding the filter paper disk indicates as an indirect measure of the ability of that compound to inhibit the microorganism. Chlorhexidine was used as positive control. The plates were incubated at 37°C for 24 hours and observed for clear zone surrounding the disc.

GC-MS analysis

GC-MS analysis was carried out employing the following condition: column BP5MS with length 30.0 m × 0.25 mm in diameter and 0.25 µl film thickness. Helium was used as carrier gas with 37.1 kPa pressure, 19.8 ml/min total flow and 0.80 ml/min column flow. The linear velocity was 32.4 cm/sec with 3.0 ml/min purge flow. GC was performed in the splitless mode and the split ratio 20:1. The oven temperature was programmed from 50°C with and increased to 300°C at 3°C/min and held for 10 min. The injection temperature was 250°C and ion-source temperature was 200°C.

Table 1. Average fermentation temperature of cocoa beans

Day	Spontaneous (°C)	*Blastobotrys* sp. (°C)	*Candida* sp. (°C)
0	28.8	28.0	28.0
1	29.7	30.0	29.5
2	36.0	36.0	35.5
3	41.3	40.0	43.0
4	41.7	39.5	42.5
5	35.0	35.0	42.5
6	33.3	34.0	36.5
7	33.0	31.5	33.5

RESULTS

Temperature and pH

In this study, each crude extract from respective fermentations was observed for its antibacterial-activity. Cocoa beans were fermented for seven days. During the fermentation period the temperatures (Table 1) and pH (Table 2) were observed simultaneously for every 24 hours. Figure 1 illustrates the average fermentation temperature of cocoa beans while Figure 2 represent the average fermentation pH of cocoa beans. For spontaneous fermentation, the temperature increased gradually from 28°C to the highest at day four, which was 41.7°C. and started to decline at day 5 until the end of fermentation. Meanwhile, *Blastobotrys* sp. fermentation and *Candida* sp. fermentation have showed its highest temperature both at day three, 40.0°C and 43.0°C respectively. The temperature of all three fermentation systems exhibited a maximum value in the middle of the fermentation period and started to decline as the fermentation ends. The initial pH (3.0) of fresh cocoa pulps was similar to all three fermentations. Trend of increment in pH was observed as the fermentation days increase for *Blastobotrys sp*. fermentation. However, for spontaneous and *Candida* sp. fermentation a slight decreased in pH value (Table 2) was observed in day five. In all cases the pH increments were gradual again throughout the fermentation. At the end of the fermentation duration, all pH generally maintained at 7 for all three fermentations.

GC-MS analysis

GC-MS analysis from the crude extracts yielded five compounds that were detected (Table

Table 2. Average fermentation pH of cocoa beans

Day	Spontaneous (°C)	*Blastobotrys* sp. (°C)	*Candida* sp. (°C)
0	3.00	3.00	3.00
1	3.00	3.00	4.00
2	4.00	3.68	4.24
3	4.54	4.29	4.49
4	4.82	5.25	4.81
5	4.38	5.91	4.06
6	7.66	6.79	7.29
7	7.97	7.54	7.68

3), namely caffeine, theobromine, gamma-tocopherol, stigmasterol and beta-sitosterol. Among these compounds detected, caffeine and theobromine were the two compounds that showed the highest percentage in early fermentation process. In general, caffeine and theobromine content (in percentage) decreased as the fermentation continues to day three but rose again when it reached day seven for all three fermentations. Spontaneous fermentation showed the highest percentage of caffeine and theobromine with 74.59% and 37.71% at early fermentation period respectively. However, in *Candida* sp. fermentation theobromine was not detected as the fermentation end. Stigmasterol and beta-sitosterol were another two major compounds markers in the GC-MS analysis in this study. The percentage of these two compounds implies that they were reduced at day three and regained the concentration as the fermentation reached day seven. These phytosterols showed the same guise for all three fermentations along the seven fermentation days. Gamma-tocopherol content in the spontaneous-fermentation was not detected in the early spontaneous fermentation but emerged at the end of fermentation. Meanwhile, for other two fermentations (*Candida* sp. and *Blastobotrys* sp. fermentation) the presences of gamma-tocopherol were at moderate concentration at early fermentation until day three and eventually regained the concentration as the fermentation day continues.

***In-vitro* susceptibility test**

This study was carried out to evaluate the antibacterial properties of the crude cocoa beans extracts by means of Kirby-Bauer disc diffusion susceptibility test. Diameter of the clear zones (in mm including disk diameter) of the crude extracts (Table 4) indicates the inhibition growth against several foodborne pathogens for crude extracts from three fermentations. The results showed that crude extracts for all three fermentations have higher inhibition zones in early fermentation against *B. cereus*, *B. subtilis*, *E. coli*,

Table 3. Percentage of targeted active compounds in fermented cocoa beans

Targeted active compounds (%)	Control (Spontaneous-fermentation)			*Blastobotrys* sp. fermentation			*Candida* sp. fermentation		
	Day 0	Day3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
Caffeine	74.59	18.26	38.45	46.64	20.26	30.59	44.15	18.23	54.04
Theobromine	37.71	17.59	26.26	35.47	19.22	25.66	34.08	16.53	ND
γ-tocopherol	ND	ND	3.63	0.95	0.43	1.29	1.11	0.88	5.38
Stigmasterol	0.51	0.16	1.46	0.69	0.28	0.70	0.58	0.49	1.64
β-sitosterol	1.07	0.22	1.74	1.31	0.41	1.17	0.95	0.64	3.04

ND, not detected

Table 4. Average diameter of inhibition zone (in mm) of fermented cocoa bean extracts

Extract Bacteria	Spontaneous Fermentation(mm)			*Candida* sp. fermentation(mm)			*Blastobotrys* sp. fermentation (mm)		
	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7	Day 0	Day 3	Day 7
Bacillus cereus	13.0	10.0	9.5	12.5	9.0	8.0	12.5	8.0	8.0
Bacillus subtilis	12.5	10.0	10.0	12.0	10.0	10.0	12.5	9.5	9.5
Escherichia coli	12.0	8.0	7.5	11.5	8.5	8.0	11.0	10.0	8.0
Klebsiella pneumoniae	12.5	8.5	8.5	13.0	9.5	9.0	13.0	8.5	8.5
Listeria monocytogenes	NA	7.0	7.0	NA	7.0	7.0	NA	7.0	7.0
Pseudomonas aeruginosa	NA	6.5	6.5	NA	6.5	6.5	NA	7.0	7.0
Salmonella enterica	10.0	NA	NA	10.0	7.5	7.0	13.0	8.5	8.5
Staphylococcus aureus	12.0	7.0	7.0	12.0	7.0	7.0	12.0	7.0	7.0

NA – no antibacterial activity.

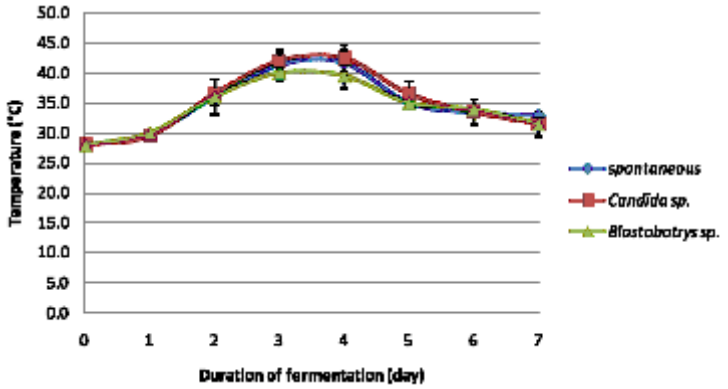

Fig. 1. Average fermentation temperature of cocoa beans

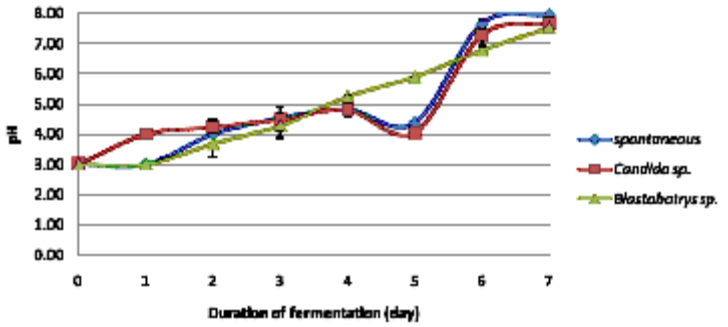

Fig. 2. Average fermentation pH of cocoa beans

K. pneumoniae, and *S. aureus* but the inhibition became weaker as the fermentations continued. On the other hand, *L. monocytogenes* and *P. aeruginosa* appeared to have hight resistance to the extracts of which no antibacterial activity were observed on the early fermentation period for all three fermentations and generally implied modest inhibition at day three and seven of fermentation. Concurrently, the crude extracts from the spontaneous fermentation of sampling day three and seven appeared to be resistant against *S. enterica*. The inhibition activity against *S. enterica* by the crude extracts of *Blastobotrys* sp. and *Candida* sp. fermentations at early fermentation executed potent inhibitory however it diminished as fermentation ends. This inclination was collateral for all extracts against *S. aureus.*

DISCUSSION

The compelling inhibitory activity of the extracts of fermented cocoa beans fermented with *Blastobotrys* sp. and *Candida* sp. as starter cultures have yet to unleash its competency. The fresh cocoa pulps were heaped in the present of yeast (*Blastobotrys* sp. and *Candida* sp.) for seven days. Samplings were taken at day zero, three and seven. Sampling populations were oven-dried as to achieve even drying throughout the process. Afterwards, the dry cocoa beans were dehusked and grinded for further analysis. During the fermentation, the measurement of temperature and pH were taken simultaneously every 24 hours. The temperature of the fermented cocoa beans for all three fermentations started approximately at 28.0°C. In the spontaneous fermentation, the temperature increased gradually until it reached its highest at day four (41.7°C) and moderately decreased as the fermentation reached the end. Meanwhile for the *Blastobotry* sp. and *Candida* sp. fermentation reached its maximum temperature both at day three which was 40.0°C and 43.0°C respectively. The increase in the temperature was also regularly reported in other cocoa fermentations (Schwan and Wheels, 2004; Jesperson *et al.*, 2005; Papalexandratau *et al.*, 2011; Ouattara *et al.*, 2014).

The rise in temperature was a result of exothermic reaction by alcoholic fermentation of yeast to produce ethanol and carbon dioxide (Gálvez *et al*., 2007) and appearance of lactic acid bacteria and acetic acid bacteria (Schwan and Wheals, 2004). At the same time aerobic spore-forming bacteria starting to grow once the acid bacteria decreases (Schwan and Wheals, 2004). The rising of temperature is also important in cocoa fermentation in order to kill the beans (Gálvez *et al*., 2007). During our fermentation monitoring, the highest temperatures ranged from 40.0°C to 43.0°C. This temperature range parallel with study by Ouattara *et al*., (2008). However, the temperature started to decline as the fermentation days increased plausibly due to degeneration of microbial activity is starting to take place (Thompson *et al*., 2001; Schwan and Wheals, 2004; Ouattara *et al*., 2014). This trend conformed for other two fermentations. The initial pH (3.0) of fresh cocoa pulps was similar to all three fermentations basically due to the high concentration of citric acid (Nielsen *et al*., 2006). This condition is favorable for yeast to grow due to high sugar content and its pH (Thompson *et al*., 2001; Nielsen *et al*., 2006). Study by Ouattara *et al*. (2008) reported the fluctuation of pH in their fermentation ranged from 3.5 to 5.5. This is also corresponding to study by Ganeswari *et al*. (2014) where the pH begins at 4. This low pH value was known to be contributed by the presence of citric acid. However, a slight pH value decreased was detected for spontaneous and *Candida* sp. fermentations at day five (Table 2). This changes might be consequently due to the difference in microflora activity of the microbial community in the system since the mass size of the cocoa beans may influence the fermentation process subsequently differ in their metabolic vitality (Schwan and Wheals, 2004; Saltini *et al*., 2013). Similar trend in pH changes were reported in studies by Sanchez *et al*. (1984), Dzogbefia *et al*. (1999) and Ouattara *et al*. (2014).

The GC-MS analysis was conducted to detect the percentage-content of available compounds during the fermentation systems of different starter cultures. Numerous studies have reported several significant bioactive groups in the cocoa and cocoa products including polyphenols (epicatechin, catechin and procyanidin) (Afoakwa *et al*., 2008; Arlorio *et al*., 2008; Ariza *et al*., 2014; Nsor-Atindana *et al*., 2012; Hii *et al*., 2009; Kim *et al*., 2014), methylxanthines (theobromine, caffein and theophylline) (Caudle *et al*., 2001; Mhd-Jalil and Ismail, 2008), peptides (albumins, globulins, prolamin, and glutenin) (Voight *et al*., 1993; Mhd-Jalil and Ismail, 2008) lipids and sterols (Schwan and Wheals, 2004; Lim, 2012), fiber (Paoletti *et al*., 2012) as well as minerals (magnesium, copper and selenium). In this study, five targeted active compounds (Table 3) were detected in all three fermentation systems; caffeine, theobromine, gamma-tocopherol, stigmasterol and beta-sitosterol. In this findings, caffeine and theobromine were the major compounds in cocoa as supported by Maleyki and Ismail (2008). The percentage detected-content of these compounds decreased in the middle of the fermentation however it increased back as the fermentation ends. Nevertheless, in *Candida* sp. fermentation the undetectable content of theobromine was still vague and unclear. Study by Adamafio *et al.* (2011) and Bentil *et al*. (2015) reported that the usage of fungi (*Aspergillus niger*) able to bio-detheobrominate the concentration of theobromine in cocoa waste to be used as animal feed. In the study by Adamafio *et al*. (2013) other yeast used was identified as *Candida krusei* as the bio-detheobrominator. This perhaps elucidates the non-traceable of theobromine in *Candida* sp. fermentation. However, based on Adamafio *et al*. (2011), this method is not feasible in conventional agricultural practices and therefore not applied by local farmers. Cocoa beans are prosperous with plant sterols including beta-sitosterols and stigmasterols specifically in cocoa butter (Steinberg *et al*., 2003). Fedeli *et al*. (1966) reported that beta-sitosterols and stigmasterols have been identified in the cocoa butter (Lim, 2012). These two are phytosterols that occur naturally in plant which able to diminish the intestinal absorption of diet and biliary cholesterols (Fernandes and Cabral, 2007; Lengyel *et al*., 2012; Botelho *et al*., 2014). These sterols also effective in lowering circulating cholesterol levels (Thompson *et al*., 2005). Stigmasterol and beta-sitrosterol were numerously reported in previous cocoa beans studies; nonetheless no study has reported the exact amount or values for these plant sterols. Study by Singh *et al*. (2012), reported that several plant sterols from *Withania somnifera, Euphorbia hirta*

and *Terminalia chebula* were tested against pathogens and found that it is susceptible against *E. coli* and *Enterobacter aerogens* (Singh *et al*., 2012). The fluctuations of both sterols in all three fermentations are still unclear thus study needed to be conducted further on this matter. On the other hand, gamma-tocopherol was not detected in the spontaneous fermentation. Plausible explanation on this matter could be because of the absence of yeast starter culture in the control fermentation. This is conformed to the study of antioxidant properties in fermented soybean by Hubert *et al*. (2008), where the tocopherol content in the fermented soybean experienced some losses compared to the unfermented soybeans. Study by Erickson *et al*. (1973), cocoa butter was rich with vitamin E such as alpha-tocopherol, beta-tocopherol, and gamma-tocopherol (Jahurul *et al*., 2013). Cocoa butter is one of the important ingredients in producing chocolate (Zyzelewicz *et al*., 2014). Based on the result, generally the additional of starter cultures have improved the content of bioactive compounds in the cocoa beans fermentation.

The antibacterial potentialities of the crude extracts from all three fermentations were screened using the method of Kirby-Bauer disc diffusion susceptibility test (Das *et al*., 2010). Based on the result (Table 4), *B. cereus* and *B. subtilis* were susceptible to all crude extracts of different sampling days from all three fermentations. The diameter of clear zones was generally higher for some extracts in the early fermentation process and eventually reduced to the end of fermentation. The inhibition potential reduced perhaps due to the excessive appearance of *Bacilli* at later stage of the fermentation suppressing the antibacterial activity of the extracts (Ardhana and Fleet, 2003; Papalexandratou *et al*., 2011). However, Lima *et al*. (2011) have mentioned that the role of *Bacillus* sp. in fermentation system is still unclear although some report suggested that they contribute to the yield of pectinolytic enzymes (Ouattara *et al*., 2008). The same trend goes to inhibition against *E. coli*, *K. pneumoniae*, and *S. aureus* where inhibition was greater at early fermentation stage of which microbial metabolic activity is about to begin. Research by Smullen *et al*. (2007) mentioned that the unfermented cocoa extracts have displayed a greater antibacterial activity than fermented cocoa extract against *Streptococcus mutans* Ingbritt. This could be the plausible reason of the antibacterial potential where the fermented beans consist lesser low molecular weight phenolics compared to the unfermented cocoa beans (Smullen *et al*., 2007). Howbeit, the resistance of *L. monocytogenes* and *P. aeruginosa* against all crude extracts were observed at early fermentations though some inhibition were marked at the end of fermentations. Calatayud *et al*. (2013) have reported that cocoa extract in the biofilm displayed bactericidal effects against *L. monocytogenes* whereby higher concentration of cocoa extract is required in food-application compared to broth media due to the food matrix. Similar study conducted also showed that cacao pulp crude extract was inactive against *P. aeruginosa* ATCC 27853 (Panganiban *et al*., 2012). Several reports have also emphasized the potential antibacterial activity of cocoa extracts that perhaps due to high concentration of flavonoids (Cushnie *et al*., 2005). Nevertheless, further study needs to be implemented as to discover the mechanism of bactericidal of the crude fermented cocoa extracts and compound/s that are responsible in the inhibition against foodborne pathogens.

CONCLUSION

The pHs of cocoa beans during fermentation ranged from pH 3.00 to 7.97 and its temperature started at 28°C and ended at 33°C. pH and temperature of these fermentations showed no outstanding distinction between each other. Caffeine and theobromine content decreased in spontaneous fermentation, *Blastobotrys* sp. and *Candida* sp. fermentation, but caffeine increased in *Candida* sp. fermentation. Gamma-tocopherol, stigmasterol and beta-sitosterol increased along fermentation process. Fermentation process is suggested to be less than seven days as caffeine content could be increased. Fermentation of cocoa beans in additional of starter cultures *Candida* sp. and *Blastobotrys* sp. have showed potential bactericidal effect compared to the control fermentation.

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