UNDERSTANDING BIOCHEMISTRY OF COCOA FERMENTATION TO ARREST BEAN ACIDITY IN PAPUA NEW GUINEA COCOA

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ABSTRACT

The dominant microbial species observed in a commercial fermentation of Papua New Guinea cocoa were Yeast, Acetobacter, Lactobacillus and Bacillus species. Maximum growth of different species of microbial was observed starting from day 2 of the fermentation. The microbial activities and their rate of metabolism during the fermentation correlated to total acid production, rate of pulp sugar utilization, oxygen concentration and temperature regime. Better understanding of cocoa biochemistry would enable devising of better interventions to arrest acid production during the fermentation to reduce residual bean acidity to improve the overall cocoa flavour.

Key words: *Cocoa microbiology, fermentation and biochemistry*

INTRODUCTION

Cocoa beans are essential raw material in chocolate production. They are produced as seeds in pods of the plant *Theobroma cacao*. The first stage in processing of the bean is fermentation. The beans are removed from pods and placed in mass in heaps or boxes where fermentation developed naturally. Fermentation is regarded as being essential for initiating the reaction that leads to the formation of substance that confer characteristic of chocolate flavour (Lehrian and Patterson, 1983).

Fermentation involves two distinct processes. First, microorganism grow in the pulp surrounding the bean, leading to a physical solubilisation of the pulp and the generation of metabolic end products and enzymes that have the potential of diffusing into the beans and affecting its chemical composition. Secondly, the environment created by microbial growth causes death of the beans and initiates an internal autolytic process that is conducted by endogenous enzymes. Numerous studies in different countries have demonstrated the contribution of yeast, lactic acid, acetic acid bacteria and other bacteria to the process (Ostovar and Keeney, 1973). Different studies conducted intended to determine the essential of microbial species to the development of good chocolate quality and to control fermentation conditions such that the growth of desirable species may be encouraged or alternately, the growth of undesirable species may be prevented (Hansen, 1975 a, b; Sanchez *et al.*, 1985). Another goal has been to link the presence of particular microorganism with key chemical and biochemical changes with the pulp and bean, such as changes in turning of the beans being related to the sensory properties of chocolate (Lopez and Quesnel 1973b; Hansel 1975 a, b; Passos *et al.*, 1984b). Despite numerous studies that have been conducted, a clear link between the microbial ecology of fermentation, biochemical changes within the beans and bean quality have not been established, though the important of microbial activities is precursor to flavour development.

Generally, beans produced from South East Asia countries including PNG are deemed to be inferior in quality than those produce from Africa. The decrease in appeal in use is the result of development of an unacceptable acidic character, which in some way, is connected to the microbial ecology to fermentation (Chong *et al.*, 1978; Karen *et al.*, 1983). Genetic materials have shown to have very limited influence on bean acidity problem. Bean acidity is measured in pH and Titratable Acids (TA). Detailed investigation confirmed a very strong correlation ($r=0.98^{***}$) between pH and total TA (Chong *et al.*, 1978). A study of the citric acid cycle and carboxylic acid carried out to determine the cause of the low pH of cocoa, indicated that carboxylic acid (acetic and lactic) appears to be the group of acids that would contribute most to low pH (Rohan and Stewart, 1965).Traditional box fermentation produced beans that have excessive acidity (pH 4.4-4.7). Chocolate derived from these beans were not entirely acceptable, hav-

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ing bitter taste and lacking chocolate strength (Shepherd and Yap, 1984).

To understand the relationship between the microbial ecology and biochemistry of fermentation, it is important to have quantitative information about the growth of individual species of microorganism throughout fermentation, as well as some knowledge about their biochemical proprieties. The concentration of metabolites and potential chocolate flavour precursors produced by individual microbial species during the fermentation will depend upon the quantitative extent of their growth (Ardhana, 1990).

Many variables are considered to affect the content of cocoa bean fermentation. These include the scale and physical conditions of fermentation, sanitation of fermentation containers and surrounding environment, degree of bean mixing during fermentation, maturity of beans at harvest and cultivar of the beans (Rohan 1963; Minifie 1980). The literature suggested the participation of a range of microbial species in the fermentation including yeast, fungi and bacteria, which is influence by different factors including environment, location and country and fermentation practices.

Cocoa is an important export commodity of Papua New Guinea, however some quality attributes of the bean are considered inferior to those produced in some other countries and there is an urgent need to address these problems. One such initiative involved obtaining more knowledge about the fermentation process and practices and understanding the involvement and influence of microbial activities and its effect on cocoa flavour. This would lead to development of improved processing techniques to improve the quality of PNG cocoa. This paper intends to identify the general ecology of microbial species present in fermentation of PNG cocoa and its influence on general cocoa biochemistry, in particular, bean acidity and to make recommendations on how the residual acidity can be reduced to improve overall cocoa flavour.

OBJECTIVES

Identify general profile of microbial species in commercial fermentation of Papua New Guinea cocoa, and its influence on fermentation pattern and cocoa biochemistry; with an attempt to arrest acid production to reduce residual acidity of the bean to improve overall flavor attributes of cocoa.

MATERIAL & METHODS

Experimental Design

Fermentation process

Cocoa pods of mixed cultivar were harvested in the field and their pods were removed and bagged before they were transported to the fermentry. The wet beans were fermented inside a standard commercial fermentation box (120 x 90 x 90) cm and dried on a conventional commercial dryer. The dried beans from each of the 25 fermentation replicates conducted were collected, bagged for quality assessment.

Sample collection and preparation

The bean acidity, sugar concentration of the pulp, and sample for flavour assessment were prepared following the standard cocoa quality laboratory procedure (PNGCCRI, 1995). The oxygen concentration was measured using an oxygen logger. The laboratory mercury thermometer was used to measure temperatures of the fermenting mass inside the fermenting box.

Flavour assessment

Organoleptic evaluation was conducted by the trained taste panel following the procedure described by BCCCA (1996); Sukha (2001).

Microbial analyses

Microbial isolation and enumeration was determined following procedures described by Carr and Passmore, (1979)

RESULTS

Figure 1, shows that all microbial activities reached their maximum level during day 2 of fermentation, before decreasing afterwards. The Yeast population increased between days 2-3 before decreasing sharply until it disappeared at day 4 of fermentation. The Acetobacter followed a similar pattern as Yeast, but continuo to maintain its population at a low level towards the end of the fermentation. As for Lactobacillus species, its population decreased after day 2 of fermentation, but maintained a relatively high

Figure 1: *Profile of average microbial population of commercial cocoa fermentation*

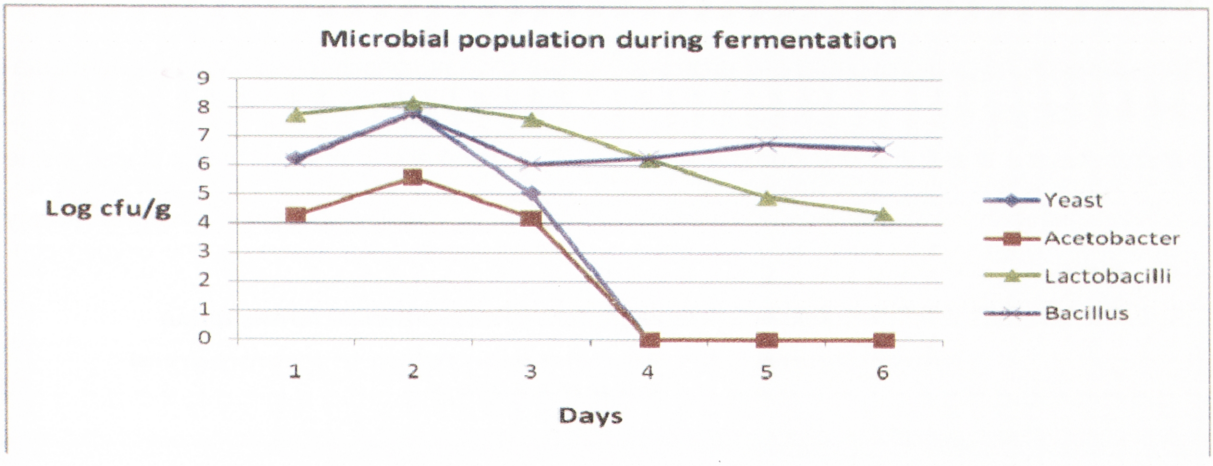
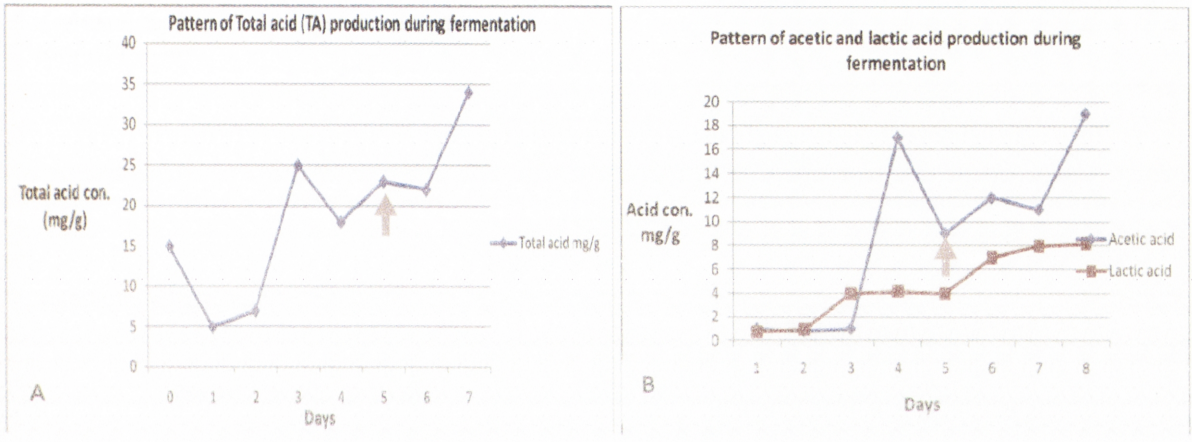


Figure 2: *Pattern of average total acid production (a) and major acids: Acetic and Lactic acid (b) in a commercial fermentation*



population toward the end of fermentation unlike Yeast and Acetobacter. The Bacillus population decreased after day 2 of fermentation, before increasing from day 3 onwards and maintained a large population throughout the rest of fermentation.

Figure 2 a, shows the average total acid (Oxalic, Citric, Ethanol, Succinic, Lactic and Acetic) concentrations produced during the fermentation. The individual concentration of minor organic acids is shown in Figure 3. The total acid concentration began to increase from days 1 – 6, with a sharp increase between days 2 - 3 coincided to the increase microbial population and activities (Figure 1). Among the acids produced, acetic (range 0.6 - 18.5 mg/g wet beans) and lactic acid (range 0.5 - 8 mg/g wet beans) were dominant throughout the fermentation, thus become the potential candidates responsible for high level of residual acids found in the beans. Acetic acid was produced in large quantity than lactic acid, showing a sharp increase from day 3 - 4 (range 0.7 - 16.5 mg/g wet beans) before decreasing (8.5 g /mg wet beans) in day 5, and increased afterwards to reach its maximum level (18.5mg /g wet beans) at the end of the fermentation. As for Lactic acid, it shows a gradual increase in concentration, but remains at a lower level than acetic acid throughout the fermentation.

For average minor organic acid concentration ***(Fig. 2);*** Succinic and Oxalic acids were present in small quantities with an average concentration ranging from 0.1 - 2mg/g wet beans. Ethanol was present in large quantity with an average concentration ranging from 0.1 - 17.5 mg/g wet beans during the fermentation. However, its concentration fluctuated at each day of the fermentation and this can be related to the acid being used mainly for microbial activities. The citric acid concentration decreased starting from days 1 – 3 (from 10 to 1 mg/g) before increasing towards the end of the fermentation with acid concentration of 3 mg/g wet beans.

Note: Higher the Titratable acidity (TA) value, the higher the bean acidity.

The average acid profile of the fermenting beans ranged from 0.05 - 0.26 mg/g beans. From the start, this was gradual, followed by a sharp decrease in pulp TA after day 3 of fermentation; corresponding to a sharp build up of acid concentration in the bean kernel; an indication of acid migration from the pulp to the kernel.

production as the result of microbial activities (Fig.5)

The oxygen concentration during the fermentation (Fig. 6) ranged from 18 - 75 % saturation. At day 1, the oxygen concentration was high (70 % saturation) for fresh beans placed inside the fermentation box, but decreased as the fermentation condition becomes anaerobic at day 2-4 of fermentation. However, the oxygen con-

Figure 3: Average minor organic acid concentration in a commercial fermentation

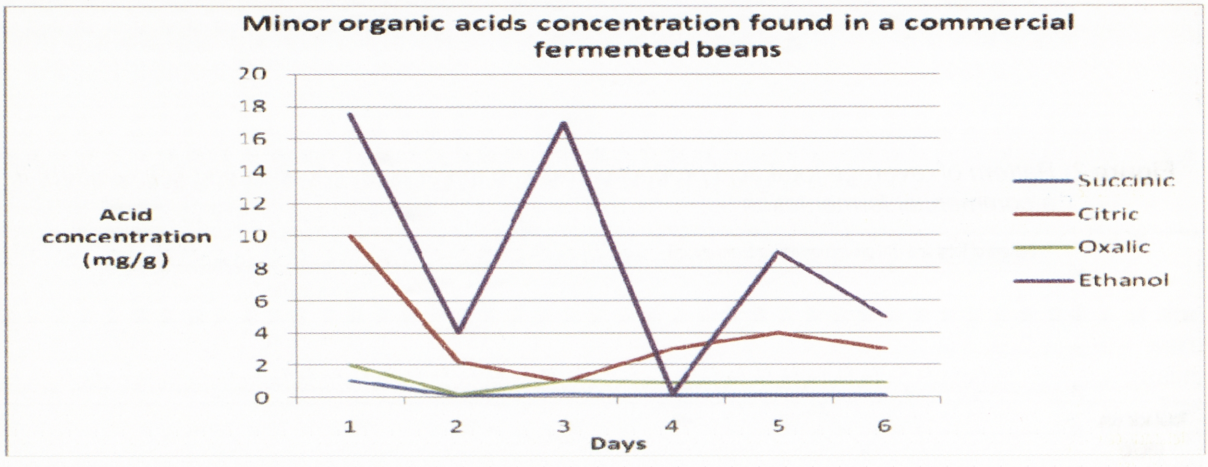
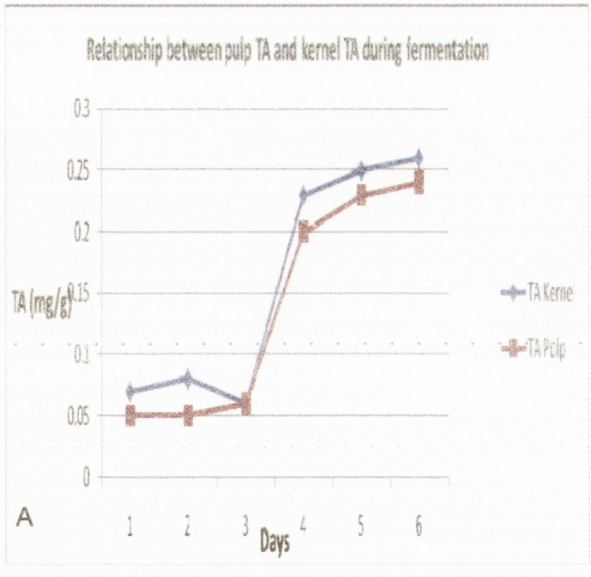
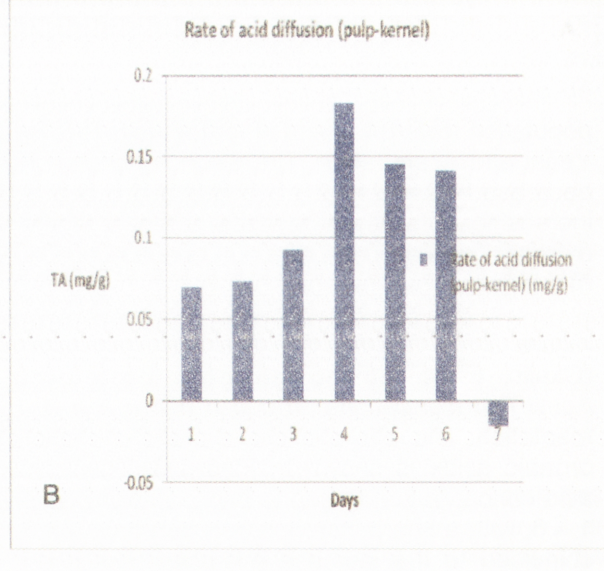


Figure 4: Relationship between average pulp and kernel TA (a) and rate of acid diffusion between bean pulp and kernel (b).





The pulp sugar production decreased during fermentation with the highest concentration (82mg/g wet beans) at the beginning of the fermentation and reached the lowest concentration (7mg/g wet beans) on day 3, before showing a slight increase towards the end of the fermentation; indicating a possible sugar

centration increased after day 4, reaching the maximum concentration (75 %) at the end of the fermentation as the result of maceration (physical solubilisation of pulp) and daily turnings of beans, introducing aeration into the fermentation box. Aeration is required for oxidation reactions to take place to produces acids that is required to cause the death of the bean.

***Figure 5*: Profile of Pulp sugar production during the fermentation**

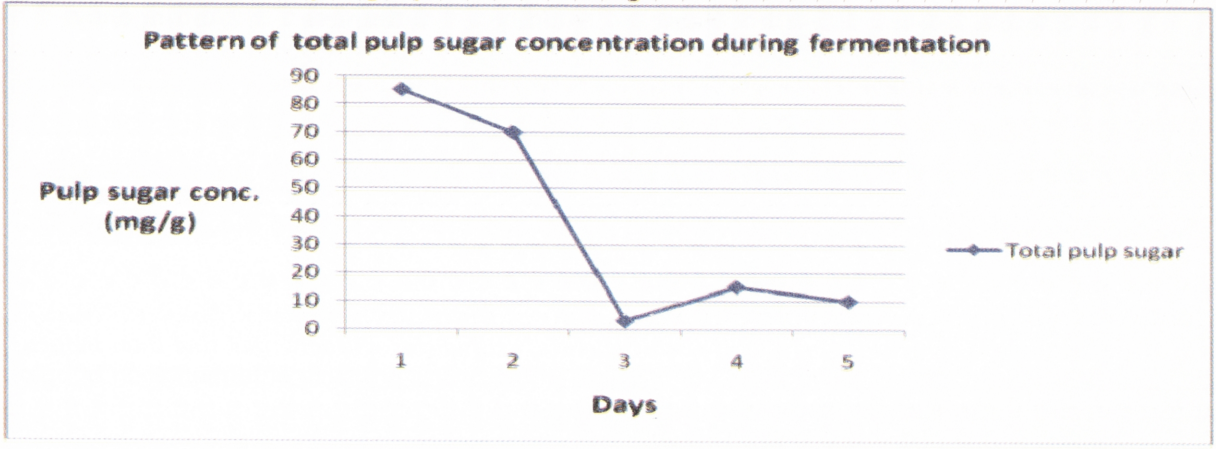
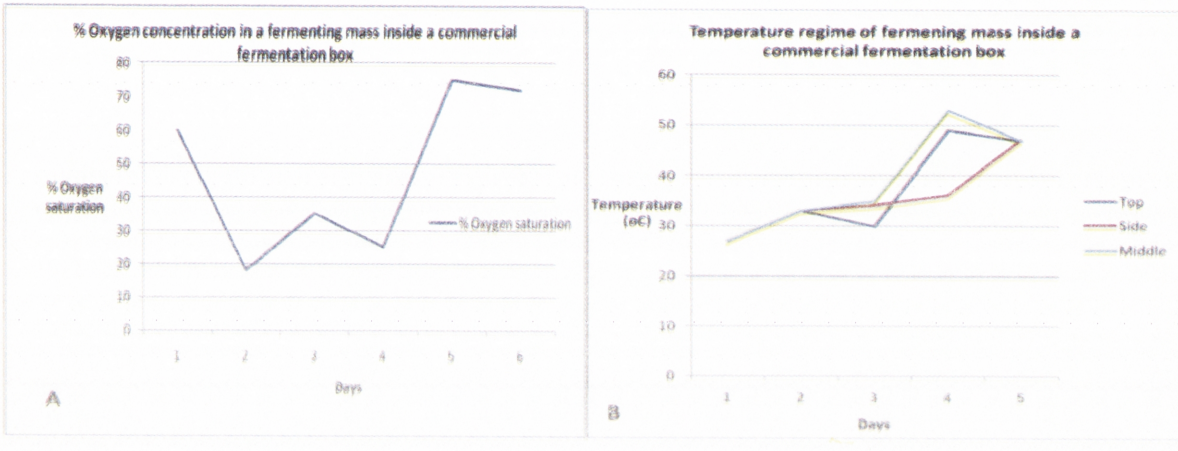


Figure 6: Oxygen saturation (%) (a), and temperature regime (b), during the fermentation



The temperature range of fermentation mass at the top, sides and middle of the fermenting mass was between 27 - 49, 27 - 47 and 27 - 53 ^{0}C respectively (Fig. 6b). The temperature of the fermenting mass at all parts of the box increased during the fermentation, reaching their maximum temperatures at day 4, before decreasing towards the end of the fermentation. A slow increase in temperature was observed for fermenting mass at the sides of the box during the initial phase of fermentation before the temperature increased rapidly from day 4 - 5. The temperature profile of the fermentation indicated that the maximum temperature of 53 ^{0}C was reached in the middle of the fermentation mass with lower temperatures recorded at the top and sides of the fermentation box.

The average flavor attributes of commercial fermented beans generated was compared against Ghana cocoa, which is considered as universal standard. PNG cocoa produced low level of chocolate flavour intensity with pronounced acidity, bitterness and astringency (Table 1).

DISCUSSION

Acid production during the fermentation period coincided with microbial activities. The microbial activities and their rate of metabolism during the fermentation correlated to total acid production, rate of pulp sugar utilization, oxygen concentration and temperature regime as shown by the result of this study. By understanding the role and presence of each of the microbial activities during the course of the fermentation, acid production can be arrested to reduce the level of residual acid in the beans to improve the overall flavour cocoa.

Yeast

Yeast population was predominant from day 0 - 2 of fermentation with a population range of 10^{7}-10^{8} cells/g, before decreasing to a population of 10^{4} cells/g at the end of the fermentation. Similar results were obtained by Ostava and Keeney (1973). The proliferation of yeast population (Figure 1) corresponded to decrease in pulp

Table 1: **Average sensory assessment result of commercial fermented beans**

Sample	Chocolate	Acidity	Bitterness	Astringency
Commercial fermented Beans	6.0	3	1.0	2.3
Ghana (Control)	8.4	7	1.6	1.0

The flavour intensity was estimated using 0-10 scale, with 0 being weak and 10 being strong. The lower the acidity value, the higher the acidic taste in flavor scoring or vice versa.

sugar concentration (Figure 3a). High population of yeast was observed during the period when the pulp sugar concentration was high, but decreased as the pulp sugar being utilized. The dominant of yeast population in the early stage of the fermentation could be responsible for converting pulp sugar primarily into ethanol that appears to be used in the later part of the fermentation to produce carboxylic acids as well as enzymes to aid pulp maceration. The initial low temperature, high pH and aerobic condition favour yeast proliferation. The presences of high yeast population in the beginning of the fermentation could be the result of pulp being cross contaminated with remnant of yeast present inside the fermentation box from previous fermentations. Yeast is also responsible for loosening physical structure of the pulp and to some extend metabolize citric acid to give a slight increase in pH. In figure 3, it shows concentration of citric acid decreasing, coincided to the same period when the yeast population was highest. Sequence of different yeast species identified to be present at different stages of fermentation (Carr and Passmore, 1979). The pulp sugar or its metabolites are used by Acetobactor and Lactobacillus species to produce acetic and lactic acid respectively.

Lactobacillus

Lactobacillus population remains high throughout the duration of the fermentation. The maximum population was reached in day 2 of fermentation with a population range of 10^4-10^8 cells/g. They are responsible for high acetic acid production, which eventually migrate into bean, hence increasing the acid concentration of the kernel (Figure 2 & 3). Similar results were obtained by Carr and Davies, (1980) for Malaysian cocoa. Qualitative and quantitative analyses of volatile and non-volatile low molecular acids have indicated that acetic and lactic acid are primarily responsible for the excessive acidity of beans. Individual species of Lactobacillus varies through out the course of fermentation of which some play predominant role than others. Both acids are products of fermentation of pulp sugar.

Acetobacter

The Acetobacter growth started in day 1 of the fermentation, with a population range of 10^4 - 10^6 cells/g. The maximum population was reached in day 2, before decreasing and represents less than 1 % of the total microflora population during rest of the fermentation (Figure 1). Acetic acid was prominent during fermentation of cocoa beans at estates in Ghana and Malaysia by days 3-5 and present at a population of 10^6-10^8 cells/g. They were present at the commencement of the fermentation being more prevalent on the beans in Ghana than those in Malaysia (Carr and Davies, 1980). Their growth coincide with conversion of ethanol into acetic acid, aeration of the bean mass by turning and collapse of the physical structure of the pulp, which also promoted aerobic condition. The oxygen concentration in the fermenting mass fluctuated due to daily turning of the beans as well as being utilized for oxidative reaction. The oxygen concentration started to increase in day 2, reaching a maximum concentration of 75 % saturation at day 5 and remain at relatively high concentration throughout the rest of the fermentation (Figure 5 a). The diminished of Acetobacter population towards the end of the fermentation could be attributed to their inability to tolerate the increasing temperature, which increased to 47-53 ^{0}C by days 4-5 (Figure 5b). Similar result was reported by Forsyth and Rombouts (1951). Acetic acid bacteria appears to make significant contribution to cocoa bean fermentation involving different species accounting for high concentration of acid, though lower than lactic acid (Figure 2b).

Bacillus

The Bacillus species appears on day 2 of fermentation with an initial population of 10^6 cells/ g, and continuo to increase and reached a

maximum population of 10^8 cells/g in day 2, before decreasing, and maintained reasonably high population of 10^6-10^7 cells/g towards the end of fermentation (Figure 1). Similar profile of Bacillus population was observed by others studies during the fermentation (Ostavar, K., 1971; Ostavar and Keeney, 1973). The contribution of Bacillus to quality of bean is not fully understood, but their contribution may not be significant. However, with an increase of Bacillus population towards the end of the fermentation that coincided to high acid production (Figure 2), their role needs to be further investigated.

Pattern of acid production

The initial decrease in pulp acidity between day 0-1 (Figure 3a) could be result of pulp sweating; acid in the pulp are lost through sweating. Bean acidity started to increase from day 1 of fermentation and this is when acid migration from pulp to kernel started (Figure 3 a & b). Large amount of total acids and acetic and lactic acids (9-10 mg/g) were produced towards the end of the fermentation (Figure 2 a & b shown by arrows), which may be an ideal period to arrest the acid production to reduce residual acid in the bean.

Acid migration

Total acid concentrations build up from day 1 and continue to increase during the fermentation with a sharp increase between days 2 and 3. The concentration of minor acids (Figure 3) may not contribute significantly to total acidity as they were presented in low concentration throughout the fermentation except for Ethanol. The acid migration from the pulp to the kernel started at day 1 of the fermentation, showing a sharp increase after day 2 and reached the maximum rate at day 4, before decreasing slightly and remains at a reasonably higher rate towards the end of the fermentation. The importance of acid production is, it contributes towards causing death of the beans.

Pulp sugar concentration

The pulp sugar started to decrease from day 1 of fermentation concided to yeast activity. In the initial stage of the fermentation (day 0-1), the yeast was responsible for metabolising the pulp sugar to ethanol, which is then utilised by the Lactobacillus and Acetobacter species to produce mainly large quantity of lactic and acidic acid respectively. Besides yeast, the other microbial activities started from day 1 of the fermentation and this is when the acid production commenced.

Oxygen and temperature regime

The initial aerobic condition of the fermenting mass could be the result of freshly harvested beans been saturated with oxygen. The aerobic condition changes to anaerobic from days 2-4 of fermentation, as the result of rigid compact structure of the fermenting mass formed inside the confinement of the fermentation box after the collapse of pulp, which limit oxygen flow into the fermenting mass. The fermentation condition changes to aerobic after day 4 of the fermentation as the result of physical solubilisation of pulp. The slight increase in oxygen concentration between days 3-4, could be the result of daily turning of the beans. Under aerobic condition, it favours proliferation of Acetobacter resulting in high acetic acid production, while Lactobacillus population is not being affected since it favours aerobic condition.

Temperature regime

The temperature of the fermenting mass can be related to the microbial activity, as fermentation is an exothermic reaction. Higher temperature was measured for all sides (top and sides) of the fermentation mass between days 3-5 coincided with proliferation of Acetobacter population. The temperature at the centre of the fermenting mass increased steadily throughout the fermentation. Lower temperatures were measured at the sides and top of the fermenting mass due to beans exposed to the external environment. Slower temperature increase was also observed at these sides of the fermenting mass compared to centre of the fermenting mass, which is less affected by the external environment. The fluctuating in temperature shown throughout the fermentation could be the result of daily bean turning.

CONCLUSION

The results of this study indicated that maximum microbial population were observed from days 1 - 3, which coincided with sharp increase in acid production and migration of acid produce from the pulp into the kernel. During the same period, there was a sharp decrease in total pulp sugar indicating the conversion of pulp sugar by yeast to ethanol or other metabolites which was used by other microbial population to produce acids as the main byproducts. Besides, yeast also

produced enzyme to aid pulp maceration. The study highlighted several important aspects of fermentation process that can be manipulated to reduce residual bean acidity, while retaining the overall flavor attributes of cocoa. The acid production can be arrested by reducing the fermentation days from 7 to 5 days. On average, large quantity of total acids (84 %) were produced from day 3-7 of which (45 %) was produced between day 5-7 (Figure 2 a & b). From the total acid produced, large amount (66 %) migrated from the pulp into the kernel (Figure 4 a & b) as the result of high level of microbial activities. The high temperature and oxygen concentration in the fermenting mass (Figure 6 a & b) correlated to increase microbial activities; converting ethanol and other metabolites (by products of pulp sugar) into organic acids. Heat and acid produced during the fermentation combined to kill the bean. The bean death causes loss of cell membrane and integrity of intercellular compartments resulting intra and intercellular mixing of water-soluble compartment. This reaction is a prerequisite to development of flavor precursors, which develop into the chocolate flavor plus other ancillary flavours. Therefore, the fermentation process can only be arrested towards the end of the fermentation and the most ideal time would be at day 5. This is when fermentation can be stopped to reduce residual bean acidity by preventing further migration of acid into the bean kernel. At that point, fermentation process is complete and the large amount of volatile acids produce is expected to remain in the pulp that can easily be removed by drying as compared to acids locked inside the kernel. In the later case, it will be difficult to remove most of the acids especially the none-volatile acid. The 5 days fermentation has shown to proceduce similar quality attributes as 7 days fermentation (Hollywood, 1994). The fermenting mass also needs to be turned and mixed properly daily during the fermentation to maintain homogeneity. This will allow all beans to be properly fermented because of temperature variation observed at different sides of the fermentation mass. Similarly, adequate mixing and turning would be required to ensure adequate aeration inside the fermenting mass especially during the anaerobic phase (before day 4) to facilitate oxidative reactions to generate sufficient heat and acid to kill all the beans to reach full fermentation status. Maximum temperature of 48-50 ^{o}C is required to cause the death of the beans. The temperature range between 47 - 53 ^{0}C was reached for all sides of the commercial fermentation, which is sufficient to cause the death of the bean.

Furthermore, residual acidity can be further reduced by applying slow drying initially to give sufficient time for acids within the kernel to make its way out to the bean pulp to be removed before the shell hardens. Commercial dryer temperature can easily be adjusted to meet this requirement, while initial slow drying can be applied for klin dryers used by majority of smallholder farmers. This requirement suits the solar dryers. This process adjustments would improve chocolate flavour and reduce bean acidity to reach quality attributes near to Ghana cocoa (Table 1) since high level of bean acidity usually tainted chocolate flavour.

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