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Contribution in the Improvement of Cocoa Bean Fermentation by using a Starter Microbial from Three Strains

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Abstract
Fermentation of cocoa beans is a critical quality control point because of aroma precursor's generation. Control fermentation as a practice to limit the quality variability of spontaneous fermentation was undertaken. Three strains of microorganisms namely *Saccharomyces* sp., *Lactobacillus* sp and *acetobacter* sp, were tested as starter culture during fermentation of Trinitario cocoa variety. Beans were carefully mixed with starter culture containing *Saccharomyces* sp. ($0.75x10^8$ cells), *Lactobacillus* sp. ($0.45x75x10^8$ cells) and *acetobacter* sp ($0.3 x10^8$ cells), box fermented and dried. Modification in temperature and pH were measured by using standards instruments. Physical quality assessment of sun-dried cocoa beans was record according to standards. Results show that pH and fermentation index meet the standards. Fermentation time was reduced to 3 days but mass beans temperature did not meet standards values even though cocoa beans were classified as ''good fermented''. Despite these preliminary encouraging trials, further studies need to be done in order to use this starter during traditional fermentation.

Keywords: Quality, Cocoa Bean, Starter, Microorganisms, Control Fermentation

Introduction
Cocoa (*Theobroma cacao*) bean is the basic material of chocolate industries [1] and aroma is the most important commercial attribute. Standardization of the marketable quality is a challenge regarding aroma. Fermentation of cocoa beans is the critical quality control point because of aroma precursor's generation [1]. Nowadays, researchers point out control fermentation as a practice to limit the quality variability of spontaneous fermentation. Fermentation is a dynamic process of heat and mass transfer resulting from microbial activities. Yeasts and lactic bacteria at the onset of the process and acetic bacteria at the end are the main type of fermenting microorganisms [2, 3]. Inoculation of cocoa beans with fermentative microorganisms (yeasts, lactic and acetic bacteria) as starter with multiple potentialities could ensure the control of fermentation stage; reducing (or eliminate) by these constraints associated with high commercial quality beans production. Therefore, this study is a contribution in improving the market quality of cocoa beans produce in Cameroon. It aims at using a starter culture composed of yeast, lactic and acetic bacteria as fermentative microorganisms in order to produce marketable cocoa beans with physicochemical, nutritional and organoleptic reproducible quality.

Material and Methods
Source of Cocoa Beans
Mature cocoa pods (yellow-orange in color) of trinitario variety were harvested from a rural farm in the equatorial raining forest of the South Region of Cameroon. The pods were manually harvested, put in new plastic bags with banana leaves and then transported to the laboratory where they were kept for two days at room temperature until the beans were removed from the pod and the pulp surrounding the seed fermented.

Origin of Strain
Strains used in this study were selected from a box fermenting medium according to the classic microbiological methods. Yeast, lactic and acetic bacteria with good capacity in growing at different temperatures and pH and in presence of their metabolites was selected.

Starter Preparation and Utilization

Microorganisms used as starter are *Saccharomyces* sp, *Lactobacillus* sp., *Acetobacter* sp. strains. They were inoculated and selected in their selective agar media and mixed in a proportion of 50(Yeast)/30(lactic acid bacteria)/20(acetic acid bacteria).

Methodology

Spontaneous Fermentation

Pods were manually open using a wooden stick. Fresh beans (11.735Kg) were poured into a wooden box (size: length 30 cm, wide 30 cm and depth 30 cm; made with *Ayous* species) perforated at the bottom then covered with banana leaves. Beans were manually turned upside down every 2 days to ensure the homogeneity of the fermentation. Color changing from white to brown with strong acidic smell marked the end of fermentation. Fermented beans were subsequently sun dried under controlled conditions until reaching a moisture content of 7.5 (% d.b.). Dry beans were finally packaged in sealed polyethylene bag and kept at -80ºC for future treatments and analyses.

Control Fermentation

Wooden box and all material were first washed with clean water and rinsed with alcohol. They were dried in sun for three hours. Pods were washed and allow drying before opening by hitting perpendicularly with a wooden stick. Beans (1.4kg) were then weighed using a calibrated scale and pour into the fermenting box containing sterile banana leaves at the bottom to trap heat. Beans were carefully mixed with starter culture (1.5ml) containing *Saccharomyces* sp. ($0.75x10^8$ cells), *Lactobacillus* sp. ($0.45x75x10^8$ cells) and *acetobacter* sp ($0.3\ x10^8$ cells). The fermenting box was then close after recovering cocoa beans with sterile banana leaves. After 3 days of fermentation, beans were subsequently sun dried until reaching a moisture content of 7.5 (% d.b.). The colonies observed were expressed as the number of colony forming units in terms of Log CFU/g. It should be mention that operators were equipped with disinfected latex gloves, masks and caps to cover their hair.

Microbial and Physical Parameters

Enumeration of Microbial Population

The microflora dynamic during fermentation was monitored by isolation of microorganism on samples taken every 24 hours using classic methods of counting in Petri dishes. 0.1ml of decimal solutions were plated on different selective agar media and incubated at 30°C for 48 to 72 hours for yeasts and lactic bacteria and 3 to 5 days for acetic bacteria. Sabouraud Chloramphenicol agar medium was used for yeast detection. GYC Penicillin Nystatin Agar Specific Medium was used for the isolation of acetic acid bacteria. The lactic acid bacteria were isolated on Man Rogosa Sharpe's selective gel medium (MRS). The colonies observed were expressed in terms of Log CFU/g.

Physicochemical Analyses

Temperature and pH

Beans mass temperature was monitored by inserting thermometer (Couper-Atkins) in the middle hole of bean mass for 5 minutes, to have a stable value. pH was recorded on samples taken every 24 hours. Pulp and cotyledon were manually separated and then crushed and homogenized. Each part was immerged into 90 ml of distilled water and filtered. The pH of the filtrate (25ml) was recorded using a calibrated pH meter.

Fermentation Index

The method of Romero-Cortes *et al*., [4] with some modifications was used. Fermentation index (IF) value was the ratio of absorbance read on spectrophotometer at 460 nm and 530 nm.

Physical Quality Assessment of Sun Dried Cocoa Beans

Moisture Content and Cut Test Analysis

Moisture content was assessed using moisture meters and cut test analysis was undertaken as per standard conditions [5].

Results and Discussion

Characteristic of Starter Culture

Screening based on resistance to stress (high temperatures and pH, as well as growth in different concentrations of intrinsic metabolites), and then high capacity of growth and metabolites production in optimal conditions (alcohol (yeasts), acetic acid (AAB) and lactic acid (Bacteria lactic acid) allow to select strains whose characteristics are as follows.

Table 1: Characteristic of starter culture

Origin	**Box fermentation of cocoa bean of trinitario variety**		
Isolation medium	Sabouraud chloramphénicol agar medium	. GYC Penicillin Nystatin Agar Specific Medium	Man Rogosa Sharpe's selective gel medium (MRS)
Morphology	Ovoid; medium in size; in a short chain	- Gram bacillus	- Gram bacillus
Catalase	+	-	-
Orientation	*Sacharomyces* sp	*Lactobacillus* sp	*Acetobacter* sp

Microorganism Dynamics During the Fermentation Process

Time variation in yeast, lactic acid and acetic bacteria during control and spontaneous fermentation is shown in Table 2. The microorganisms load is high in control process. There are two stages in microorganism dynamics during spontaneous fermentation. The initial stage (0 to 96h) dominated by yeast and lactic acid bacteria (LAB) and the final stage dominated by acetic acid bacteria. In control fermentation, microorganisms are high at the onset of fermentation and decrease then after. The dynamic of microorganism process stop early in control fermentation.

Table 2: Microbial load expressed in Log CFU of microorganisms as a function of fermentation time

	Control fermentation			**Spontaneous fermentation**		
Code	Y_1	ALB_3	AAB_2	-	-	-
Time (h)	Yeast (LogCFU /g)	Acid Lactic Bacteria (LogCFU /g)	Acid Acetic Bacteria LogCFU /g)	Yeast (LogCFU /g)	Lactic acid Bacteria (LogCFU /g)	Acid Acetic Bacteria LogCFU /g)
0	18.13	17.62	17.22	6.51	6.21	-
12	13.71	11.67	9.38	nd	nd	nd
24	14.88	9.31	11.41	9.19	6.62	4.70
48	14.73	9.15	8.70	11.16	10.80	-

72	6.42	11.92	12.70	9.03	9.03	2.42
96	-	14.26	-	6.80	8.17	9.77
120	-	-	-	6.62	7.18	6.62
144	-	-	-	8.93	6.12	6.11

Heat and Mass Transfer Kinetics During the Fermentation Process

Time variation in temperature and pH during the fermentation process is shown in figure 1. In spontaneous fermentation, temperature increases (Figure 1a) gradually from 28ºC to reach a maximum (47ºC) over 120 h of fermentation and decreases then after to 40ºC at the end of the process. In control process, temperature increases during the 24h of fermentation and then decrease but did not reach 47^0C. In spontaneous fermentation, pulp's pH (Figure 1b) decreases initially during 60 hours of fermentation and increase then after. Cotyledon's pH decreases during all the fermentation period. In control fermentation, pulp's pH increases rapidly and steadily during the entire process while cotyledon's pH decreases slowly and gradually.

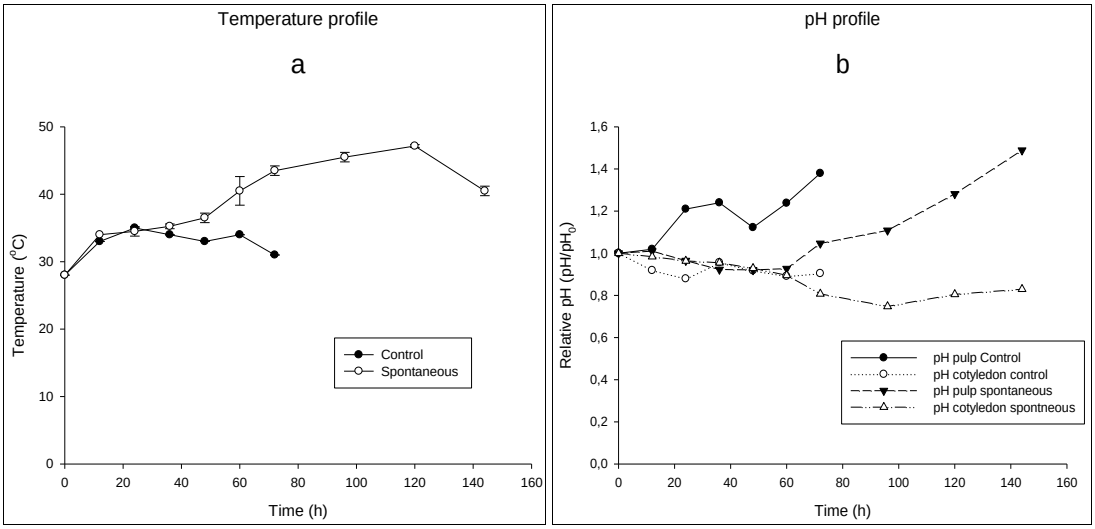

Fig 1: Dynamics of bean mass temperature (a) and pH (b) of the pulp and cotyledon during spontaneous and control fermentation

Fermentation Index

Time variation in fermentation index is shown in figure 2. Fermentation index increases progressively in control and spontaneous fermentation. The values obtain in control fermentation are the highest. Fermentation index value greater than 1 is obtained after 120h of fermentation in spontaneous fermentation and 72h in control fermentation indicating that cocoa bean mass is sufficiently fermented after 120h and 72h in spontaneous and control fermentation respectively.

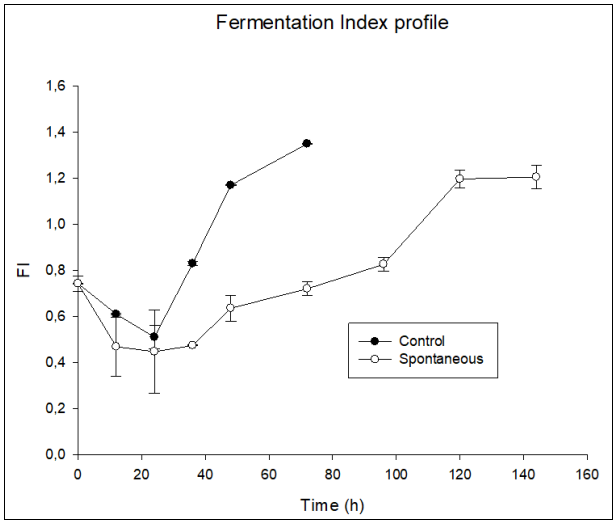

Fig 2: Time variation in fermentation index during spontaneous and control fermentation

Physical Quality Assessment of Sun Dried Cocoa Beans Commercial Quality Standards

The moisture contents of spontaneous (144h) and control fermented (72h) and dried beans are 7.5 % and it is closer to the value of 7% approximately required by manufacturer. The results of cutting test for spontaneous fermentation are 98.83 ± 0.28% of well-fermented grains and 1.66±0.57% of violet grains (underfermented). Cutting test score obtained for control fermentation is 60 ± 0.1% of well-fermented grains and 30 ± 0.3% of violet grains (underfermented). This percentage respects the standard value of ≥ 60%. Thus classifying it as ''good fermented''.

The microbial dynamic recorded in spontaneous study is in agreement with the results reported in the literature [6, 7, 2]. In control fermentation, microorganisms inoculated decrease over the time. This trend of behavior is reported by many authors [8, 9]. The decrease in microbial population can be due to shortage of nutrients or physico chemical modifications in the fermenting medium [2, 6]. The microorganisms load is high in control process and the pulp's pH increase rapidly and steadily in contrary to spontaneous fermentation where decreasing phase is experienced before increasing phase. This may be due to the high initial load of microorganisms inoculated resulting in a rapid breakdown of citric acid by yeast and/or lactic acid bacteria in order to metabolize pulp's sugars [10, 11, 12]. Microbial activity during fermentation produced heat which is a latent parameter recorded as temperature. Temperature did not reach 47^0C in control fermentation. One explanation can be the low quantity of beans mass used which were not

sufficient to develop and maintain critical heat. Nevertheless, the pH values recorded in spontaneous and control fermentation are in the range of values reported by Calvo *et al* [2] for a good fermentation. Fermentation index value is greater than 1 indicating that cocoa bean mass is sufficiently fermented and the time needed to obtain these values is shorter in control than spontaneous fermentation. One objective in starter development is the reduction of fermentation time to obtain a more stable product at the end of the process [1, 9, 8]. This objective was successfully realized using our starter culture. Regarding the cutting test result, control fermented beans can be classified as good fermented but the percentage of well-fermented grains recorded (60%) is in the boundary. This means that enough still to be done. Optimal conditions for a good rise in temperature should be determinate. Studies on the genetic stability of the starter microflora would be also appropriate.

Conclusion

Microorganisms load is high in control process. pH and fermentation index meet the standards during control fermentation. Fermentation time was reduced to 3 days but mass beans temperature did not meet standards values. Nevertheless, control fermented cocoa beans can be classified as ‘‘good fermented’’. Despite these first encouraging trials, further studies need to be done in order to fulfill all the standards. The way of using this starter during traditional fermentation in such a manner that starter microflora supplant the natural microflora imposing itself during the fermentation process is a prospect.

References

1. Schwan R, Wheals AE. The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr. 2004; 44:205-221.
2. Castro-Alayo EM, Idrogo-Vasquez G, Siche R, Cardenas-Toro FP. Formation of aromatic compounds precursors during fermentation of *Criollo* and *Forastero* cocoa. Heliyon. 2019; 5:e01157. Doi: 10.1016/j.heliyon.2019
3. Indarti E, Widayat HP, Zuhri N. Effect of Fermentation Container and Thickness of Bean Mass During Fermentation Process of Cocoa Bean (*Theobroma Cocoa* L).1st Syiah Kuala University Annual International Conference 2011, Banda Aceh, Indonesia, November 2011. Syiah Kuala University, 2011.
4. Romero-Cortes T, Salgado-Cervantes MA, García-Alamilla P, García-Alvarado MA, Rodríguez-Jimenes G, del C, *et al*. Relationship between fermentation index and other biochemical changes evaluated during the fermentation of Mexican cocoa (*Theobroma cacao*) beans. Journal of the Science of Food and Agriculture. 2013; 93(10):2596-2604.
5. ISO 2451. Cocoa beans - Specification and quality requirements – Sampling, 2017.
6. Afoakwa EO, Kongor JE, Takrama JF, Budu AS. Changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma cacao*) beans. International Food Research Journal. 2013; 20(3):1215-1222.
7. Ouattara DH, Ouattara HG, Droux M, Niamke SL. Analysis of Environmental Conditions Affecting Acids Production in Lactic Acid Bacteria Involved in Ivorian Cocoa Fermentation. Scholars Academic Journal of Biosciences. 2019; 7(4):158-165. ff10.21276/sajb.2019.7.4.1ff. ffhal-02320665
8. Lefeber T, Janssens M, Camu N, De Vuyst L. Kinetic Analysis of Strains of Lactic Acid Bacteria and Acetic Acid Bacteria in Cocoa Pulp Simulation Media toward Development of a Starter Culture for Cocoa Bean Fermentation. Applied and Environment Microbiology. 2010; 76(23):7708-7716. 0099-2240/10/$12.00 Doi:10.1128/AEM.01206-10
9. Sandhya MVS, Yallappa BS, Varadaraj MC, Puranaik J, Jaganmohan Rao L, Janardhan *et al*. Innoculum of the starter consortia and interactive metabolic process in enhancing quality of cocoa bean (*Theobroma cacao*) fermentation. LWT - Food Science and Technology. 2016; 65:731-738.
10. Camu N, De Winter T, Verbrugghe K, Cleenwerck I, Vandamme P, Takrama JS, *et al*. Dynamics and Biodiversity of Populations of Lactic Acid Bacteria and Acetic Acid Bacteria Involved in Spontaneous Heap Fermentation of Cocoa Beans in Ghana. Applied and environmental microbiology. 2007; 73(6):1809-1824. Doi:10.1128/AEM.02189-06
11. Apriyanto M, Sutardi, Supriyanto, Harmayani E. Study on effect of fermentation to the quality parameter of cocoa bean in Indonesia Asian J. Dairy & Food Res. 2016; 35(2):160-163.
12. Lima LJR, Almeida MH, Nout MJR, Zwietering MH. *Theobroma cacao* L, "The Food of the Gods": Quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. Crit Rev Food Sci Nutr. 2011; 51(8):731-761.