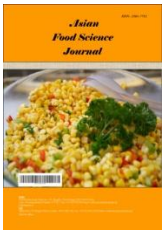

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Fermentation Practices and Microbiological Profile of Fermented Cacao Beans (*Theobroma cacao*) from Mvila Division, South Region, Cameroon

C. Saïdou [a*], B. A. Mohammadou [a], NZ. H. Ghomdim [a], L. B. Mezajoug [a], F. S. Nodem [b] and C. Tchiegang [b]

[a] University Institute of Technology, University of Ngaoundere, P.O.Box 454, Ngaoundere, Cameroon.
[b] National School of Agro-Industrial Sciences, University of Ngaoundéré, P.O.Box 455, Ngaoundéré, Cameroon.

Authors' contributions

This work was carried out in collaboration among all authors. Author CS collected the samples and carried out experiments. Authors ZHG, BAM, LBM and FSN contributed to paper writing. Authors CS, BAM, ZHG, LBM and CT reviewed and edited the paper. Author CT supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Fermentation is an important process in the production of commercial cocoa beans. Conducted in a natural way, it takes place through the action of microorganisms such as lactic acid bacteria (LAB), acetic bacteria and fungi. This generally contributes to obtaining cocoa of different grades. In order to improve the quality of commercial cocoa in the division of Mvila, the aim of this study was to assess the fermentation practices and the microbiological profile of fresh fermented cocoa beans. For this purpose, a survey on the fermentation techniques of the cocoa sector was firstly done with 75 producers. Thereafter, samples of fermented and dried cocoa beans from different producers were collected for commercial quality assessment (grades, impurities rate and water content). Producers with cocoa beans of good quality were retained and the microbial (LAB, yeast enumeration and characterization) quality of their fresh fermented cocoa were monitored after fermentation. The results obtained on the post-harvest treatment techniques of cocoa beans revealed that the technical itinerary is respected with 100% of producers who put the pots in incubation before fermentation. In addition, fermentation techniques are practiced by 87,5% of producers and take place for 6 days long (in 95 %), notably fermentation in wooden box (47.5%),

**Corresponding author: Email: saidouclement2015@gmail.com;*

fermentation in heaps (32.50%). According to the commercial quality, the localities of Melane and Nkoemvone have good quality (Grade=1) cocoa with a rate of brown beans of 95.4% and 94% respectively. Lactic acid bacteria, acetic bacteria and yeast were presents in all fresh cocoa beans with the final load of 1.17×10^7 CFU/g, 1.87×10^5 CFU/g and 5.60×10^6 CFU/g respectively. The fermentative capacity revealed that among the 13 isolates of yeasts, 11 were alcohol producers. This study shows that these microorganisms are responsible for the good fermentation of cocoa beans. It would be judicious to make molecular identification of these strains and to use them as a starter for the controlled fermentation of cocoa.

Keywords: Post-harvest treatment; natural fermentation; fermented cocoa beans; commercial quality.

1. INTRODUCTION

From the twenty-two species that constitute the genus *Theobroma* (family *Sterculiaceae*), *Theobroma cacao* is one of the most important agricultural export commodities in the world and forms the backbone of the economies of some countries in West (i.e., Ivory Coast and Ghana) and central Africa (i.e., Cameroon) [1,2]. World cocoa production is about 4.3 million tons and is valued at billions of dollars. Among it, West Africa alone accounted for ~71% of global cocoa supply [1,3].

In Cameroon during the last decades, production has been growing up and the government expected to reach 500,000 t by encouraging the setting up of new cocoa plantations [1]. This initiative should be associated with the improvement of the cocoa bean production and marketable quality. In addition, the economy of most developing countries, based primarily on their agricultural resources, is strongly dependent on the often rigorous and rigid quality standards set by developed countries.

Cocoa beans are the basic raw material for the production of chocolate [3,4]. Raw cocoa beans are inedible because of their bitter and astringent flavour and unpalatable and unpleasant taste; they must be cured before they can be processed into good-tasting and full-flavoured cocoa and chocolates [1,5–7]. Technological pre-treatment involves fermentation of the fresh cocoa pulp-bean mass, followed by drying of the fermented cocoa beans and subsequent roasting of the fermented dry cocoa beans [1–3,5,8]. It is therefore certain that the fermentation of cocoa beans plays an important role in the physicochemical composition and aroma profile of cacao-based products [5,6]. Nowadays, indicators of well fermented and dried quality beans are brown color, low astringency and bitterness, absence of off-flavors and, during the quality control, a cut test has been considered as a good indicator when determining the degree of fermentation of cocoa beans [1,6,9]. So, based on cut test score, many authors stated that in order to assess the degree of fermentation, cocoa beans can be divided into four categories: i) fully fermented beans with brown color (characteristic of well-fermented cocoa); ii) fully purple beans iii) slaty beans and iv) defectives beans [9].

Cocoa bean fermentation is one of the few remaining spontaneous microbial processes that occur at the farm through traditional practices by the action of various endogenous microorganisms, including yeast, lactic acid bacteria and acetic acid bacteria. They degrade the mucilage pulp, releasing numerous secondary metabolites with organoleptic characteristics [4,5,10]. In Cameroon, many fermentation practices exist through different localities and differs between smallholders. These variabilities influence Cameroonian cocoa market quality and cause misquotation and loss of profit. Similarly, Grade 2 cocoa, representing 95% of production, has led to a discount of 200 to 250 pounds on the London Stock Exchange [11]. However, one of the solutions to limit this problem is the control of cocoa fermentation that is generally carried out by inoculating the mass of fresh beans with selected microbial starter. Moreover, this study is the first step in the development of a microbial starter for the fermentation of cocoa in the Department of Mvila and aims to determine the characteristics of fermentation practices and to isolate, from the beans fermented by the producers, the microorganisms of interest for good cocoa fermentation.

2. MATERIALS AND METHODS

2.1 Survey on the Fermentation Practices

A cross-sectional descriptive study was conducted from July to November of 2021 in five localities of the Mvila Department because of the

important production and processing of cocoa beans in Cameroon [12]. These localities included: Bityli, 2°56'00''N; 11°11'00''E; Biyenyen, 2°83'00''N; 11°13'00''E; Melane, 3°00'01''N; 10°55'12''E; Metipkwale, 3°08'00''N; 11°09'00''E; Nko'emvone, 2°49'10''N; 11°08'10''E). The majority of the village residents belong to the *Bantou* tribe. A semi-structured questionnaire for interviews with producers or cocoa transformers were done and questions were based on the post-harvest cocoa operations, fermentation practices (i.e., fermentation containers, fermentation conditions, stirring frequency, fermentation time, etc.) and criteria for the appreciation of fermented cocoa beans. Were included in this study (i) the residents of the village and producers of fermented cocoa beans (ii) have knowledge and skills on cocoa processing. For the cocoa fermentation processing discussion was conducted with 75 (15 producers per localities; 5 localities) producers, the data obtained were compared and consensus process was carried out. The interviews were conducted by trained enumerators, a resident of the village. French language and wherever possible vernacular (*bulu*) language was used.

2.2 Evaluation of the Commercial Quality of Cocoa Beans Produces

The water content: It was determined as described by the AFNOR [13].

***The graining*:** It measures the number of healthy or normal cocoa beans contained in a given mass of beans. Therefore, 100 beans from each sample were weighed and the total weight of the beans is then calculated.

***Cut test*:** The quality of the fermented and dried beans was evaluated by the cut test technique which measures the fermentation index and is based on color and odor as criteria [1,9,12]. Briefly, one hundred dried cocoa beans were cut the long way through the center utilizing a penknife. Both parts of each bean were analyzed in full sunshine as indicated by the cross-sectional shade of the beans. Perceptions were made for bug harm, shape pervasion, germination as well as of the shade of the beans (record, completely purple and completely brown). Slaty bean qualities incorporate rubbery cotyledon, blackish shading and protection from cutting. Purple beans happen when the aging has been ended rashly. Deficient beans are the amount of sprouted beans, plagued beans and level beans. The percentage count of each color attribute was used to calculate the cut test score as described by Niemenak et al. [1].

***Rate of impurities*:** It consists in quantifying (in g) the foreign bodies (grains of sand, pieces of wood, seeds of palm, etc.) in a given mass of samples (g).

2.3 Microbial Analysis of Fermented Cocoa Beans Samples: Enumeration, Isolation and Phenotypical Characterization of LAB, Yeast and Acetic Bacteria

2.3.1 Enumeration of LAB, yeast and acetic bacteria

Enumeration of these microorganisms has been done using the microbiological analytics technics as described by Guiraud [15]. Briefly, serial dilutions (10^{-1} to 10^{-7}) of 25 g of each sample were done in 225 mL of saline water (0.85%, *w/v*). Thereafter, one hundred microliters aliquots of the appropriate dilutions were surface-plated on the specific media: (i) MRS agar [16] and (ii) Sabouraud supplemented with chloramphenicol (0.05 g. L^{-1}) [4] respectively for enumeration of lactic acid bacteria and yeast. The cultured were incubated at 37ºC and 25ºC for 72 h respectively. Concerning acetic bacteria, the following media with composition described below was used (g.L^{-1}): glucose (20 g); yeast extract (8 g); peptone (5 g); agar (12 g); calcium carbonate (7 g) supplemented with ethanol (5 mL) which is added after sterilization. Thereafter, the cultures obtained were incubated at 37ºC for 72 h.

2.3.2 Isolation and phenotypical characterization

For bacterial and fungal isolation, the previous cultures obtained were used. For each Petri dishes and for each sample, a representative's colonies were randomly selected for their purification and characterization. Therefore, individual colonies were streaked twice on nutrient agar and characterized through phenotypical tests. All isolates including bacteria and yeast were subjected for Gram stain, catalase, oxidase and CO_2 from glucose production. For yeast isolates production of ethanol was also done. All these tests were performed as described by Guiraud [15].

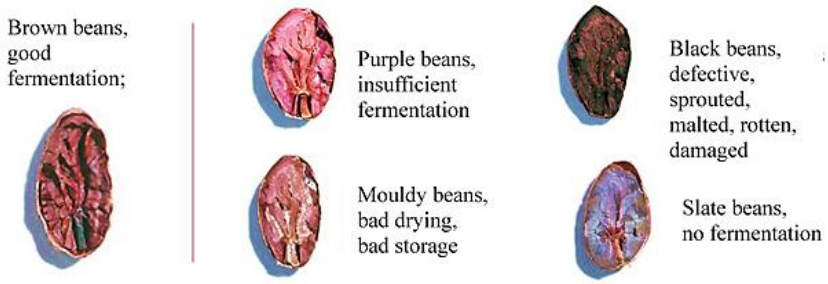

Fig. 1. Appreciation of cocoa beans through cut test [14]

2.4 Statistical Analysis

Data from survey were entered in MS Excel, and analyzed using XLStat version 2016.02 software windows. Descriptive analysis was done to provide general information on the characteristics of fermented cocoa beans processing. Analyzed data are presented using percentages, table or bar charts. For the analytical part, data were expressed as means ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Fermentation Practices of Cocoa Beans

The post-harvest processing techniques of cocoa beans considerably influence the physicochemical, organoleptic and nutritional qualities of the final product. In this study, cocoa bean processing itineraries were evaluated for 75 producers from different localities in the Department of Mvila and the data are shown in Table 1. The data obtained show that 87.5% of the producer's ferment and dry their beans while the others (12.5%), after shelling, immediately sell or dry the fresh beans. We noted that three main varieties i.e., *Criollo* (36.1%), *Forastero* (34.5%) and *Trinitario* (29.4%) are commonly used for production. However, the cultivar used during post-harvest operations, particularly during fermentation, is not considered by all the producers surveyed. They mentioned that non-consideration for the cultivar is linked to the lack of financial resources, of qualified personnel, and of adequate material and equipment for fermentation. In fact, the fermentation technique varies with the producer, the locality, and it takes place either in wooden box (47.5%), or in heaps and covered with tree leaves (32.5%) or in a plastic bag (20%). The duration of fermentation varies from 4 days (2.5%) to 8 days (2.5%) with a maximum (95%) fermenting time of 6 days. During fermentation period, the beans are continuously turned each 24 hours (12.8%) or 48 hours (87.2%) and this could improve the fermentation time and quality. Indeed, research accomplished by Limia et al. [2] mentioned that turn of the cocoa beans mass was carried out in 9 out of the 13 fermentations from several producing Countries including Trinidad, Ivory Coast, Brazil, Ghana, Indonesia, the Dominican Republic, and Nigeria and the fermentation duration varied between 4 and 7 days. The predominance of fermentation time of 6 days found in this study has also been reported by researchers [2,4]. The previous authors recommend the fermentation in heaps using banana leaves and using wooden box during 6 days for the production of a good-quality raw cocoa because this allows the contamination rate of cocoa by fermentative microorganisms.

The predominance of these three particular cultivar has been mentioned by several authors [2–4,12]. Similarly, the use of the three fermentation techniques or methods including wooden box, heaps and covered with tree leaves (32.5%) and plastic bag has been documented [1,2,4]. Other findings mentioned that in addition to the three methods of cocoa beans fermentation listed before the used of baskets, or drying platforms are also frequently used [2]. This could be explained by the variability of cultural practices related to each geographical population.

3.2 Commercial Quality of Cocoa

After the diagnosis of post-harvest cocoa treatments, 75 samples were collected from each producer in order to evaluate their marketable quality and the results according to their appearance (color, odor, etc.) are illustrated in Table 2. The reference of the Federation of cocoa trade was used for the grade classification. Globally, at the end of the post-harvest process carried out (harvesting, shelling, fermentation, drying, storage), it is noted that in the Nko'emvone and Melane localities, the cocoa

beans have a very low rate of purple, moldy, slate and defective beans and a high rate of brown beans (91.2%) characteristic of very good quality (Grade 1). Slaty beans are an indicator of improper fermentation of cocoa and this demonstrates that fresh cocoa beans fermentation in these localities was processed properly in all treatments. It also indicates that the pods were harvested at a suitable stage of maturity.

On the other hand, in Biyenyen and Bityli localities, the cocoa beans have a very high rate of purple, moldy, slate and defective beans and a low rate of brown beans: these beans were of very poor quality. Purple beans are due to insufficient fermentation, moldy beans are due to poor drying and storage, and slate beans are due to lack of fermentation. In both localities, numerous producers do not respect good practices related to the harvest and post-harvest treatment of cocoa with a negative's effects on the marketable quality of the beans. Moldy beans reveal visible fungal growths, characteristic of poor drying, poor storage and even physicochemical characteristics of the cocoa.

It has been showed that fermentation techniques including time and containers could affect the fermentation step and particularly the aspect of the cocoa beans. For example, Tagro et al. [4] observed that cocoa fermented in plastic box showed the higher percentage of purple beans ranged from 33% to 45% for 4 and 5 days of fermentation than those fermented for 6 days. In contrast, the same authors reported a significant decrease from 9.33 to 7.17% of defectives beans and from 12.33 to 2.17% of purple beans for duration from 4 to 6 days of fermentation in wooden box. Researchers mentioned that the presence of certain species of molds could promote the presence of mycotoxins which are metabolites harmful to health. Therefore, their absence would reflect a good fermentation and drying of beans.

Table 1. Distribution of producers according to fermentation practices of fresh cocoa beans

Fermentation practices	Terms and conditions	Frequency (%)
Cultivated varieties	*Criollo*	36.1
	Trinitario	34.5
	Forastero	29.4
Respect of the fermentation conditions	Yes	0.0
associated to the varieties	No	100.0
Fermentation technic	Fermentation in wooden box	47.5
	Fermentation in heaps	32.5
	Fermentation in plastic bag	20.0
turning frequency	48 h	87.2
	24 h	12.8
	96 h	0
Fermentation time	6 days	95.0
	8 days	2.5
	4 days	2.5

Table 2. Distribution of cotyledons colors of fermented and dried cocoa beans

Localities	Brown beans (%)	Purple beans (%)	Moldy beans (%)	Slate beans (%)	Defective beans (%)	(%) Grade
Biyeyem	2.3	4.38	2.66	85.5	5.16	OG
	91.22	3.20	2.45	1.51	1.62	G1
Bityli	2.5	11.83	3.05	78.57	4.05	OG
	74.5	8.72	4.57	5.47	6.74	G2
	93.44	3.41	1.41	0	1.74	G1
Nkoemvone	94.53	2.41	1.22	0	1.84	G1
Melane	95.45	3.05	1.50	0	0	G1
	81.49	9.63	3.14	0	5.74	G2
Metipkwale	79.59	8.78	3.57	3.21	4.85	G2
References	≤ 80%	≥7%	≥3%	≥3%	≥3%	G1
	≤ 75%	<7%	≥4%	≥8%	≥6%	G2

G1 and G2: Grade 1 and 2 respectively; OG: Out of grade

In general, the distribution of samples according to their marketable quality and locality show that quality was not closely associated with a specific variety of cocoa but with the production locality with a maximum of Grade 1 samples obtained in the locality of Nko'emvone (53.3%), followed by Meleng and Metipkwale (60.0%) and finally Biyenyen (46.7%). This could be due to the conduction of the fermentation process in these two localities. Concerning the variations observed among these localities, it is well documented that the heap size, pod storage after harvest, fermentation time, number and time of turning during fermentation affect the quality of the fermented cocoa beans [1]. Lagunes et al. [6] also reported that a greater aeration of the mass due to the disappearance of the mucilage enables acetic acid bacteria to develop and intervene. Therefore, the high percentage observed from the Nko'emvone (53.3%), and Meleng and Metipkwale localities (Fig. 2) could be explained by the high percentage of producers respecting the post-harvest treatment techniques itinerary. In contrast, beans of poor marketable quality (out of grade) were mostly found in Biyenyen (46.7%).

Samples of superior quality (Grade 1) were selected randomly and representatively from producers in different localities for determination of impurity rate, graining, moisture content and data are resumed in Table 3. The impurity rate, water content and graining values were respectively range from 2.5 to 3.75 %, from 5.1 to 8.6 g/100 g DM and from 95.1 to 101.4 g/100 cocoa beans. Concerning weight of beans, our results are not in consistent with those reported by Niemenak et al. [1]. We note that all localities showed weight lower than 1.05 g which represents minimum value acceptable by chocolate makers [1]. According to these workers, beans less than 1.05 g have high ratio testa on cotyledons and cannot be shelled easily.

Concerning the water content of the cocoa bean samples, the authors mentioned that good cocoa should have a water content less than 8% in order to limit fungal development and cause changes in the nutritional composition of the beans [4]. These different quality criteria are influenced by the fermentation stage which is very important.

3.3 Microbiological Characteristics of Fermented Fresh Cocoa Beans

The microbiological characteristics of fresh fermented cocoa beans according to producers and localities were evaluated and the data are shown in Table 4. We noted that the microbial load varies with the locality, the microorganism founded and even the fermentation method with values (in CFU/g) of 1.3×10^4 to 9.3×10^6 for yeasts, 1.2×10^4 to 6.5×10^6 for acetic bacteria and 1.7×10^5 to 9.9×10^9 for lactic acid bacteria. For acetic bacteria, there was no variation with the locality studied and fermentation method. On the other hand, for yeast and lactic acid bacteria, the highest values were noted in the Nkoe'mvone localities with microbial loads reaching 10^7 and 10^9 for these two germs respectively. Regarding the fermentation mode, the highest values were recorded in the wooden box and heap fermentations.

The quality of commercial cocoa beans, the principal raw material for chocolate production, relies on the association of several factors that include the type of planting material, the agricultural practices, and the post-harvest processing [2,4]. Among these, the fermentation of the cocoa beans is still the most relevant since it is the process whereby the precursors of the cocoa flavor arise [2]. In the present study, three main microorganisms such as yeast, lactic acid and acetic bacteria were found and enumerate in the fresh fermented cocoa beans. These microbial groups have been described in the literature as agents of cocoa fermentation [2,4–6, 17]. In this study, it was found that the microbial load of acetic bacteria remained relatively lower compared to other microorganism present in the medium during cocoa fermentation. Other researchers reported that a successful fermentation requires a succession of indigenous yeasts (i.e., *Saccharomyces, Pichia, Hanseniaspora, Issatchenkia*, etc.), lactic acid bacteria (i.e., *Limosilactobacillus fermentum, Lactiplantibacillus plantarum*, etc.) and acetic acid bacteria (i.e., *Acetobacter, Gluconoacetobacter*) respectively [2,4–6]. On the other hand, fermentation in wooden box and heaps possessed the higher microbial loads including yeast, lactic acid and acetic bacteria regardless the locality. These results are in agreement with those of the literature [4]. This could be explained by the fact that crate fermentation allows for easy mixing and handling, increasing the contact surfaces of the microorganisms with the energetic substrate and also allowing for a high yield of brown beans [14]. However, these values are lower than those found by Bang et al. [18] who showed microbial loads of lactic acid bacteria in crate, tarp and heap fermentations of 2.10^9; 9.10^{10} and 1.10^8

CFU/g respectively. Similarly, the fungal loads obtained were lower than those of the same authors. These differences would be due to climatic conditions, microorganisms involved, physico-chemical parameters (pH and temperature) of the fermenting environment, the biochemical composition of the cocoa (pulp and beans) and the cocoa variety used [4–6]. Physicochemical and biochemical factors of fermentation are also related to the harvest period, the degree of maturity of the pods, the quantity and quality of the pulp [3].

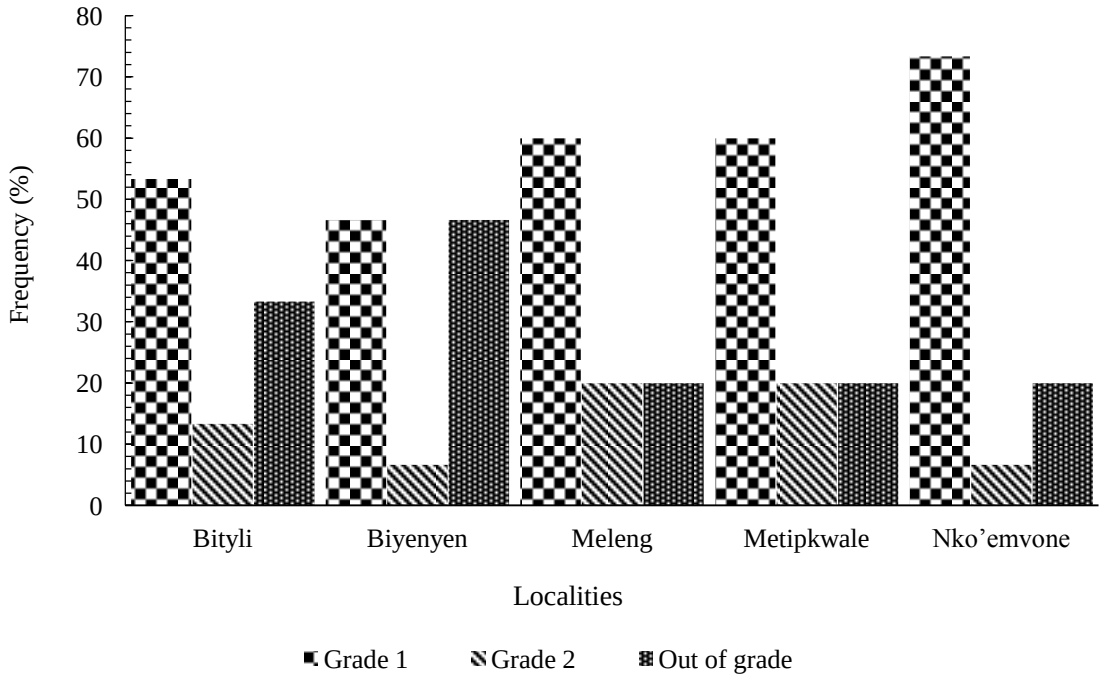

Fig. 2. Distribution (in %) of cocoa grades in the different production localities of the Mvila Department

Table 3. Physico-chemical quality of market cocoa beans according to production areas

Locations	Impurity rate (%)	Water content (g/100 g DM)	Graining (g)
Bityli	2.62 ± 0.04	8.57 ± 0.55	97.41 ± 2.12
Biyenyen	3.71 ± 2.0	7.42 ± 0.23	97.07 ± 2.39
Melenane	3.50 ± 1.15	5.09 ± 1.31	98.12 ± 1.75
Metipkwale	3.75 ± 2.23	7.67 ± 0.08	97.14 ± 3.07
Nko'emvone	2.50 ± 1.82	7.47 ± 0.16	98.75 ± 2.58

DM: Dry matter

Table 4. Microbial load (CFU /g) of different fresh fermented beans

Locations	Sources	Yeast	Acetic bacteria	Lactic acid bacteria
Bityli	Wooden box	$1.4 - 5.6 \times 10^6$	$1.9 - 4.6 \times 10^5$	$1.2 - 5.9 \times 10^7$
	Heaps	$1.3 – 7.2 \times 10^4$	$1.2 – 2.2 \times 10^5$	$1.8 – 5.3 \times 10^5$
Biyeyem	Heaps	$2.3 - 4.5 \times 10^6$	$2.1 - 5.8 \times 10^5$	$1.7 - 4.4 \times 10^8$
	Bag	$1.7 - 6.4 \times 10^5$	$1.2 - 2.7 \times 10^5$	$4.2 - 6.1 \times 10^6$
Melane	Bag	$3.1 - 5.4 \times 10^6$	$2.1 - 4.1 \times 10^5$	$1.1 - 4.2 \times 10^8$
	Wooden box	$1.8 - 3.3 \times 10^5$	$1.2 - 6.1 \times 10^5$	$1.7 - 4.4 \times 10^5$
Nko'emvone	Wooden box	$6.6 - 9.3 \times 10^6$	$1.9 - 2.6 \times 10^5$	$5.1 - 9.9 \times 10^9$
Metipkwale	Heaps	$3.5 - 1.8 \times 10^4$	$2.6 - 3.9 \times 10^5$	$2.8 - 3.3 \times 10^6$

Globally, the low microbial loads were observed from cocoa fermented in a plastic bag regardless the germs studied. This observation has also been mentioned by other authors [4]. This could be explained by the influence against to the germination of fungal spores and bacteria induced by the plastic nature of material of plastic box. Plastic containers could be presented as a limiting microbial growth material against the microbial proliferation during this fermentation. Nevertheless, the highest microbial contamination level measured in cocoa beans fermented in heaps using banana leaves may be because of the direct contact with the air and the ground. In addition, the biological composition of banana leaves could be considered as an additional substrate for each microbial proliferation.

Concerning yeast, several studies mentioned that the growth and activity of yeast play a major role on the aromatic quality of cocoa through the synthesis of numerous secondary metabolites (i.e., ethanol, carbon dioxide, glycerol as side-products, etc. [3,5,6,10,19]. The development of fungi in all fermented cocoa samples may be explained by the sweet mucilage indicated by high concentration of carbohydrates surroundings of cocoa seeds and the initial acidic pH that were highly conducive to filamentous fungi growth [4]. Therefore, the increase in pH of fermented cocoa was because of the decrease in pulp citric acid concentration about 55% and to a migration of ethanol, lactic acid, acetic acid, 2,3-butanediol acetate, 2-phenyl ethanoate and ethyl dodecanoate and other many organic acids produced by microbial activities from the outside to the inside of cocoa seeds [4,6,7].

Indeed, Jamili et al. [19] established that the addition of a yeast inoculum in the fermentation could improve the quality of cocoa beans compared to non-inoculated beans. Similarly, the Cut test revealed that beans fermented without yeast were purple in color, not entirely brown, and the chocolate prepared from these beans was more acidic and lacked characteristic chocolate flavors. Acetic bacteria species oxidize the ethanol produced by the yeasts into acetic acid and the lactic acid produced by the LAB into acetic acid and acetoin; subsequently, the acetic acid is overoxidized into carbon dioxide and water [5]. For this purpose, 13 yeast isolates were selected and their ability to produce ethanol after 48 hours of incubation was evaluated and the data showed that 7 strains were positive with high values obtained with strains Y2, Y3, Y81 and Y9 (Fig. 3). Our findings are similar to those of Vuyst and Weckx [5] reporting that the production of CO_2 by yeast was maximal after 2 days of fermentation, followed by a progressive decrease in production due to the presence of acetic bacteria converting the organics released.

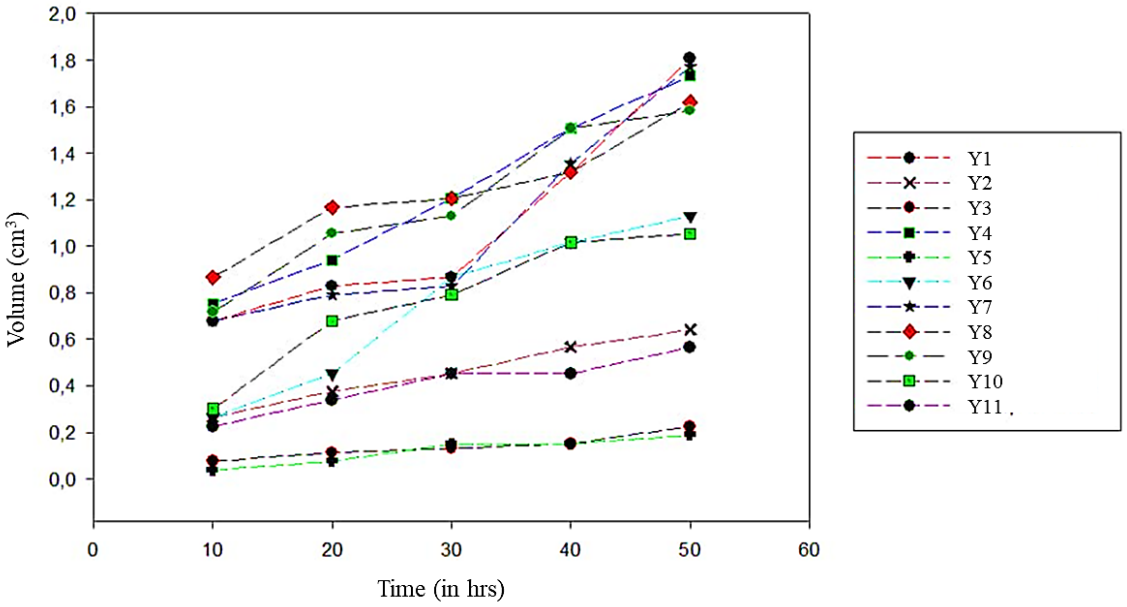

Fig. 3. Ethanol production capacity of isolated yeasts

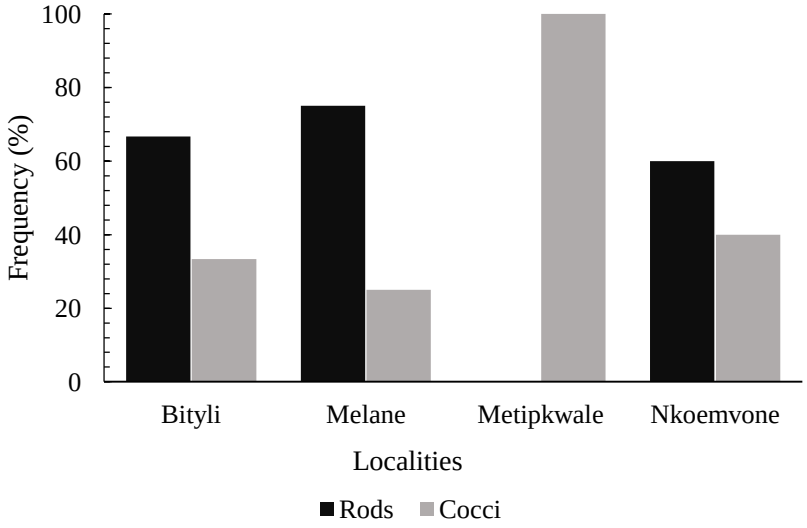

Fig. 4. Morphological characteristics of isolated lactic acid bacteria

Concerning LAB, 12 isolates were selected in a representative and random way, globally, they were all homofermentative. For cell morphology, Fig. 4 shows that rod-shaped bacteria were mostly isolated regardless of the locality except in the locality of Metipkwale where only round-shaped bacteria were obtained. These different tests suggest that these different isolates would belong to the genus *Streptococcus, Lactococcus, Pediococcus, Leuconostoc* and *Lactobacillus* [2,7].

4. CONCLUSION

Wooden boxes, banana leaves and plastic bags are the main tools used by producers in the Mvila department for the fermentation of mixtures of the *Forastero, Criollo* and *Trinitario* varieties of beans. These natural fermentation practices, which take place from 4 to 8 days is due to the presence of microorganisms including yeasts, acetic and lactic acid bacteria. They are responsible for a range of commercial quality (i.e., grades, impurities, graining) of cocoa beans of this area. It therefore judicious to make molecular identification of these strains and to use them as a starter for the controlled fermentation of cocoa.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding authors on request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Niemenak N, Ariel J, Effa P. Physical and chemical assessment quality of cocoa beans in south and center regions of Cameroon. SYLLABUS Rev. Sci. Ser. 2014;5:27–33.
2. Lima LJR, et al. *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;8398. DOI: 10.1080/10408391003799913.
3. Afoakwa EO, Quao J, Takrama J, Budu AS, Firibu KS. Chemical composition and physical quality characteristics of

Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. J. Food Sci. Technol. No. ICCO 2009; 2011.
DOI: 10.1007/s13197-011-0446-5.

4. Tagro G, Dadie T, Koffi KPB, Ban-koffi L, Kedjebo KD. Performance of different fermentation methods and the effect of their duration on the quality of raw cocoa beans. Int. J. Food Sci. Technol. 2010;45: 2508–2514.
DOI: 10.1111/j.1365-2621.2010.02424.x.
5. De Vuyst L, Weckx S. The cocoa bean fermentation process: From ecosystem analysis to starter culture development. J. Appl. Microbiol. 2016;121:5–17.
DOI: 10.1111/jam.13045.
6. Lagunes S, Loiseau G, Luis J, Barel M, Guiraud J. Study on the microflora and biochemistry of cocoa fermentation in the Dominican Republic. Int. J. Food Microbiol. 2007;114:124–130.
DOI: 10.1016/j.ijfoodmicro.2006.10.041.
7. Ozturk G, Young GM. Food evolution: The Impact of Society and Science on the fermentation of cocoa beans. Comprehensive Reviews in Food Science and Food Safety. 2017;16:431–455.
DOI: 10.1111/1541-4337.12264.
8. Kouakou BJ, Irie BZ, Dick E, Goran KEN, Kouame B. Characterization of cocoa (*Theobroma cacao* L.) fermentation techniques in main production areas in Côte d'Ivoire. Int. J. Innov. Appl. Stud. 2018;24(2):728–735.
9. León-Roque N, Abderrahim M, Nuñez-Alejos L, Arribas SM, Condezo-Hoyos L. Talanta Prediction of fermentation index of cocoa beans (*Theobroma cacao* L.) based on color measurement and artificial neural networks. Talanta. 2016;161:31–39.
DOI: 10.1016/j.talanta.2016.08.022.
10. Afoakwa EO, Takrama J, Budu A. Effects of pulp preconditioning on total polyphenols, o-diphenols and anthocyanin concentrations during fermentation and drying of cocoa (*Theobroma cacao*) beans. J. Food Sci. Eng. 2013;3(3):235–245.
11. CICC, Conseil Interprofessionnel du Cacao et du Café "Rapport annuel des activités," Cameroun; 2017.
12. Saïdou C, Tchemtchoua E, Mahama A, Mohammadou BA, Abolo D, Ali A, Njintang NN. Post-harvest system and quality of cocoa beans in the Southern Region of Cameroon. Eur. J. Nutr. Food Saf. 2021; 13(12):1–17.
DOI: 10.9734/ejnfs/2021/v13i1230466.
13. A.F. de N. AFNOR. Recueil des normes françaises des produits dérivés des fruits et légumes, jus de fruits. Receuil de l'AFNOR, Vol. 1ère Editi. 1982; 327.
14. Barel M. Impact du traitement post-récolte. Edition Quea Page; 2013.
15. Guiraud JP. Microbiologie alimentaire, RIA. Paris; 2003.
16. De Man JC, Rogosa M, Sharpe ME. A medium used for the cultivation of Lactobacilli. J Appl Bacteriol. 1960;23: 130–135.
17. Dadie T, Koffi KPB, Ban-Koffi L, Kedjebo KD, Guehi TS. Original article Performance of different fermentation methods and the effect of their duration on the quality of raw cocoa beans. Int. J. Food Sci. Technol. 2010;45:2508–2514.
DOI: 10.1111/j.1365-2621.2010.02424.x.
18. Bang KL, Ouattara H, Karou TG, Guehi ST, Nemlin JG, Diopoh JK. Impacts de la fermentation du cacao sur la croissance de la flore microbienne et la qualite des feves marchandes. Agron. Africaine. 2013;25(2): 159–170.
19. Jamili, Yanti NA, Susilowati PE. Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia. Biodiversitas. 2016; 17(1):90–95.
DOI: 10.13057/biodiv/d170113.

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