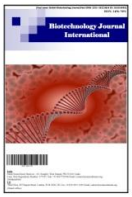

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Technical Sheet of the Preparation of Traditional Cassava Starters Used for *Attieke* Production in Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BZBIA, BKJP, KKA, CWH and KAC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RKN and DKM managed the analyses of the study. Author BZBIA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study was to identify the different methods of preparing the traditional cassava starters used for attiéké production in Côte d'Ivoire, allowed the recounting of five different production methods.
Study Design: Sampling of cassava starters were collected from attiéké producers de four areas of south of Côte d'Ivoire. The cassava starters obtained with the braised cassava were collected from attiéké women producers of Grand-Lahou, those obtained directly from fresh cassava were collected from attiéké producers in the region of Bonoua, and those obtained with the cassava cooked at water were collected from attiéké women producers of Abidjan and Jacqueville. Place and Duration of Study: University of Nangui Abrogoua, Abidjan, Côte d'Ivoire (between March 2018 and June 2019).

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Methodology: Traditional cassava starters are produced with the bitter or sweet variety of cassava, either freshly preserved without cooking until fermented, boiled in boiling water or braised over a wood fire.
Conclusion: This study highlighted five methods of preparing traditional cassava starters used in Côte d'Ivoire for the preparation of attiéké, a food derived from cassava. This is the starters from fresh preserved uncooked cassava that derived from fresh braised cassava and those (three) from fresh cassava cooked with boiling water either with the casing or without the casing after cooking and without the shell during cooking.

Keywords: Starters; cassava; fermentation; Côte d'Ivoire; attiéké.

1. INTRODUCTION

Cassava is one of the world's most important food crops, with an annual world production of about 277.957 million tonnes in 2017 (Mt) [1]. In Côte d'Ivoire, cassava is the second most important food crop after yams and before plantains. There are two types of cassava: sweet cassava, consumed as a porridge or paste (placali) and bitter cassava used to make attiéké. Several products derived from manioc are marketed, including gari, attiéké, cossettes, starch, tapioca, fufu, raw flour and fermented dough [2]. In Côte d'Ivoire, attiéké is a food product that originates from the tradition of the lagoon people [3] and it is the principal shape of the transformed of cassava [4].

Attiéké is the main product of bitter cassava processing in Côte d'Ivoire. Initially consumed in the south of the country attiéké is nowadays produced and consumed throughout the Ivorian territory, and in the West African sub-region [5-6]. Attiéké is the product obtained after peeling, cutting, washing, crushing, fermenting, pressing, sieving, drying, winnowing and steaming of fresh cassava roots (*Manihot esculenta* Crantz) [7].

The production of attiéké requires the use of a traditional cassava starters [8-9]. The rate of ferment incorporated into the fermenting paste varies between 4 and 10% of the total mass of peeled roots [10-11]. The fermentation time of this dough varies from 15 to 24 hours or 24 to 48 hours respectively according to [3,12]. It is during fermentation that the organoleptic characteristics of attiéké develop.

The organoleptic characteristics of attiéké depend not only on the variety of cassava, whether it is bitter (rich in hydrogen cyanide) or sweet (practically free of hydrogen cyanide) used for its production [13] but also and especially, the type of traditional cassava starters used for the fermentation of the crushed cassava dough. These types of traditional cassava starters incorporated into cassava dough for attiéké production influences the final product quality [3]. Indeed, there are different types of traditional cassava starters according to their origin. Commonly three types of traditional cassava starters (i.e. fresh with the peel, braised with the peel and boiled without the peel) are used in Côte d'Ivoire for the preparation of attiéké [14,15]. These different types of cassava starters used for the preparation of attiéké have been the subject of several studies of cassava starters [16,17,18], but no technical sheet concerning the establishment and the regrouping of their technique of use has not yet been made. It is in this perspective that this study was initiated to identify and enhance the techniques of preparation of different traditional cassava starters.

2. MATERIALS AND METHODS

2.1 Materials

Traditional cassava starters were collected from attiéké producers in four cities of Côte d'Ivoire. The *Alladjan* starters locally called braised cassava starters collected among women producers of attiéké and traditional cassava starters of Grand-Lahou. The *Abouré* starters locally called raw cassava starters collected among women producers of attiéké and traditional cassava starters of Bonoua. the *Ebrié* starters and *Avikam* starters collected among women producers of attiéké and traditional cassava starters respectively of Abidjan and Jacqueville.

2.2 Methods

2.2.1 Methods of preparation of traditional starters obtained from braised cassava

Fresh cassava roots are braised on charcoal over low heat for 10 to 20 minutes. After

cooking and cooling on the free air during 30 min to 1 hour, the roots of cooked cassava are preserved in food bags or plastic bags to ambient temperature (28 to 32°C) for the fermentation. At the end of 48 to 72 hours, these roots of fermented cassava are sold on the markets with producing traditional food from the cassava (Fig. 1). This type of traditional cassava starter that was produced in the region of Grand-Lahou is nowadays produced and used everywhere in Côte d'Ivoire.

2.2.2 Methods for the preparation of traditional starters obtained from raw cassava

Fresh cassava roots are washed and cut, then stored in food or plastic bags at ambient temperature (28 to 32°C) for fermentation. At the end of 72 to 96 hours, these roots of fermented cassava are sold on the markets with producing traditional food from the cassava (Fig. 2). This type of traditional cassava starter is produced specifically in the Bonoua region.

2.2.3 Methods of preparation of traditional starters obtained from boiled cassava

The preparation of starters obtained from boiled cassava can be done in three modes: Roots of fresh cassava are washed with clean water, peeled, are cut out and washed, then cooked again on charcoal to fire at the end of 10 to 20 min. After cooking and cooling with the free air during 30 min to 1 hour, the roots of cooked cassava are preserved in food bags or plastic bags to ambient temperature (28 to 32°C) for the fermentation in a store of stock of the equipments of preparation of cassava starters or attiéké. At the end of 72 hours, these roots of fermented cassava are sold on the markets with producing traditional food from the cassava (Fig. 3). This type of traditional cassava starter that was produced by the native peoples of Abidjan and Dabou is nowadays produced and used everywhere in Côte d'Ivoire.

Roots of fresh cassava are washed with clean water and then cooked directly with the casing in a pot containing water on charcoal over low heat after 10 to 20 minutes. After cooking and cooling in the open air, the barks of the roots of cassava either are removed on pulp (Fig. 4) or preserved with cooked pulp (Fig. 5) and stored in jute bags with the ambient temperature (28 to 32°C) for fermentation. At the end of 72 hours, these fermented cassava roots will be washed in basins and cut into small pieces, then added to the cassava paste as it is ground in the mill. These two types of traditional cassava starters are produced specifically in the Jacqueville region.

2.2.4 Isolation of microorganisms from traditional cassava starters

The isolation of fungi was carried out according to the agar dilution method. 10 g from each cassava starter sample, were homogenized with 90 mL of buffer peptone water (AES Laboratory, France) and serial decimal dilutions (10^{-1} to 10^{-6}) were performed. Enumeration of mesophilic aerobic germs (MAG) were done according to AFNOR Standard (NF V08-051, 1999). Plate Count Agar was used for MAG and incubated at 30°C for 24-72 hour. Coliforms were carried out according to AFNOR Standard (NF ISO4832: 2006) applied to the lactose agar biliated with the purple crystal and the neutral red. Numeration of lactic bacteria according to the standard ISO 15214:1998 applied to Man Rogosa Sharpe agar, who were incubated anaerobically for 24 to 48 hour at 32°C in a jar containing a candle. The enumeration of yeasts and moulds was carried out according to the Standard NF (ISO 6611, 2004) applied to the Sabouraud agar to Chloramphenicol and incubated at 30°C for 24 to 72 hour in Petri dishes. *Bacillus* according to the method described by Buttiaux et al. [19] applied to Plate Count Agar containing 1% starch. For preliminary identification, *Bacillus* colonies were isolated and characterized by their morphological properties and appearance. The Petri dishes containing between 15 and 300 colonies were counted in CFU g^{-1}.

2.2.5 Identification of *Bacillus* strains

2.2.5.1 Isolation of chromosomal DNA

Chromosomal DNA was prepared from overnight culture on agar Mossel. Isolation and purification were conducted with a kit (Instagen Matrix Bio-Rad, USA) according to the manufacturer's instructions.

2.2.5.2 16S rDNA amplification

To amplify the 16S rDNA gene, a primer pair (Table 1) hybridizing to two conserved regions was used for the identification of Bacillus strains. PCR mixture consisted of 0.2 mM of each primer (16R1522 and 16F27), 20 µL of 1X Master Mix (5PRIME Hot MasterMix 2,5X DOMINIQUE Dutscher, France), 1 µL of DNA and H2O in a final volume of 50 µL.


Fresh cassava


Cassava braised


Cassava starters

Fig. 1. Starters obtained from braised cassava


Fresh cassava


Diced raw cassava


Raw cassava starters

Fig. 2. Starters obtained from the raw cassava roots with the peel


Fresh cassava


Roots of Fresh cassava peeled


Cassava starters

Fig. 3. Starters obtained from boiled cassava without the peel

Fresh cassava | Boiled cassava with the peel | Cassava starters

Fig. 4. Starters obtained from cassava boiled with the peel and fermented without the peel

Fresh cassava | Boiled cassava with the peel | Cassava starters

Fig. 5. Starters obtained from cassava boiled with the peel and fermented

Table 1. Primers used for gene amplification

Primer name	Tm (°C)	%GC	Oligonucleotide (5'--- 3')
16R27	57,3	50	AGAGTTTGATCCTGGCTCAG
16F1522	44,7	60	AAGGAGGTGATCCAGCCGCA

Amplification conditions consisted of 94°C initial denaturation for 2 min, 35 cycles of 94°C for 1 min, 58°C for 30 seconds (hybridization), 65°C for 2 min (extension) and final extension at 65°C for 7 min before cooling at 4°C in PCR thermocycler 2720 Thermalcycler type (AB Applied Biosystems, Syngapore). Ten (10) µL of the amplified products of PCR were analyzed by electrophoresis in 1% of agarose gel stained with ethidium bromide.

2.2.5.3 Sequencing and phylogenetic analysis

PCR products were purified and quantified by electrophoresis in 08% (w/v) agarose gel. The 16S rDNA obtained was sent to the sequencing

platform of Cochin Eurofins MW operon (France). DNA sequences were determined by chain-termination method [20] using automatic ABI 3730Xl sequencing kit 96 capillary DNA Analyzers. The sequences obtained were compared with sequences in the database of National Center for Biotechnology Information using the BLAST program and its taxonomic browser server (http://www.ncbi.nlm.nih.gov/blast) was helped to find the affiliation of strains

2.2.6 Enzymatic activities

Enzymatic activity of *Bacillus* strains was performed using API-ZYM system according to the manufacturer's instructions. The results were analyzed with a reading table of API-ZYM system.

3. RESULTS AND DISCUSSION

The load of the GAM (9.52 log (UFC/g)) most significant in the three types of cassava starters, was followed that of *Bacillus* (6.54 log (UFC/g)), of the lactic bacteria and the total coliforms with similar loads of 5,93 log (UFC/g).The smallest load (2.01 log (UFC/g)) was obtained with yeasts and moulds (Table 2).

Bacillus subtilis was the most identified species with a frequency of 30% followed by *B. amyloliquefaciens* (18%) and *B. methylotrophicus* (10%) (Fig. 6). The frequency of identification of *B. toyonensis* and *B. cereus* was 6% each. *B. vallismortis* and *B. pumilus* species with a frequency of 4% were the least identified. The frequency of unidentified species (*Bacillus spp*) was 22%.

Table 2. Microbial loads Isolated from traditional cassava starters

	Microbial loads (log10 UFC/g)					
Type of starters	**MAG**	**Lactic bacteria**	**total Coliforms**	**Thermotolerance Coliforms**	***Bacillus***	**Yeasts and Mouds**
Bolied	7,44	5,8	5,8	4,12	6,54	2,22
Raw	8,9	5,93	5,93	4,7	6,33	3,33
Braised	9,52	5,76	5,76	5,2	5,67	2,01

MAG: Mesophilic aerobic germs

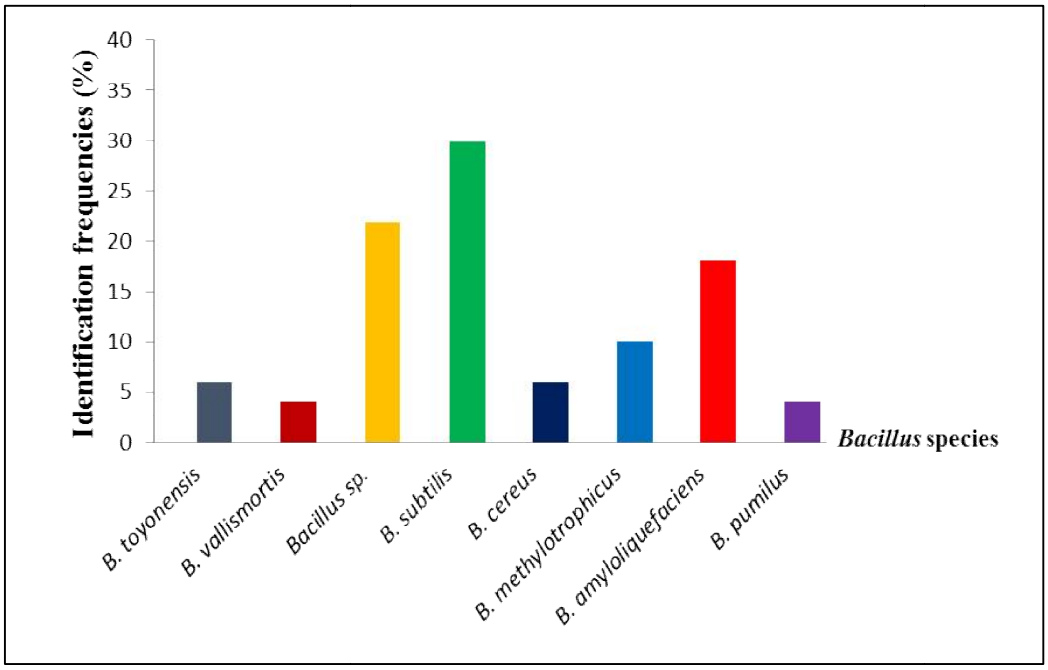

Fig. 6. Isolation frequencies of Bacillus species from traditional cassava starter

Table 3. Enzymes produced by *Bacillus* species isolated from traditional cassava starters

	Number and frequency of Bacillus strains [N(F)]			
Enzymes	***Ebrié* starter**	***Alladjan* starter**	***Abouré* starter**	**Total**
PAL	28(56)	10(20)	3(6)	41(82)
EST	35(70)	12(24)	3(6)	50(100)
ESTLIP	31(62)	12(24)	2(4)	45(90)
LIP	5(10)	2(4)	0(0)	7(14)
LEUAR	19(38)	8(16)	3(6)	30(60)
VALAR	6(12)	3(6)	0(0)	9(18)
CYSAR	6(12)	3(6)	0(0)	9(18)
TRYP	3(6)	2(4)	0(0)	5(10)
α-CHY	7(14)	1(2)	0(0)	8(16)
PAC	25(50)	6(12)	2(4)	33(66)
NAPP	26(52)	10(20)	2(4)	38(76)
α-GAL	9(18)	3(6)	0(0)	12(24)
β-GAL	12(24)	5(10)	0(0)	17(34)
β-GLUC	5(10)	1(2)	0(0)	6(12)
α-GLU	35(70)	12(24)	3(6)	50(100)
β-GLU	31(62)	11(22)	3(6)	45(90)
NAβG	2(4)	1(2)	0(0)	3(6)

Enzymes : PAL: Phosphatase alcaline ; EST: Estérase (C4) ; ESTLIP : Estérase lipase (C8) ; LIP: Lipase (C14) ; LEUAR : Leucine arylamidase ; VALAR : Valine arylamidase ; CYSAR : Cystine arylamidase ; TRYP : Trypsine ; α-CHY: Alpha- Chymotrypsine ; PAC : Phosphatase acide ; NAPP : Naphtol phosphohydrolase ; α-GAL : Alpha-galactosidase (mélibiase) ; β-GAL: Béta-galactosidase (lactase) β-GLUC : Béta-glucuronidase (hyaluronidase) ; α-GLU : Alpha-glucosidase (maltase) ; β-GLU : Béta-glucosidase (cellulase) ; NAβG : N-acétyl-béta-glucosaminidase (chitinase) ; N: Number; F: Frequency.

Table 3 indicates the enzymes produced by the *Bacillus* species isolated from traditional cassava starter. Among the enzymes sought, seventeen enzymes were produced by at least one or more *Bacillus* species whereas two enzymes, esterase (C4) and alpha- glucosidase, were produced by all *Bacillus* species isolated. A high percentage (90%) of *Bacillus* isolated, produced beta-glucosidase and esterase lipase, 82% produced alkaline phosphatase and 76% produced naptol phosphohydrolase

4. DISCUSSION

This study results showed that the traditional cassava stater was colonized by a variety of fermentative and pathogenic microorganisms. It is about Mesophilic aerobic germs, *Bacillus*, lactic bacteria, total coliform, thermotolerance coliform, yeasts and moulds. The presence of these microorganisms in traditional cassava starter was reported by several studies [18-21]. The results of our study corroborate those of Djeni et al. [22] who found higher loads of these same germs in cassava starters from the Ebrié, Adjoukrou and Alladjan ethnic groups. The presence of the germs, in particular *Bacillus*, lactic bacteria, yeasts and moulds is very important because they take part in the fermentation of cassava. The species of *Bacillus*, lactic bacteria, yeasts are very important because they produce different enzymes which contribute to the softness and detoxification of cassava dough [23-24].

Seven species of *Bacilllus* have been identified from traditional starter of cassava analyzed, namely B. toyonensis, B. vallismortis, B. subtilis, B. cereus, B. methylotrophicus, B. amyloliquefaciens and B. pumilus. These species differ from those reported by Assanvo et al. [25] and Ehon et al. [26] in their study based on the microflora of the traditional starter of cassava for the production of attiéké. Indeed, these authors reported three species of Bacillus, namely B. sphaericus, B. brevis and B. coangulans. Contrary to these authors, the species identified in this study have been reported by other authors during the fermentation of cassava roots. B. subtilis, B. cereus, B. pumilus and B. amyloliquefaciens were identified by Amoa-Awua and Jakobsen [27] during the process of the cassava roots fermentation [13] identified the presence of B. amyloliquefaciens and B. cereus from Adjoukrou traditional cassava starter. Bacillus species can produce α-amylase which can hydrolyze starch and release the sugars for the production of organic acids mainly lactic acid.

But, the capacity to produce lactic acid was also observed for *Bacillus* strains in fermentation medium [28-29]. The lactic acid production by Bacillus spp. would be useful during of the fermentation of cassava dough, because the of the acidification step which determines the final acidulous taste of attiéké [26]. B. subtilis (30%) was the important isolate among the seven species identified in this study. This result corroborates those of Adewumi et al. [30] and Azokpota et al. [31] during the production of gari. Bacillus are found to produce some products such as vitamin B3, B12, K and digestive enzymes such as amylase, protease and lipase [8]. Bacillus species have shown overall a good α-glucosidase (100%) and β-glucosidase (94%) synthesis ability. β-glucosidase is the main enzyme responsible for the natural degradation of the cyanogenetic glucosides of cassava into glucose and acetone cyanohydrin (Djoulde et al., 2005). According to Mkpong et al. (1990), 65% of homology was observed between β-glucosidase activity and that of linamarase. Concerning α-glucosidase, it is involved in the hydrolysis of isomaltose resulting from the degradation of starch by α- amylase.

4. CONCLUSION

In this study, there are five methods of preparing traditional cassava starters used in Côte d'Ivoire for the preparation of attiéké. This is the starters from cassava braised, starters from raw cassava roots with the peel, starters from cassava boiled without the peel, starters obtained from cassava boiled with the peel and fermented without the peel and starters obtained from cassava boiled with the peel and fermented. Six microorganisms were isolated in the cassava starter studied among which only Bacillus, lactic bacteria, yeasts and molds really participate in the fermentation of cassava. The enzymatic capacity of Bacillus were studied and seven species of Bacillus (*Bacillus subtilis, Bacillus cereus, Bacillus pumilus, Bacillus amyloliquefaciens, Bacillus methylotrophicus, Bacillus vallismortis and Bacillus toyonensis*) were identified. *Bacillus subtilis* was the most present among the seven species. Several species of *Bacillus* have shown a capacity to produce various enzymes notably osidases, phosphatases, lipases and proteases. Among the osidases, β-glucosidase and α-glucosidase were the most produced by the species. All species were able to produce esterase (C4) and alpha-glucosidase enzymes. It should also be noted that some species have been able to produce up to 15 enzymes.

In perspective, this study was conducted on the three types of cassava ferments, in particular cassava starters from the *Ebrié, Abouré* and *Alladjan* (respectively starters from cassava braised, starters from raw cassava roots with the peel and starters from cassava boiled without the peel). It will be more extensive on the two other types of cassava starters i.e. starters obtained from cassava boiled with the peel and fermented without the peel and starters obtained from cassava boiled with the peel and fermented that have never been the subject of scientific study. Also, it will be a comparative study on the probiotic activities of bacillus species that have not yet been explored.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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