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Technological, biochemical and microbiological characterization of fermented cassava dough use to produce cassava stick, a Gabonese traditional food

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Abstract

Cassava stick is a fermented food made with cassava roots and widely consumed by Gabonese populations. It manufacturing processes are made traditionally, are not often mastered and then affect the product quality. This study aims to determine various manufacturing processes of Gabonese cassava stick and appreciate physicochemical and microbiological aspects of the dough used for production. A survey was carried out among ten producers and fifteen samples were collected for the analysis. The physicochemical and microbiological parameters were determined using standards methods. Bacillus strains have been isolated, characterized biochemically and identified by PCR technique. The investigated parameters values varied from producer to another. The average values of pH, dry matter content and ash content of collected samples were respectively 4.23±0.28; 47.32±2.66 % (w/w) and 1.19±0.49 % (w/w). The total proteins and carbohydrates content were respectively 1.39±0.32 and 90.41±4.03 % (w/w) dry matter. The lipids were detected as traces in all samples. Microbiological analysis of fermented cassava dough showed average values of TMAF, Bacillus, LAB and yeast of 6.82±0.51, 5.34±0.35, 5.84±1.06 and 3.95±0.97 log CFU/g of dough respectively. The samples were not contaminated by Salmonella or Shighella. Bacillus subtilis strains known for theirs bacteriocins production were isolated on fermented cassava dough and could have an important role in spontaneous cassava fermentations considering theirs biochemical properties.

Keywords: *Microbial, technological, biochemical, characterization, fermented cassava, stick, Gabon*

Introduction

Cassava root is one of the most important food components in many African communities. Indeed, a variety of cassava-based products have been developing. The more popular of them are *agbélimawé, attiéké* and *gari* in West Africa, *nshima* in East Africa and *chikwangue* Central Africa (E. T. Agbor *et al.*, 1995; G. Amani *et al.*, 2007). Unfortunately, these cassavas products are processed in arduous conditions through a strenuous process relying on the producer experience, which could affect their quality.

Cassava stick is the most popular local food in Gabon resulting from the fermentation of tuberous cassava roots (PRASAC, 2013). It is commonly known in Gabon as *mouyondo* (for *Adouma* ethnic group), *ovondoh* (for *Benga* ethnic group), *mboung* (for*Fang* ethnic group), *mughume* (for *Massango* ethnic group), *mpita* (for *Nzebi* ethnic group), *mulembu* (for *Punu* ethnic group), *mopiti* (for *Tsogho* ethnic group), etc. Similar products are also found in other Central Africa communities. Cassava stick is known as *chikwangue* in Republic of Congo, *miondo*, *mitumba* or *bobolo* in Cameroon, *mubangui* in Democratic Republic of the Congo and *mungbélé* in Central Africa Republic and in Chad (E. T. Agbor *et al.*, 1995; G. Amani *et al.*, 2007). This food is becoming popular as well it is exported in other parts of the world. In Ouagadougou cassava stick is appreciate by local communities. Unfortunately, its processing technology is characterized by empiric steps which are very difficult to control (M. Sotomey *et al* D. D. Eric-Alain, 2001). The organoleptic characters vary from one ethnic group to another, one producer to another and one production to another. Moreover, the good hygiene practices during the manufacturing process of cassava stick are not often respected.

The process of many cassava products in Africa frequently involves spontaneous fermentation, a very sensitive step. Previous studies have shown large presence of various germs during fermenting cassava dough. Lactic bacteria (LAB), yeast, *Bacillus*, *Enterococcus* and mould appear as dominant microflora (J. Assanvo *et*

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al., 2006; N. T. Djeni *et al*., 2015; F. Guira *et al*., 2016). Thus, the lack of good hygiene practice during cassava stick production constitute a risk factor of food contamination by pathogenic microorganisms such as *Bacillus cereus*, *Staphylococcus aureus*, *Yersinia*, *Shigella*, *Samonella*, *Clostridium* and their toxins.

Up to date, there is few data on Gabonese cassava stick characterization for it valorization. Considering the importance of this food for our populations and the world's growing demand for food and also to avoid or limit food borne diseases, it is crucial to improve its safety and provide standardization of its manufacturing process. The purpose of the present study is to determine the technological, biochemical and microbiological data of cassava dough used to manufacture Gabonese cassava stick.

Material and methods

Sampling

Approximately 250 g of cassava dough used to produce Gabonese cassava stick were collected among different producers of ten (10) ethnic groups in seven (7) localities of Gabon (*Akébé, Akournam, Bitam, Ekouk, Kango, Oyane, Plein-niger*) and 3 districts of Ouagadougou city-Burkina Faso (*Goughin, Kalgondhin, Zone une*). Cassava stick production was followed to establish production diagrams. A total of 15 samples were collected in sterile conditions.

Physico-chemical analysis

The physico-chemical analysis was conducted according to standard methods. Then, the pH values of the samples were measured with an electronic pH meter (CONSORT P901, Belgium) using a mix of 10 g of each sample in 50 mL of distilled water. Total titrable acidity was determined as a percentage of lactic acid equivalents in fresh matter (Eq. lactic acid/100g). Then 10 g of product are mixed with 50 ml of distilled water in an Erlenmeyer and 10 ml of the dilution were then titrated against 0.1 N KOH using phenolphthalein as indicator.

Proximate composition determination

Proximate analysis of samples was conducted using conventional procedures described by the Association of Official Analytical Chemists (AOAC, 1984). Dry matter was determinate by drying at 105±2 °C overnight. Ash content by incineration at 550 °C for 12 h, crude protein by the Kjeldahl method; and crude fat content by Soxhlet extraction using hexane as solvant. Total carbohydrate content was determined by the phenol sulphuric acid method (M. Tollier and J. P. Robin, 1979). The starch content is determined using the colorimetric method (S.C. Jarvis and B. K. Walker, 1993). The energy value was calculated using the method described by A. L. Merrill and B. K. Watt (1955).

Microbial analysis

Except of *Samonella* and *Shighella,* all numerations was done using of 10 g of each sample mixed in 90 ml of sterile diluent (physiological water). Tenfold serial dilution was prepared and spread-plated for microorganisms count. Thus, 1 mL of each dilution was inoculated. For *Samonella* and *Shighella* determination, 25 g of each sample was mixed in 225 ml of sterile buffered peptone water.

Total Aerobic Mesophilic Flora was enumerated on Plate Count Agar (Liofilchem, Italy) incubated at 30°C for 3 days according to ISO 4833 (2003) standard.

Lactic acid bacteria (LAB) were counted on Man Rogosa and Sharpe Agar (Merck, Germany) incubated anaerobically at 37°C, for 3 days according to ISO 15214 (1998) standard.

Yeasts and molds were counted on Sabouraud Chloramphenicol Agar (Liofilchem, Italy) after incubation at 25°C for 4 to 5 days according to ISO 7954 (1988) standard.

Coliforms were enumerated on Violet Red Bile Lactose (VRBL) (Liofichem, Italy), incubated at 37°C for total coliform and 44°C for Fecal Coliform for one day according to ISO 4832 (2006) standard.

Salmonella and *Shighella* were sought on agar Salmonella-Shighella (SS) agar after a pre-enrichment on buffered peptone water (BPW) and enrichment on Rapapport Vassiliadis Soja (RVS) according to standard method.

Bacillus spp. were determined by spreading 0.1 mL of pasteurized dilution series sample on the surface of Plate Count Agar (Liofilchem, Italy) according to S. Kastner *et al.,* (2007). The inoculated plates were incubated at 37 °C for one to two days.

Characterization of Bacillus *sp. isolates*

For characterization and identification of *Bacillus* sp., suspected colonies on Plate Count Agar (PCA) resulting from series of dilution samples were picked. Then pure colonies were obtained after 3 repetitive sowing on Luria Bertani (LB) Agar. The isolates were first selected based on characterization of the colonies form and the cell morphology then Gram test, oxidase activity, catalase activity, production of acetoin (VP) and sporulation for additional identification tests. Then, gaz production from glucose was performed using Kliger Iron Agar (Liofilchem, Italy). Carbohydrate fermentation were determined by the API 20E (API, BioMerieux, France) as described by the manufacturer.

Molecular identification of Bacillus sp. Isolates

Isolate *Bacillus* strains were grown on LB agar at 37°C for 24 h. Then, total cellular DNA was extract according to QIAamp® DNA Mini and Blood Mini Handbook (Third Edition, 2010; QIAgen).

Table I: Primers and PCR products

Species/Strain	Primer Sequence	Target	PCR products	Reference
***Bacillus subtilis* group (*subtilis, licheniformis, amyloliquefaciens, pumilus, atrophaeus*)**	*5'-AAGTCGAGCGGACAGATGG-3'* *5'-CCAGTTTCCAATGACCCTCCCC-3'*	16S rDNA gene	595bp	(Wattiau, 2001)[17]

bp: pairs of bases

DNA amplification was conducted in a DNA thermal cycler TC-412 (Serial No.: 137370-2, UK) in order to determine the species affiliation. A specific primer *Bsub5F/Bsub3R* provided by Eurofins MWG Operon (Germany) was used (P. Wattiau *et al.,* 2001). Then 50 µl as volume was used: 30 µl of Taq PCR Master Mix Kit (QIAgen, France), 4 µl of each specific-primer, 8 µl of sterile water (QIAgen, France) and 4 µl of DNA extract.

The amplification profile was 94 °C for 5 min, 94 °C for 45 sec, 60°C for 45 sec, 72 °C for 1 min and 72 °C for 10 min, 30 cycles.

The presence of PCR products was determined by gel electrophoresis in 1.5 % agarose gel containing ethidium bromide. Electrophoresis in Tris-Borate EDTA was performed at 100 volts, 50 mA for 1 h. Highlight of specific bands was revealed using UV plate, then photographed.

Data processing and statistical analysis

Each sample was analyzed in triplicate. Microbial counts were converted to log cfu mL^{-1}. All the results were subjected to analysis of variance (ANOVA) using SPSS (version 20.0). Means, standard error of means and the least significant difference between the means were determined. The degree of significance was fixed to P = 0.05.

Results and discussion

Cassava stick process

Results from our investigations showed that several processes are used to produce Gabonese cassava stick in communities. Initially cassava roots are processed to obtain crude dough. Then this crude dough is treated to become a ready to cook thick and smooth dough. Finally after conditioning, dough is steam cooked. Cassava stick processes are condensed in figure 1.

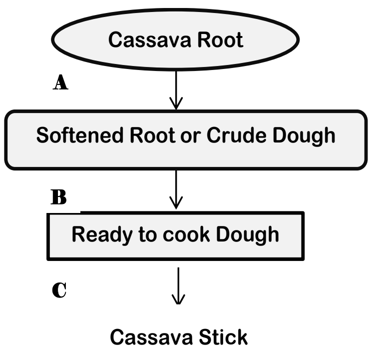


Figure 1: Cassava stick process diagram

Variations of Gabonese cassava stick production steps

A. Three (3) alternatives have been found during the cassava root transformation into crude dough. The cassava root is sometime peeled before be soaking and reciprocally. Cassava root can also be use directly to produce crushed dough without soaked. Figure 2 described crude dough production processes from cassava roots.

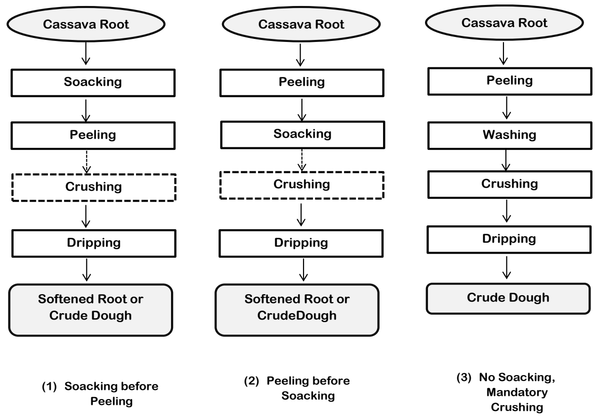


Figure 2: Softened root or Crude dough alternatives producing process

B. Crude dough or softened root is treated in two various ways according to their storage duration. In the large urban areas as Libreville (Gabon), the producers use crude dough from rural area. This crude dough retained for a long time undergoes more processing than non-conserved crude dough as descried in figure 3.

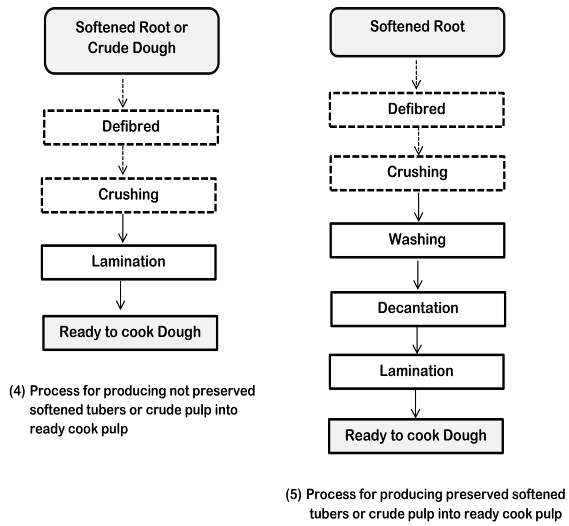


Figure 3 : Ready to cook dough alternatives producing process

C. Two (2) stick cooking processes have been identified. A process with one single cooking and another process with two cooking as outlined in figure 4.

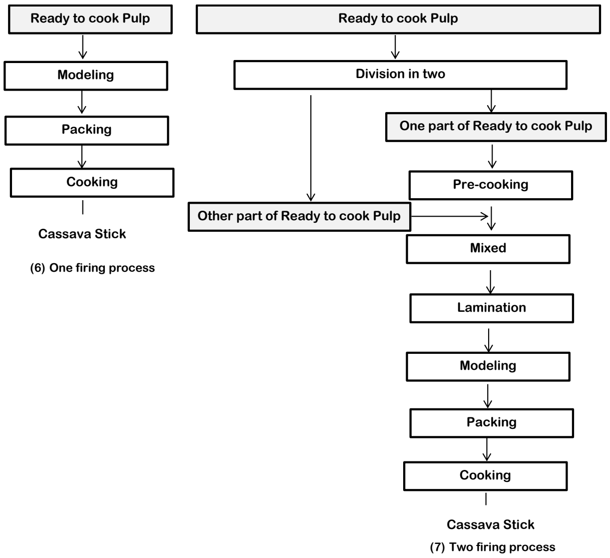


Figure 4 : Cooking process alternatives

In urban areas such as Libreville (Gabon) and Ouagadougou (Burkina Faso), cassava stick producers avoid some operations units deemed as too long and tedious. They use as raw material kept dough in warehouses. In Ouagadougou, all producers do not conduct lamination step because it is difficult, too long and unprofitable.

The varying duration of the spontaneous fermentation time is one of particularities of cassava stick production processes. For the production of *Nzebi* cassava stick also called "*mpita*", the fermentation last for 7 days instead of 3 or 4 days. This very important step of cassava stick production can be done in natural environments such as edges of rivers or ponds (S. C. Kobawila *et al.,* 2005) instead seals and tubs. In remote regions, there is a lack of water supply and the sanitation level is relatively precarious. Households do not have conventional latrines. That is why people opted for defecate in nature. Hence there is a risk for the spontaneous natural fermentation to be contaminated with fecal matter and influence organoleptic characteristics of the finished product.

Obamba cassava stick from lengthily stored dough in warehouses is the most commercialized stick in Libreville. *Obamba* cassava stick and "*macongo*" stick of Fang ethnic group (B. Delpêche, 1995) have the particularity to undergo two cooking process as described in figure (7). The table II shows origin, producer ethnic group, production steps and the use of cassava dough about cassava stick production for each sample.

Table II : Overview of cassava dough preparation characteristics, mode and context

Id	Origin		Ethnic group	Raw material → Variants of Cassava stick production steps	particularity of dough production
	Country	Locality			
E1	Gabon	Plein-niger	Omiènè	Soft yellow roots → (A1) (B4) (C6)	Not soaked roots
E2				Soft white roots → (A1) (B4) (C6)	Not soaked roots
E3		Akournam	Nzebi	Bitter white roots → (A3) (B4) (C6)	-
E4				Bitter white roots → (A3) (B5) (C6)	7 days of roots steeping
E5		Akébé	Obamba	Roots→Retained crude dough→(B5)(C7)	Pre-cooking followed by mixing with other uncooked dough
E6				Roots → Retained crude dough	Unprocessed retained crude dough
E7		Kango	Punu	Bitter white roots → (A2) (B4) (C6)	-
E8		Ekouk		Soft yellow roots → (A2) (B4) (C6)	-
E9				Soft white roots → (A2) (B4) (C6)	-
E10		Oyane	Fang	Soft white roots → (A2) (B4) (C6)	-
E11		Aniezock		Bitter white roots → (A2) (B4) (C7)	Pre-cooking followed by mixing with other uncooked dough
E12				Bitter white roots → (A2) (B4) (C6)	-
E13	Burkina Faso	Goughin		Bitter white roots → (A2) (B4) (C6)	Not lamination of dough
E14		Kalgondé		Roots→Retained crude dough→(B4)(C6)	Not lamination of dough
E15		Zone une	Téké	Roots→Retained crude dough→(B4)(C6)	Not lamination of dough

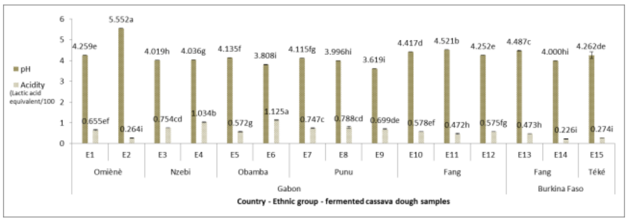
Id: Sample identifier; (-): No transformation particularities

The manufacture chain analysis of Gabonese cassava stick shows many biochemical and microbiological danger

Biochemical and microbiological aspects of dough use to produce cassava stick in Gabon

Biochemical aspects of cassava dough used to produce cassava stick

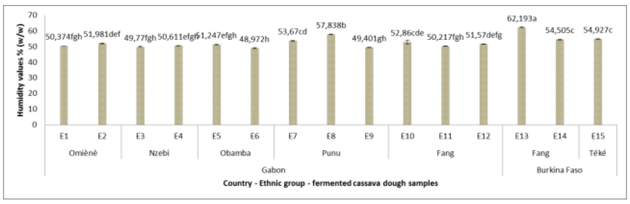
The pH values of cassava dough both in Gabon and Burkina Faso is ranged between 3.62±0.03 and 5.55±0.06 with an average of pH 4.23±0.29. Their acidity was from 0.23 % to 1.12 % as lactic acid with an average of acidity 0.62±0.20 %. There is significant difference ($P<0.05$) among cassava dough samples used to produce cassava stick according to their pH and acidity parameters. These acid values are similar to other fermented cassava dough use to produce attiéké in Ivory Coast and Burkina Faso (N. T. Djéni et *al.*, 2015; F. Guira et *al.*, 2016). These values reflect organic acids production during microbial fermentation (N. T. Djeni et *al.*, 2015). Acidity mainly depends on fermentation process. During processing of Gabonese cassava stick homemade, fermentation unfolds throughout processes of root soaking and dough storage. The variability of acidity values is tribute to the fermentation processes time or yet the presence of some microbial flora with high acidifying ability.



The same letter for the same parameter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 5: pH values and titrable acidity of fermented cassava dough samples

Moisture content is ranged between 48.97±0.16 and 62.18±0.29 % (w/w), with an average of 52.28±2.66 % (w/w) (Figure 6). There is significant difference (P<0.05) in the moisture content among cassava dough samples. This difference is tribute to producers which face difficulty to run dripping of cassava dough. These results show that moisture levels are similar to those of traditional cassava inocula called "magnan" and fresh cassava mash from Ivory Coast (N. T. Djeni et *al.*, 2015) [6]. M. M. Dédédji et *al.* (2008) showed that moisture values may be constant with the mechanization of the process.

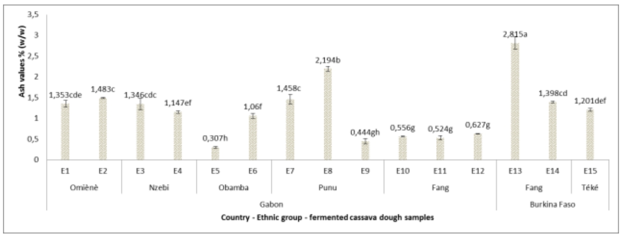


The same letter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 6: Dry matter content of fermented cassava dough samples

Empirically, Gabonese producers use pressing with big stone that exerts strong pressure on filled polystyrene bags with cassava dough. The moisture values of the cassava fermented dough would enable bacterial overgrowth.

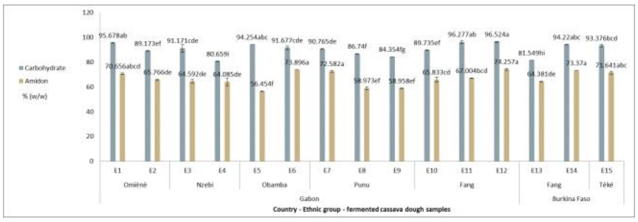
Ash content is ranged between 0.30±0.03 and 2.82±0.21 % (w/w) of dry matter, with an average of 1.19±0.49 % (w/w) (figure7). There is a significant difference (P<0.05) in the content of ash among fermented cassava dough production. A. I. Ihekoronye et *al.* (1985) and E. Avouampo et *al.* (1995) found an average of ash content of 2.4 % and 1.22 ± 0.06 % respectively in the peeled roots and cassava flour. The variation of ash content may be due to edaphic environment and dust impurities in the environment where fermented cassava dough is produced.



The same letter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 7: Ash content of fermented cassava dough samples

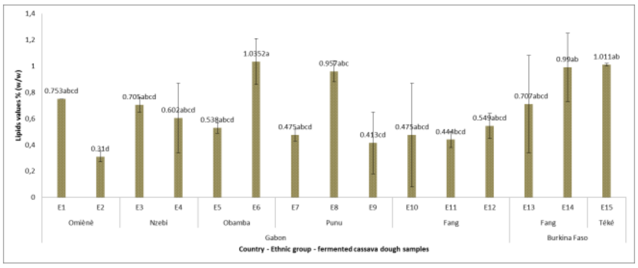
The total carbohydrates content is ranged between 80.66±0.31 and 96.52±0.42 % (w/w) of dry matter, with an average content of 90.41±4.03 % (w/w) (Figure 8). Starch content in fermented cassava dough is ranged between 56.45±0.11 and 74.26±1.05 % (w/w) dry matter; with an average of 66.83±4.75 % (w/w) of dry matter (Figure 8). There is a deference (P<0.05) in the contents of carbohydrates and starch in investigated fermented cassava dough. It appears that fermented cassava dough still contained significant levels of starch which is not metabolized during fermentation. A. Brauman (1996) has showed that starch is little degraded during cassava fermentation while the reducing sugars would be widely consumed. In fact, owing to lack of fermentation processes mastery by producer, the cassava dough is exposed to an environmental microbial flora with amylase capacities that may change from one production to another.



The same letter for the same parameter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 8: Carbohydrate and starch content of fermented cassava dough samples

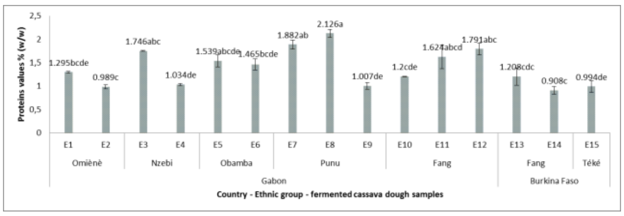
The content in lipids is low in fermented cassava dough as shown in figure 9. Similar results were revealed for cassava roots, fermented cassava dough use for attieke production (D. R. Djoulde et *al.*, 2007; N. T. Djeni et *al.*, 2015; F. Guira et *al.,* 2016). These results confirm some previous work which proved that cassava roots have a low content in lipids (A. I. Ihekoronye et *al.*, 1985; F. M. Mehouenou et *al.*, 2006; O. Lasekan et *al.*, 2016; F. Guira et *al.*, 2016).



The same letter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 9: Lipid content of fermented cassava dough samples

The content in proteins is ranged between 0.98±0.05 and 2.13±0.09 % (w/w) of dry matter, with an average of 1.2±0.14 % of dry matter (m/m) (Figure 10). There is a significant difference (P<0.05) in the protein content of fermented cassava dough. This difference may be tribute to several factors including the fermentation process and the difference in raw materials used. Although yeasts which have an important role during fermentation are source of proteins (A. Bekatorou et *al.*, 2006), fermented cassava dough use for cassava stick as well as other cassava derived products contain relatively low level of proteins content (Huch born Kostinek, 2008; F. M. Mehouenou et *al.*, 2016 and F. Guira et *al.* 2016). However, some authors such as D. R. Djoulde et *al.* (2003) showed that the use of *Lactobacillus plantarum* and *Rhizopus oryzae* as fermentation starter lead to increase the protein content in cassava products.



The same letter indicated no statistical difference (p=0.05) between the values obtained for the different fermented cassava dough samples

Figure 10: Protein content of fermented cassava dough samples

The variability of proximate composition may depend on cassava roots varieties used, soil conditions in growing district, environment and production process.

Microbiological aspect of dough use to produce cassava stick

The microbiological analysis results of the fermented cassava dough use to produce cassava stick from Gabon and Burkina is presented in figure 11.

The number of Total Aerobic Mesophilic Flora (TAMF) is ranged between 4.26 and 7.92 log CFU/g of dough, with an average of 6.82±0.51 log CFU/g of dough. The Presumptive lactic acid bacteria (LAB) levels varied from 3.32 to 6.48 log CFU/g of dough, with an average of 5.84±1.06 log CFU/g of food. These high microbial loads are justified by the fermentation and would highlight the acidity value of cassava dough. The LAB importance as the dominant flora has been put in evidence by A. Brauman (1996) and W. K. A. Amoa-Awua et *al.* (1996). LAB are responsible of the production of organic acids such as acids lactic, propionic, acetic, fumaric, mallic, oxalic, tartaric etc. (N. T. Djeni et *al.*, 2014). It results in the environment acidification. The microorganisms load found is less than those of J. Assanvo et *al.* (2006) and F. Guira et *al.* (2016) who found an average load of 7.76 and 9.32 log CFU/g of LAB in the fermented cassava dough used for attiéké production. The microbial amount of the fermented cassava dough is relatively similar to those of counted by D. R. Djoulde et *al.* (2007) in the bobolo dough, a typical cassava stick of Cameroon. A. C. Kakou (2000) also obtained microbial amounts that are closer of our results with 5.78 log CFU/g for TMAF and 5.69 log CFU/g of cassava dough for LAB.

Yeasts and molds counted vary from less than 1.0 to 5.58 log CFU/g of cassava dough, with an average of 3.95 ±0.97 log CFU/g of cassava dough. These results are significantly lower than those of J. Assanvo et *al.* (2006) and F. Guira et *al.* (2016) which were in order of 7.0 log CFU/g of dough. According to A. Brauman (1996), yeasts (mainly *Candida* spp.) don't have an important role during the submerged cassava fermentation, but could influence cassava dough conservation. Furthermore, it is yeasts that are responsible of volatiles compound production (O. Lasekan et *al.*, 2015) which influence the organoleptic quality of cassava stick. The Obamba cassava stick, much sold in large agglomerations and deriving from long preserved cassava dough, is known in Gabon for its particular aroma.

Total and fecal coliforms are less represented; the complete samples have less than 10 coliform/g. *Salmonella* and *Shighella* bacteria were absent in all analyzed samples. Following these results, it can be concluded that sanitation systems and hygienic practices of cassava stick production are respected by producers. However, cassava dough is exposed during its production to many biological hazards, among which dubious quality of water and re-using storage bags. It may be explained both by the acidity (A. Brauman, 1996) and the production of bacteriocins or bacteriocins like inhibitory substance (BLIS) by certain bacteria such as Lactic Acid Bacteria or *Bacillus* (A. Savadogo et *al.*, 2006; H. Cisse et *al.*, 2016).

Bacillus level in 2/3 of fermented cassava dough samples is more than 3.00×10^5 CFU/g of dough. S. Kastner et *al.* (2007) obtained similar amounts of microorganisms for cassava dough uses to produce attieke. The presence of important load of *Bacillus* in fermented cassava dough may be due to their ability to form spores and thus to withstand more easily the environment stress. *Bacillus* genus includes pathogenic species producing toxins. It is the case of *B. cereus, B. anthracis, B. thuringiensis and B. mycoides*. The presence of these toxins in fermented product constitutes a significant toxicity risk for consumer (L. I. I. Ouoba et *al.*, 2008). However, *Bacillus* is important in some processes of food fermentation (T. Hosoi and K. Kiuchi, 2003; M. D. Bengaly, 2001). Moreover, *Bacillus* are reputed for their antibacterial and antifungal properties. A. Savadogo and A. Tapi (2011) showed that *Bacillus* bacteria isolated from Soumbala and Bikalga have the ability to produce lipopeptides such as fengycins, surfactins, iturins synthesized by Non Ribosomal Peptide Synthetases (NRPS).

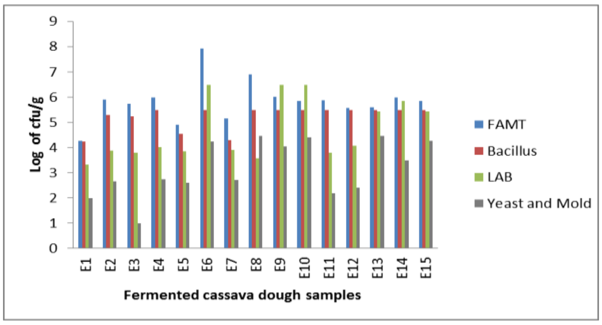


Figure 11: Average total microbial count in fermented cassava dough sample

These results confirm the presence of an uncontrolled microbial flora in the cassava dough used to produce stick. Hence, it may be possible to find in the fermented cassava dough *Bacillus coagulans*, a bacterium with cumulative properties of lactic bacteria and *Bacillus* used as probiotic or other bacteria as *Bacillus subtilis* describe by S. Abban et *al.* (2013). In the light of this, it would be important to better study about *Bacillus* genus in cassava dough.

Isolation, characterization and identification of Bacillus spp isolated in fermented cassava dough

The cultural characteristics showed variable colonies of presumptive *Bacillus* strains on PCA agar. This Presumptive *Bacillus* strains were grouped into 12 groups based on their specific colonies. They are all microscopic rod-like forms, mobile, group en chain or isolated Gram positive, catalase positive and spore forming. Table II presents a summary of the biochemical characteristics of the 12 *Bacillus* isolates. The isolates showed a high diversity concerning their sugar metabolisms. *Bacillus* strains showed protease activity. They have the ability to produce tryptophane désaminase (TDA) and arginine-dihydrolase (ADH).

Table III: Biochemical characters of the isolates

		STRAIN CODE											
		SBM1	SBM2	SBM3	SBM4	SBM5	SBM6	SBM7	SBM8	SBM9	SBM 10	SBM 11	SBM 12
biochemical characteristics	Mobiity	+	+	+	+	+	+	+	+	+	+	+	+
	Catalase	+	+	+	+	+	+	+	+	+	+	+	+
	Oxydase	+	+	+	+	+	+	+	+	+	+	+	+
	Spore forming	+	+	+	+	+	+	+	+	+	+	+	+
	Gram strain	+	+	+	+	+	+	+	+	+	+	+	+
	H2S	-	-	-	-	-	-	-	-	-	-	-	-
	Urée	-	-	-	-	-	-	-	-	-	-	-	-
	TDA	+	+	+	+	+	+	+	+	+	+	+	+
	Indole	+	+	+	+	+	+	+	+	+	+	+	+
	VP	+	+	+	+	+	+	+	+	+	+	+	+
	Gas production	-	-	-	-	-	-	-	-	-	-	-	-
	ONPG	-	+	+	-	+	+	-	+	-	-	+	-
	Citrate	+	+	+	+	+	+	+	+	+	+	+	+
fermentation of sugars	Glucose	+	+	+	+	+	+	+	+	+	+	+	+
	Lactose	-	+/-	+	-	+	+	-	+	-	-	+/-	-
	Saccharose	+/-	+/-	+/-	+	+	+/-	+/-	+	+	+/-	+	+
	Manitol	+/-	+/-	+/-	+	+	+/-	+	+	+	+/-	+/-	+/-
	Inositol	+/-	+/-	-	-	+/-	-	+/-	+/-	+/-	+/-	-	+/-
	Sorbitol	+/-	+	+/-	+/-	+/-	+	+/-	+	+	+/-	+/-	+/-
	Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-
	Melobiose	-	-	-	-	-	-	-	-	-	-	-	-
Suspect bacteria genus		***Bacillus* spp**											

Ribosomal 16S gene amplification on purified genomic DNA using PCR specific primers *Bsub5F* and *Bsub3R* (P. Wattiau *et al.,* 2001) showed highly similarity to «*Bacillus subtilis*» group which contains *B. subtilis, B. licheniformis, B. amyloliquefaciens, B. pumilus, B. atrophaeus* species. The positive amplification reaction is materializing by a single band with 595 bp as size. The amplicons of DNA extracts from SBM3, SBM6, SBM8 and SBM11 showed an intense band on a BET-stained agarose gel. These positive PCR results confirm is in accordance with the biochemical tests. The other strains that did not react positively would belong to other *Bacillus* species.

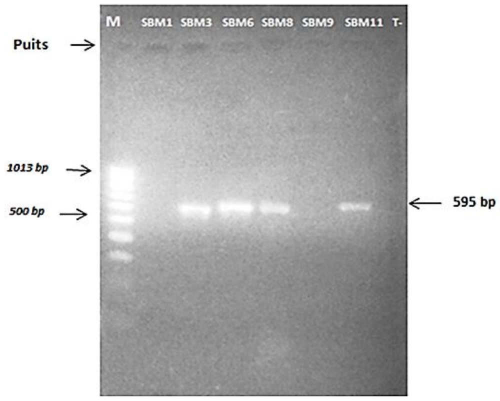


Figure 12: Electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with *Bacillus subtilis* Group Specific Primer, Lane M: 100-bp DNA molecular mass marker (hyperLadder IV), Lane T-: negative control, SBM_X: strains code

The Ascending Components Hierarchical (ACH) leads to a dendrogramme which regroup two major clusters according to their physico-chemical and microbial properties. The first cluster includes samples from Plein-Niger and Akébé districts in the Center of Libreville as well as those of Goughin, Kalgodhin and Zone 1 in Ouagadougou, Burkina Faso. In the same way the cassava doughs from Akournam district in Librevilles as well as those of Kango, Ekouks and Oyanes a few kilometers far from Libreville constitutes another cluster.

The process impact in the final product composition is also evident throughout sample E11 and E12. The cassava dough used to produce those sample come from the same localities but different diagram was used by the same producer for their production.

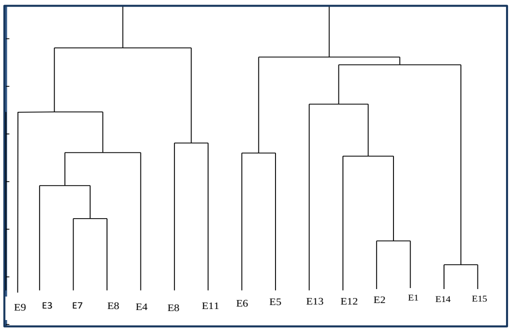
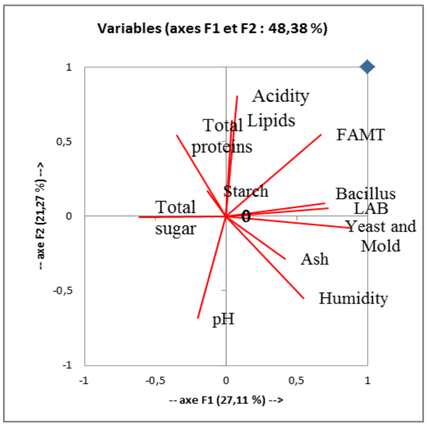


Figure 13: The Ascending Components Hierarchical (ACH)

Dendrogramme of fermented cassave dough samples according to their physico-chemical and microbial properties of fermented cassava dought samples

The Principal Components Analysis (PCA) of the samples showed a variation according to their physico-chemical composition. Figure 14 represents the scattering of the physico-chemical characteristic of cassava dough with a representativeness rate of for the axis 1 of 21,27%. According to this figue, sample E6 is the most acid when sample E7, E11, E5 have the most content in total sugar. E14 and E10 have the most content in ashes. The samples which present the important charge in microorganisms (LAB, yeast and mold, *Bacillus*) are E8, E9, E14 and E15.



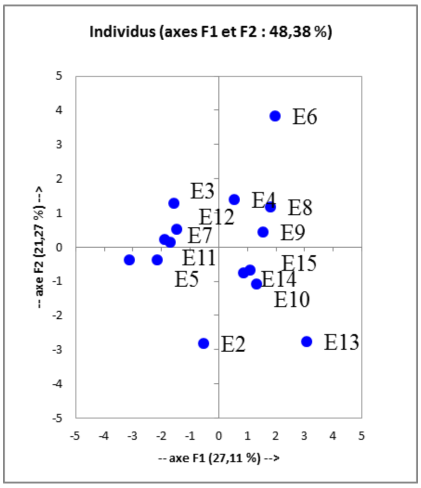


Figure 14: PCA graphical representation according to physico-chemical and microbial properties of fermented cassava dought samples

Conclusion

The cassava stick is cooked fermented dough produced from cassava roots handcrafted and widely consumed by the communities in Central Africa. For cassava stick production mastery in order to obtaining standardized and healthy products, it was important to gain a precise idea of artisanal production practices of this product in Gabon. The fermented cassava dough taken before cooking has been used in this study to highlight the causes of finished product variability and to assess microbiological hazards during the producing of the cassava stick. The absence of coliforms and *Samonella* in spite of breaches of good hygiene and manufacturing practices combined with the presence of a diversified flora could constitute a base of investigation of efficient strains adapted to the African conditions.

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