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Phenotypic characterization of yam ferments: the case of the "florido" and "bètè-bètè" varieties produced in Cote d'Ivoire

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Abstract

The issue of yam preservation and processing is at the heart of many debates. In this study, two varieties (bètè-bètè and florido) were used to produce ferments. Three types of ferment, preserved in two types of packaging (jute bag and plastic bag), were developed. The microflora found on the different ferments consisted mainly of molds, yeast, aerobic mesophilic germs (the total flora) and lactic acid bacteria. The Florido variety showed an average mold load of between 4,5 10^4 CFU/mL and 1,4 10^5 CFU/mL. Three types of mold were identified: *Fusarium, Aspergillus* and *Penicillium*. Lactic acid bacteria were counted at between 2,5 10^4 CFU/mL and 3,9 10^4 CFU/mL. Total flora recorded the highest levels at 3,1 10^7 CFU/mL and 8,7 10^7. The variability of the microflora of yam ferments may make them a potential means of bioprocessing yam-based food products or other starchy substances.

Keywords: Ferments; *Dioscorea alata*; Starch; “florido”; “bètè-bètè”.

1. Introduction

Yam (*Dioscorea spp*) is a major food crop in many tropical countries, whether in Asia, South America, Africa or particularly West Africa (Price et al., 2020; Adewumi et al., 2021). In the various production zones, yam cultivation is essentially based on varieties such as "florido", "bètè-bètè", "sao", "wacrou", "sopere", "kponan", "lokpa", "assawa", "kranglè", "kangba", "gnan" etc. (Doumbia et al, 2004). In Côte d'Ivoire, seeds of the "bètè-bètè" and "florido" varieties of the D. alata species are the most widely used in yam production. These two varieties account for 60% of direct consumption. Another species, *D. cayenensis*, includes the "kponan" and "krenglè" varieties. These varieties account for 40% of production and are intended for marketing (Minagra, 1991). They are therefore an important source of income for the people who grow them (Babaleye, 2003). Yam is the leading food crop in Côte d'Ivoire, with annual production varying from between 2.8 and 3 million tonnes over the last ten years, representing per capita consumption of over 275 kg (Gone et al, 2017). These figures place yams in first place among Côte d'Ivoire's food crops (Amani et al., 2010). Cassava comes second with over 2,450,000 tonnes (FAOSTAT, 2010), corresponding to around 123 kg per resident. Like starch products such as corn, wheat, potatoes and manioc, yam is a basic raw material used in the preparation of a range of finished or semi-finished products. It is the subject of several culinary preparations such as yam fries, croquettes, yam fritters, foufou, yam purée, braised yam; foutou, artisanal raw flour (Dury, 2000; Laria, 2005; Camara et al, 2018). In addition to these products, yam is the subject of a number of semi-industrial transformation processes resulting in by-products such as precooked flour and yam flakes (Amani et al., 2010). In addition, traditional ferments called "magnans" are obtained from cassava (Boli et al, 2023). These are used as inoculum in the cassava paste to be fermented for the production of foods such as attiéké, gari, placali and others. They are obtained from fresh, boiled or braised substrates. According to Assanvo, 2001, these ferments are home to an epiphytic microflora (lactic acid bacteria, molds

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and yeasts) with specific metabolic and physiological properties. The preponderance of these microorganisms varies according to the type of ferment (fresh, braised or boiled). They determine the organoleptic properties of the final product. However, for the preparation of the traditional fermented foods mentioned above, only cassava ferments have long been used by the local population. This could lead to a shortage or a decline in the effectiveness of these ferments in the future. Yet yam has enormous nutritional potential, notably its protein content, which averages 9.6 g/100 g dry matter (Medoua, 2005), good starch digestibility due to its crystalline structure similar to that of cereals (Kone, 2019) and, above all, a moisture content ranging from 65 to 73% (Assiri et al., 2010). These characteristics are conducive to good microbial development. In view of the above, yam could be used to produce a flora of high technological importance that could be used in the processing of agricultural products in general, and food products in particular, to develop new products. The overall aim of our work is to produce yam ferments from the "bètè-bètè" and florido varieties. Specifically, the aim is to identify the different groups of microorganisms (bacteria, yeasts and molds) present in yam ferments, and to compare them with known microorganisms in cassava ferments.

2. Material and methods

The study material consisted of two yam varieties, "florido" and "bètè bètè", both from the *Dioscorea alata* species of the Dioscoreaceae family. These yams were obtained from the Bayon market in Yopougon (Abidjan, Ivory Coast). The choice of this market was guided by the fact that most of the yam tubers coming from the production zones and destined for marketing are stored there.

The technical equipment consisted of the usual microbiology laboratory equipment. These include a STERISTEAM-CDL (Italy) autoclave for sterilizing culture media and Petri dishes. An optical microscope (CETI, Belgium), slides, coverslips and forceps were used to identify molds and bacteria. Plastic bags were used to transport the yam tubers to the laboratory, and clean containers (saucepans, knives, etc.) were used to prepare the various ferments. The media used in this study were Dichloran-Glycerol 18 (DG 18) agar for yeast and mold enumeration. This medium, to which an antibiotic (dichloran) has been added, is seeded by surface spreading. Plate Count Agar (PCA), a glucose agar with yeast extract, was used to enumerate the different bacterial genera present on yam ferments, in particular mesophilic aerobic germs (MAG). MRS agar was used to enumerate the lactic acid bacteria present in the various samples. In addition to culture media, buffered peptone water (BPW) and methylene blue were used for dilutions and for staining fresh molds on slides, respectively. Reagents such as hydrogen peroxide and N-dimethyl paraphenylene diamine (PDA) were used to test for catalase and oxidase in bacteria respectively.

2.1. Sampling

Two varieties of yam were sampled: 'bètè bètè' and 'florido'. A lot of twelve (12) tubers of each variety was collected from a wholesaler-seller at the Bayon market in Yopougon. Each lot was divided into three (03) small batches of four (04) tubers each. These three small batches are peeled and cut separately. Batch n°1 is kept fresh and again divided into two samples of two (02) tubers each. One of the samples is packed in a plastic bag and the other in a jute bag. Batches n°2 and n°3 are boiled and braised respectively. Like batch no. 1. They are then each divided into two samples, one packaged in a plastic bag and the other in a jute bag. In all, twelve (12) samples were prepared, six (06) for each yam variety (**Table 1**):

Table 1 Different types of samples

Yams varieties	Samples	Abréviations
"bètè-bètè"	Braised and preserved in jute	BbrJ
	Braised and preserved in bags	BbrS
	Boiled and preserved in jute	BblJ
	Boiled and preserved in bags	BblS
	Fresh and preserved in jute	BfrJ
	Fresh and preserved in bags	BfrS
"florido"	Braised and preserved in jute	FbrJ
	Braised and preserved in bags	FbrS
	Boiled and preserved in jute	FblJ
	Boiled and preserved in bags	FblS
	Fresh and preserved in jute	FfrJ
	Fresh and preserved in bags	FfrS

2.2. Preparation of ferments

All ferments analyzed were prepared at the CNRA site (Adiopodoumé station). The yams were peeled, washed, cut up and placed in clean containers. They were cooked in water at 100°C for 5 to 10 minutes, and cooled in the open air for 15 min before packaging. For braised samples, slices were placed on coals and carefully turned over to ensure even cooking. All samples were kept in the open air for 72 hours.

2.3. Preparation of stock suspensions and decimal dilutions

Stock suspensions and dilutions were prepared in accordance with ISO 6887, which refers to the preparation of samples, stock suspensions and decimal dilutions. Flasks containing 225 mL of buffered peptone water were autoclaved to prepare the stock suspensions. Approximately 25 g of ferment are taken from each sample (under aseptic conditions) and added to the various flasks. Each vial is manually shaken to obtain a mixture corresponding to the mother suspension. Decimal dilutions of the stock suspension were made under aseptic conditions (created by the flame of the Bunsen burner). To do this, 1 mL of the stock suspension (the sample) was taken with a sterile pipette and transferred to a test tube containing 9 mL of physiological water, corresponding to the 10-1 dilution. Using another sterile pipette, 1 mL of dilution 10-1 is withdrawn and transferred to another test tube containing 9 mL of diluent, corresponding to dilution 10-2. The same operation is repeated under the same conditions until dilution 10-7 is obtained.

2.4. Preparation of bacterial cultures

Bacterial cultures are prepared in accordance with ISO/TS 11133 and the manufacturer's instructions. For one liter of medium, the corresponding quantity of dehydrated powder is weighed and placed in an Erlenmeyer flask. One liter of distilled water is then added, and the whole mixture is carefully stirred with a metal stirrer and heated on a hot plate until completely dissolved. The media thus prepared (PCA, MRS and DG18) are autoclaved at 121°C for 15 min, then kept supercooled at 45°C in a water bath. They are poured into Petri dishes, which are then kept in cold storage at 4°C prior to inoculation. Germ enumeration and identification

Germ enumeration was carried out in accordance with ISO 21527-1:2008.

Inoculation was carried out by surface spreading of the agar. In this way, 0.1 mL of each of the dilutions indicated is placed on a petri dish containing DG18 medium. Spreading was carried out uniformly by sweeping and rotating the entire surface of the agar. The plates were dried for 10 min before incubation at 30°C for 72 hours. In the case of GAM, inoculation was carried out by incorporation into the mass. To achieve this, 1 mL of inoculum from the mother suspension was placed in the Petri dish, then covered with culture medium. The whole is slowly homogenized. The dishes are dried for 10 min before incubation at 30°C for 24 hours. Lactic acid bacteria were also inoculated by surface

spreading on MRS agar. Incubation took place at 30°C for 48 hours. Germs were isolated in accordance with ISO 21527-1:2008 (F).

Separate colonies were picked and seeded on the same medium for purification. Identification was carried out in accordance with NF X 41-500.The various fungal colonies obtained were described macroscopically and then observed under a light microscope (magnification 40) according to the key of BOTTON et al. (1990). Macroscopic examination takes into account the surface and underside of the colonies. On the surface, the characteristics observed are the appearance of the colonies (powdery, filamentous, downy, etc.), the shape (domed, star-shaped, etc.) and the color (white, green, etc.). On the reverse side, the ability of the mycelium to penetrate the agar, the color of the reverse side and the presence or absence of pigment are observed. For microscopic analysis, part of the colony is removed with forceps and placed in a drop of methylene blue on a slide. The resulting preparation is covered with a coverslip, then observed under a light microscope with a x 40 objective. The characteristics observed are the appearance of the mycelium (septate or not), the presence or absence of spores, the shape of the spores, conidial heads and conidiophores. The information obtained from macroscopic and microscopic analyses was used to determine the type of mold. Yeasts were identified by staining with methylene blue and observation with an optical microscope (GX100). The different yeast strains are characterized by the presence or absence of pseudomycelium, the type of partitioning, budding, etc. The identification of bacteria on MRS agar involved five (05) main steps:

2.5. Fresh observation

Inoculate a drop of distilled water and mount between slide and coverslip. Observation is made with a microscope, objective 40. The characteristic observed is mobility.

2.6. Gram staining

This test was performed on young cultures of bacteria isolated on MRS medium. Cells were smeared onto a slide. Crystal violet and lugol solutions were respectively applied to the smear for 1-2 minutes, followed by washing with water. Ethanol 90% was applied to the smear, which was then treated with fuschine for 1 min 30 sec. The smear was observed under an immersion microscope at magnification (X 100). Bacteria with purple staining are Gram-positive (+), characteristic of lactic acid bacteria, while those with pink staining are Gram-negative (-), characteristic of enterobacteria.

2.7. Catalase test

A colony of a culture of each isolate was placed on a slide via a loop, and a drop of hydrogen peroxide was added. The presence of catalase is indicated by the appearance of effervescence. Isolates lacking catalase are unable to degrade hydrogen peroxide; they are catalase-negative, characteristic of lactic acid bacteria.

2.8. Oxidase test

Discs impregnated with N-dimethyl paraphenylene diamine (PDA) were used for this test. The purplish-pink coloration of the initially colorless reagent indicates the presence of cytochrome oxidase in the bacteria. Lactic acid bacteria are generally oxidase-negative.

2.9. Expressions of results

The germ count N for the different types of ferment is calculated in accordance with ISO 7218-1:2007 (F) on the method of counting colonies on solid media and the expression of results. The N colony count is defined as the weighted average of successive dilutions according to the following expression:

$$N = \frac{\Sigma C}{V\,(n1 + 0{,}1\,n2)\,d}$$

ΣC: sum of colonies on all successive dilution dishes, at least one of which contains a minimum of 30 colonies and a maximum of 300; **V**: volume of inoculum applied to each dish (V = 1 ml); **n1**: number of dishes retained for the first dilution considered; **n2**: number of dishes retained for the second dilution; **d:** dilution rate corresponding to the first dilution retained.

Germ frequency is determined in accordance with ISO 7218-1:2007 (F) using the following formula :

$$F = \frac{N_c}{N_T} X\ 100$$

F: Germ frequency ; **Nc:** Number of colonies of a given species for a type of ferment; N_T: Total number of colonies of all species for this type of ferment.

3. Results

3.1. Fungal flora of yam ferments

Based on macroscopic and microscopic characteristics, the fungal flora determined on the various ferments is mainly represented by three genera (03): Penicillium, Fusarium and Aspergillus (Table 2).

Table 2 Macroscopic and microscopic appearance of isolated fungal genera

Colony appearance	**Fluffy**	**Cottony**	**sandy grain**
Colony form	Non-invasive	Non-invasive	Non-invasive
Colony color	Blue-green center White around	White/purple	White/brown
Reverse	Penetrates agar	Does not penetrates agar	Penetrates agar With age
Color of reverse	Pale to yellowish	Red or violet	Colorless to pale yellow
hyphae	septate	septate	septate
Conidial head	Brush-like shape	Absence	Gourpillon shape
Spore shape	Rounded	Fusiform	rounded
Gender detected	*Penicillium*	*Fusarium*	*Aspergillus*

3.2. Average load of microflora isolated from ferments of the "florido" variety

The average mold load ranged from 4.5 10^4 to 1.4 10^5 CFU/mL. This load appears to be higher in the braised and jute-bagged ferment (FbrJ), and relatively low in the fresh bagged ferment (FfrS). The average yeast load ranged from 1.5 10^4 to 2.5 10^4 CFU/mL. This load appears to be relatively constant. GAM had the highest load (from 9.8 10^5 to 3.1 10^7 CFU/mL), followed by lactic bacteria (from 1.9 10^2 to 2.5 10^6 CFU/mL). These results are detailed in **Table 3** below:

Table 3 Average microflora load of "florido" ferments

Ferments / **Germs**	**FbrS**	**FfrS**	**FblS**	**FblJ**	**FbrJ**	**FfrJ**
Mold (CFU/mL)	5 10^4	4,5 10^4	4,6 10^4	1,3 10^5	1,4 10^5	5,7 10^4
Yeast (CFU/mL)	4,5 10^4	2,7 10^4	3,2 10^4	1,5 10^4	1,6 10^4	1,7 10^4
Total flora (CFU/mL)	2,1 10^7	2,1 10^5	3,1 10^7	1,8 10^7	1,3 10^7	9,8 10^5
Lactic bacteria (CFU/mL)	1,5 10^6	1,2 10^2	2,5 10^6	1,6 10^6	1,9 10^6	1,9 10^2

CFU/mL: colony-forming units per millilitre

3.3. Average load of microflora isolated from "bètè-bètè" ferments

Analysis of ferments from the "bètè-bètè" variety reveals a variable preponderance of molds. Their load ranged from 4.1 10^4 to 1.6 10^4 CFU/mL. Yeasts are present in the various ferments with a relatively constant average load ranging from 1.1 10^4 to 3.5 10^4 CFU/mL. GAMs are strongly represented in the various ferments (ranging from 3.5,105 to 2.4,10^7 CFU/mL), while lactic acid bacteria have a load ranging from 1.2,10^2 to 3.9,10^6 CFU/mL.

Table 4 Average microflora load of "bètè-bètè" ferments

Ferments / Germs	BbrS	BfrS	BblS	BblJ	BbrJ	BfrJ
Mold (CFU/mL)	4,8 10^4	4,1 10^4	4,6 10^4	1,1 10^5	1,6 10^5	8,1 10^4
Yeast (CFU/mL)	2,5 10^4	1,1 10^4	3,2 10^4	3,5 10^4	2,6 10^4	2,3 10^4
Total flora (CFU/mL)	1,5 10^7	3,5 10^5	1,1 10^7	2,4 10^7	8,9 10^7	4,6 10^5
Lactic bacteria (CFU/mL)	1,4 10^6	1,2 10^2	1,5 10^6	2,6 10^6	3,9 10^6	1,9 10^2

3.4. Germ frequencies in the "florido" variety

The presumptive mold colonies identified were of the *Penicillium, Fusarium* and *Aspergillus* genera. The rate of appearance of these molds varies between 05% and 35%. The FblJ and FbrJ ferments appear to have the highest *Penicillium* rates, at 30% and 40% respectively. In addition, *Aspergillus* seem to be more preponderant in the FfrJ ferment, appearing in 35% of cases, while *Fusarium* levels are rather low in each of the ferments (25% maximum). On the other hand, yeasts have a considerable presence (45%) in the FblS ferment (Table 4).

Table 5 Occurrence frequencies (%) of sprouts in the "florido" variety

Ferments / Germs	FbrS	FfrS	FblS	FblJ	FbrJ	FfrJ
Penicillium (%)	05	05	10	30	40	10
Fusarium (%)	15	10	15	25	25	10
Aspergillus (%)	10	30	10	10	05	35
Yeast (%)	10	15	45	10	10	10
Total flora (%)	15	0,5	12	25	47	0,5
Lactic bacteria (%)	14	0,4	15	28	42	0,6

3.5. Frequency of appearance of germs in the "bètè-bètè" variety

The frequency of appearance of molds is between 05% and 35%, while that of yeasts is between 05% and 30%. *Aspergillus* and *Fusarium* are highest in the BbrJ ferment (35%) and relatively low in BbrS (05% *Fusarium*) and BblS (05% *Aspergillus*). As for *Penicillium*, its frequency of appearance is lower in most ferments, but is slightly higher in BblS (35%). In addition, yeasts, which are less prevalent in BfrS (05%), seem to be more preponderant in BbrS (30%). These results are detailed in Table 5:

Table 6 Occurrence frequencies (%) of sprouts in the "bètè-bètè" variety

Ferments / Germs	BbrS	BfrS	BblS	BblJ	BbrJ	BfrJ
Penicillium (%)	10	15	35	15	10	15
Fusarium (%)	05	15	10	25	35	10
Aspergillus (%)	10	25	05	10	35	15
Yeast (%)	30	05	25	20	10	10
Total flora (%)	16	0,3	12	16	55	0.7
Lactic bacteria (%)	25	0,4	39	19	16	0,6

3.6. Comparison between microflora of yam ferments and those of cassava ferments

The microflora of cassava ferments is made up of four main groups of germs, according to subsequent research. The group of contamination indicators giving an idea of the hygienic state of the product has not been identified. However, all other germs are present on the yam ferments studied (Table 6).

Table 7 Comparison of the microflora of yam ferments with those found on cassava ferments by Assanvo, 2001.

Ferment source / Microorganisms	**Casava** (Assanvo, 2001)	**Yam ("bètè-bètè" and "florido") from this study**
mold	Presence	Presence
Yeast	Presence	Presence
Total flora	Presence	Presence
Lactic bacteria	Presence	Presence

4. Discussion

Analysis of the various ferments reveals a high bacterial presence on the one hand, and a significant load of molds and yeasts on the other. The bacterial genera isolated include GAMs, which are strongly represented by lactic acid bacteria. The main fungal genera isolated were *Penicillium, Fusarium* and *Aspergillus*. In fact, the presence of these germs is due to microbial contamination originating in the ferment manufacturing process. The work of Boli et al, 2023 on cassava ferments showed that the level of contamination of these ferments was very high. This was justified by the fact that manufacturing techniques, in particular the manual handling of products and their storage under relatively warm ambient temperature conditions, were conducive to the maintenance of such a high microbial load. As our yam ferments have undergone the same manufacturing process as traditional cassava ferments, the manufacturing environment and the equipment used (containers and utensils) are determining factors not only for the proliferation of fungal spores, but also for the transmission of bacterial and yeast strains.

While all the above-mentioned microorganisms owe their presence to the environment and the manufacturing process, their growth and proliferation are due to the nutrient richness of yam tubers. Indeed, one of the greatest assets of fresh yam tubers is their moisture content, which, according to Manas et al., 2007, is close to 80%. According to Attaie et al, 1998, this moisture content is slightly reduced by evaporation as the yam passes over the coals, but the ferments produced from braised yam have a sufficient water content (50 to 70%) for good bacterial growth. For Omonigho and Ikenebomeh, 2000, however, this content increases during cooking in water, thus facilitating microbial growth, the germination of spores of various fungi and their development in ferments from boiled yam. Yam is also rich in digestible carbohydrates, including starch, oses and diholosides (Coulibaly et al, 2021). According to Koffi and Aboule, 2008, these substrates serve as nutrients for microorganisms, which break them down into fermentable sugars, notably lactose, galactose, glucose, ribose and mannose. Microbial enzymes such as polygalacturonases, pectin esterases, cellulases and amylases are released, and this activity is complemented by the cellulosic activity of molds.

The results also showed that the preponderance of microorganisms varied according to the type of ferment and the variety of yam processed. For example, the ferment obtained from yams boiled in water and stored in jute bags contained more lactic acid bacteria for both varieties. Indeed, boiled yam has a high water content that favors bacterial and fungal growth (Omonigho and Ikenebomeh, 2000). The jute sacking used to store the ferments allows for low aerobiosis, enabling optimum growth of lactic acid bacteria. This is the basis for the high lactic acid bacterial load in yam ferments stored in jute. This hypothesis is supported by the results of work by Assanvo, 2001, who showed that the preservation of cassava ferments in nets proved advantageous to the proliferation of lactic acid bacteria. As these germs are micro-aerophilic, their growth and activity are optimal in this preservation mode. On average, products preserved in plastic bags contain the highest yeast loads. The high presence of yeast in this type of ferment is due to the fact that the bag creates an anaerobic condition that favors the growth of yeast, which develops in the presence of oxygen as well as in its absence. In fact, work carried out on yeasts by Verna, 2001, showed that they were intensely active in the absence of oxygen. This is also due to the fact that the growth of lactic acid bacteria and molds is very limited under anaerobic conditions, so these yeasts are less exposed to competition with these germs. Molds, on the other hand, have higher loads in jute bags. This is due to the aeration of the environment. According to Preetha and Narayanan, 2020, fungis have the following physical growth factors which are water, thermal conductivity and, above all, oxygenation.

Fresh tubers preserved in jute do not contain lactic acid bacteria, as they have not undergone the physico-chemical changes brought about by cooking, as is the case with other ferments. Thus, the hardening of the yam during the 72 hours of preservation due to its gradual loss of water prevents the degradation of the yam's native starch by lactic acid bacteria, hence their lack of growth. The work of Boukhelkhal and Moulai, 2018 has shown that during its fermantation, starch presents a retrogradation, more precisely the reorganization of its constituent molecules. This arrangement facilitates its degradation by bacterial amylases (Chuzel, 1992). Some mold and yeast strains are present on fresh ferment because of their resistance to low humidity levels (Manas et al., 2007). The germs that make up yam ferments, like those of cassava ferments, are an advantage in the manufacture of fermented foods. According to Assanvo, 2001, these germs are responsible for fermentation, giving the finished product its acidic character and special organoleptic properties.

5. Conclusion

Microbiological analysis of ferments from two yam varieties, “bètè-bètè” and “florido”, revealed a high load of microorganisms. These microorganisms consist mainly of GAM, lactic acid bacteria, molds and yeasts. The microflora of the yam ferments studied is similar to that of cassava ferments, commonly known a “magnan”, which are used to make fermented foods such as “attiéké”, “placali”, “gari” and many others. Given their highly interesting microbiological potential, yam ferments could be used experimentally to manufacture these same fermented foods. Better still, these ferments could be used in the development of new food products.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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