



Physicochemical and Microbiological Characteristics of Cassava Starters used for the Production of the Main types of Attiéké In Côte D'ivoire

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Abstract

Five types of attiéké have been described in Côte d'Ivoire according to their origin of production. The type of starter added into cassava dough is one main determinant of attiéké quality therefore the physicochemical and microbial profiles of 30 cassava starters from the main production zones in Côte d'Ivoire were determined in this study. Starters produced in Dabou, Jacqueline and Grand-Lahou were more acidic. Their total acidities were respectively 9.25 ± 2.46 g l⁻¹, 6.75 ± 2.11 g l⁻¹ and 6.89 ± 1.99 g l⁻¹ with low pH (4.53 ± 1.18 , 4.42 ± 0.35 and 4.68 ± 0.26). Those from Abidjan and Yamoussoukro were less acidic with lower total acidities (5.52 ± 1.97 g l⁻¹ and 4.82 ± 1.37 g l⁻¹) and higher pH (4.95 ± 0.33 and 5.45 ± 0.49). Moisture levels of samples from Dabou, Jacqueline and Grand-Lahou were lower, while their reducing sugar and starch content were higher compared with those from Abidjan and Yamoussoukro. Starters produced in these localities contained more Enterobacteria, while the others had a high load of lactic acid bacteria, mesophilic aerobic germs, yeast, moulds and Bacillus. Multifactorial analysis revealed three main groups of cassava starters depending on the region of production.

Keywords: Cassava Starter, Fermentation, Attiéké Quality, Grands Ponts Region, Côte D'ivoire

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Introduction

Attiéké is a food product that originates from the tradition of the lagoon people of Côte d'Ivoire (Kouassi et al., 2016). Currently, production of attiéké and marketing activities for the product have been taken up by several women's groups of different ethnicities, religions, regions and nationalities. The different production sources have resulted in the availability of many types of attiéké (e.g. Garba, normal Attiéké, Agbodjama, Adjoukrou Attiéké, Attiéké of Grand-Lahou). The typology of these types of attiéké depends on their origin (Assanvo et al., 2002;

Heuberger, 2005; Djéni et al., 2014; Krabi et al., 2015) and the quality depends on the production zone and/or the ethnic group (Assanvo et al., 2006; Djéni et al., 2011; Akely et al., 2014). A recent study revealed that only five types of attiéké produced in Côte d'Ivoire are actually based on the local ancestral know-how of indigenous women of the production zone (Kouassi et al., 2016).

The production of attiéké requires the use of a biological starter that is produced traditionally from cassava roots (Assanvo et al., 2002; Bouatenin et al., 2016). The starter reduces the cassava dough fermentation time to about 15 hours (Assanvo et al., 2002; Kastner et al. 2010; Bouatenin et al., 2016) by providing the essential microbiota (Assanvo et al. 2006). This microbiota consists essentially of mesophilic aerobic germs (MAG), lactic acid bacteria (LAB), yeasts, moulds, Enterobacteria and Bacillus spp (Assanvo et al., 2002; Coulin et al., 2006; Nimaga et al., 2012). Depending on how the cassava roots are cooked, three types of cassava starters (i.e. fresh, braised and boiled) are commonly encountered (Tetchi et al., 2012; Krabi et al., 2015). According to Krabi et al., (2015), the use of the type of starter is linked to the region of production: fresh cassava starter is produced in the Abouré region in Côte d'Ivoire (Tetchi et al., 2012) while the braised and boiled cassava starters are produced and used in other attiéké producing regions (Assanvo et al., 2002; Krabi et al., 2015). Most recent work has shown that the types of starter incorporated into cassava dough for attiéké production influences the final product quality (Kouassi et al., 2016). Indeed, the cassava starter is a key ingredient in attiéké production because it not only contributes to reduce considerably cyanide content (Heuberger, 2005) by fermenting the cassava dough, but also improves the texture and the flavor of the final attiéké (Bouatenin et al., 2016). Therefore, the best is the quality

of cassava starter, the cassava dough is well fermented and the best are the quality and the safety of attiéké. Such cassava starter contain efficient fermentative germs which are able to ferment the cassava dough by producing hydrolytic enzymes involved in the fermentation (Bouatenin et al., 2013). Thus, cassava starter plays insidiously important role on the health of attiéké consumers. However, the association between the types of starters and the types of attiéké produced has not been assessed yet. The aim of the current study was therefore to evaluate the specificity of cassava starters used in the production of the different types of attiéké according to geographical origin based on the physicochemical profile and the composition of fermenting microorganisms.

Materials and methods

Sudy sites

The zones included in this study are the lagoon area of traditional and cultural production of attiéké, namely Grand-Lahou, Dabou and Jacqueville in the Grands Ponts region, Abidjan and the zone of Yamoussoukro, where production and sale of significant quantities are observed (Assanvo et al., 2006). In each of those zones, two villages were randomly selected from a repertory of 9 to 15 villages retained after an exploratory survey. Village selection criteria were the distance from the main town of the corresponding department (< 45 km) and the presence of attiéké producers' groups. Thus, the villages Braffedon and Nandibo 2 (Grand-Lahou), Akradio and Opoyounem (Dabou), Addah and Sassako-Begnin (Jacqueville), Anoumambo and Djibi village (Abidjan) (Kouassi et al., 2016) and Lolobo and Seman (Yamoussoukro) were selected.

Choice of sample suppliers

In each study location, three attiéké producers were randomly selected amongst the producers who had participated in the survey. These producers have harvested cassava for starter production directly from their fields except those based in Abidjan who supply themselves from the market. Cassava starter production was conducted according to the usual cultural know-how of traditional producers as described by Kouassi et al., (2016).

Sampling and transport of samples

On the basis of previous work (Kouassi et al., 2016), only the most widely used cassava variety in each locality was selected for cassava starters production. Thus, the varieties Improved African Cassava (IAC) and Yavo were retained for all the lagoon zones and Yamoussoukro, respectively. A total of 30 cassava starter samples (5 production zones × 2 villages surveyed × 3 starter producers) were collected for microbiological and physicochemical analysis. Ready-to-use cassava starter samples (500 g) were aseptically taken from producers into food bags, placed into thermal container with dry ice and transported to the laboratory for subsequent analysis.

Physicochemical analysis

Titrate acidity and pH (Djéni et al., 2011), dry matter and moisture contents (AOAC, 1990), starch content (Hassid et Neufeld, 1964), residual sugar (Dubois et al., 1956) and reducing sugars (Bernfield, 1955) of samples were determined.

Microbiological analysis

The preparation of the analytical unit, the stock solution and the

decimal dilutions were carried out as described by Djéni et al., (2011). Enumeration of mesophilic aerobic germs (MAG) and lactic acid bacteria (LAB) 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 hr. On Man Rogosa Sharpe agar, LAB were incubated anaerobically for 24 to 26 hr at 32°C in a jar containing a candle. Enterobacteria were counted on violet crystal and neutral red bile glucose agar according to NF ISO 03 453, 1995. The enumeration of yeasts and moulds was carried out according to the Standard NF (ISO 6611, 2004). Yeasts were grown on Sabouraud agar and incubated at 30°C for 24 to 72 hr in Petri dishes. The Potatoes Dextrose Agar was used for the isolation of moulds and Petri dishes were incubated at 30°C for 24 to 72 hr. Bacillus counts were performed on Mossel Selective Agar. The Petri dishes containing between 15 and 300 colonies were counted in cfu g⁻¹.

Statistical analysis

One-Factor Analysis of Variance was used to compare the physicochemical and microbiological parameters of the different samples. Initially, the equality of variance was verified through the Levene test (Suffert, 2007; Tchuigoua, 2011). In case of a significant difference, $P \leq 0.05$ (Vessereau, 1992), multiple comparisons were carried out using a Duncan test. Microbiological data were transformed with log (cfu g⁻¹+1) to obtain equality of variance. Principal component analysis (PCA) and Hierarchical agglomerative clustering (HAC) were performed, in addition. PCA allowed grouping the different types of samples analysed, according to their physicochemical and microbiological compositions. HAC allowed agglomerating cassava starter samples based on the similarity of their physicochemical and microbiological compositions. The dendrograms were obtained applying a Ward algorithm for obtaining more distinct classes (Bruynooghe, 1978).

Results and discussion

Physicochemical parameters of cassava starters of selected production zones

The physicochemical parameters of the cassava starters of each production zone assessed are presented in Table 1. Starters produced in the zones Dabou, Jacqueville and Grand-Lahou demonstrated lower moisture contents (60.75 ± 5.40 , 60.68 ± 3.64 and $61.71 \pm 4.03\%$, respectively) compared with those in Abidjan ($66.45 \pm 5.70\%$) and Yamoussoukro ($70.74 \pm 4.35\%$). The starter produced in Dabou showed the highest titratable acidity (9.25 ± 2.46 g l⁻¹) while the one produced in Yamoussoukro had the lowest (4.82 ± 1.37 g l⁻¹). The pH of the starters assessed ranged from 4.42 ± 0.35 (Jacqueville) to 5.45 ± 0.49 (Yamoussoukro). The total sugar content was found to be high and similar for samples from the zones Abidjan (10.68 ± 2.71 g 100 g⁻¹), Jacqueville (11.29 ± 1.67 g 100 g⁻¹) and Grand-Lahou (11.17 ± 1.92 g 100 g⁻¹) compared with the low value observed in cassava starters of the zone of Dabou (6.66 ± 1.76 g 100 g⁻¹). Cassava starters from the zones of Abidjan and Yamoussoukro had similar and relatively low reducing sugar content compared with those of Dabou, Jacqueville and Grand-Lahou (Table 1). A general high content of starch (the main component of cassava starter) was ranging from 6.31 ± 2.52 g l⁻¹ for the zone of Yamoussoukro to 12.12 ± 4.58 g l⁻¹ for the zone of Grand-Lahou.

	Moisture (%)	Total acidity (g l ⁻¹)	pH	Starch (g l ⁻¹)	Total sugar (g 100 g ⁻¹)	Reducing sugar (g 100 g ⁻¹)
Abidjan	66.45 ± 5.70a	5.52 ± 1.97ab	4.95 ± 0.33b	8.98 ± 5.21ab	10.68 ± 2.71a	0.15 ± 0.11a
Dabou	60.75 ± 5.40b	9.25 ± 2.46c	4.53 ± 1.18a	9.82 ± 6.11ab	6.66 ± 1.76b	0.42 ± 0.24b
Jacquerville	60.68 ± 3.64b	6.89 ± 1.99b	4.42 ± 0.35a	10.28 ± 5.16a	11.29 ± 1.67a	0.51 ± 0.15b
Grand-Lahou	61.71 ± 4.03b	6.75 ± 2.11b	4.68 ± 0.26ab	12.12 ± 4.58a	11.17 ± 1.92a	0.44 ± 0.20b
Yamoussoukro	70.74 ± 4.35c	4.82 ± 1.37a	5.45 ± 0.49c	6.31 ± 2.52b	7.99 ± 1.63c	0.11 ± 0.09a

Table 1: Physicochemical parameters of starters

Fermenting germs of cassava starters of selected production zones

With the exception of the cassava starter of the zone of Yamoussoukro, which did not contain mould or *Bacillus* (Table 2), all fermenting germs assessed were observed in the cassava starters of the other production zones. The microbial population consisted of LAB, MAG, yeasts and moulds predominantly. The total load of fermenting

germs was statistically higher in the cassava starters of the zones of Dabou, Jacquerville and Grand-Lahou compared with the total load of fermenting germs observed in cassava starters of the zones of Abidjan and Yamoussoukro. However, for Enterobacteria, higher loads were observed in the starters obtained from Abidjan and Yamoussoukro (1.83 ± 1.41 and 1.85 ± 1.84 log (cfu g⁻¹ +1)) than in those of all other production zones (0.22 ± 0.51 to 0.54 ± 1.26 log (cfu g⁻¹ +1)).

	Lactic acid bacteria	Mesophilic aerobic germs	Enterobacteria	Yeasts	Moulds	Bacillus
Grand-Lahou	6.78 ± 0.30ab	7.90 ± 0.26a	0.22 ± 0.51a	6.43 ± 1.07a	5.27 ± 1.07a	3.71 ± 1.83a
Jacquerville	6.76 ± 0.29ab	7.83 ± 0.32a	0.24 ± 0.56a	6.45 ± 1.08a	5.26 ± 1.06a	3.89 ± 1.45a
Dabou	6.90 ± 0.32b	8.01 ± 0.45a	0.54 ± 1.26a	6.88 ± 0.27a	5.82 ± 0.41a	3.49 ± 2.58a
Abidjan	6.35 ± 0.89a	6.56 ± 0.76b	1.83 ± 1.41b	4.95 ± 0.95b	2.35 ± 2.61b	1.62 ± 2.41b
Yamoussoukro	6.35 ± 0.77a	5.42 ± 0.95c	1.85 ± 1.84b	4.86 ± 0.49b	0.00c	0.00c

Table 2: Fermenting germs of cassava starters [log (cfu.g⁻¹ +1)]

Grouping of cassava starters of selected production zones according to their physicochemical and microbiological components

The correlation between the biochemical and microbiological parameters identified resulted in four axes of eigenvalues (Table 3). The two main axes 1 and 2, which constitute 96.49% of the total variability, allowed graphical representation by hierarchical ascending classification (HAC) (Fig. 1) and by principal components analysis (PCA) (Fig. 2). It appears that of the 12 parameters that contributed significantly to the formation of the plane (axes 1 and 2), the parameters LAB, MAG, Enterobacteria, yeasts, moulds, *Bacillus*, reducing sugars, moisture and pH were positively correlated with the first axis (83.80%), while starch and total sugars were positively correlated with axis 2 (12.69%).

Three groups of cassava starters were distinguished in both the dendrogram (Fig. 1) and the PCA plot (Fig. 2). Group 1 consisting of

cassava starters of the zones of Dabou, Jacquerville and Grand-Lahou was characterized by high contents of starch, reducing sugars, total sugars and a high titratable acidity, while moisture content and pH were low. In the same group, the total fermenting microbiota was found to be high with, however, low loads of Enterobacteria. The second group representing the cassava starter of the zone of Abidjan was distinguished by high values for pH and moisture content, and low levels of starch, reducing sugars and titratable acidity. High loads of Enterobacteria and mean loads of other fermenting germs were observed in group 2. The cassava starter of the zone of Yamoussoukro (group 3) demonstrated a high pH and moisture content, and low levels of starch, total sugars, reducing sugars and total acidity. An absence of moulds and *Bacillus*, low loads of MAG, LAB and yeasts but a high load of Enterobacteria were furthermore observed in this group.

	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	10.055	1.523	0.250	0.171
Variability (%)	83.795	12.695	2.084	1.426
Cumulative (%)	83.795	96.490	98.574	100.000
LAB	8.998	5.119	5.008	2.744
Mesophilic aerobic germs	9.778	0.041	6.271	0.280
Enterobacteria	9.125	0.098	30.547	2.647
Yeasts	9.225	4.081	3.534	0.785
Moulds	9.799	0.028	5.668	0.011
Bacillus	9.761	1.054	0.647	0.474
Starch	7.577	8.989	5.599	50.948
Total sugar	0.436	62.549	0.038	1.854
Reducing sugar	9.009	1.280	25.944	5.655
Humidity	9.790	0.041	3.498	3.623
Total acidity	7.234	16.286	9.675	0.187
pH	9.266	0.435	3.571	30.792

Table 3: Correlation between the physicochemical and microbiological parameters and the axes, after the PCA relative to the cassava starters

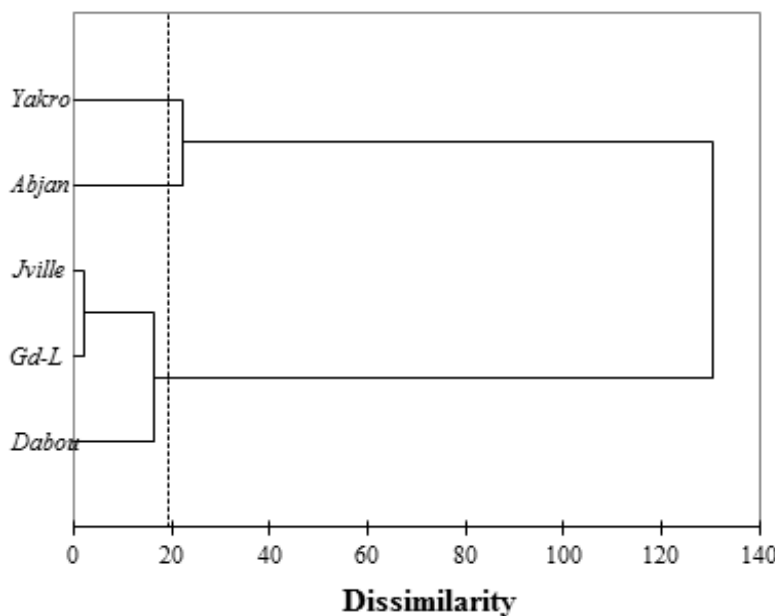
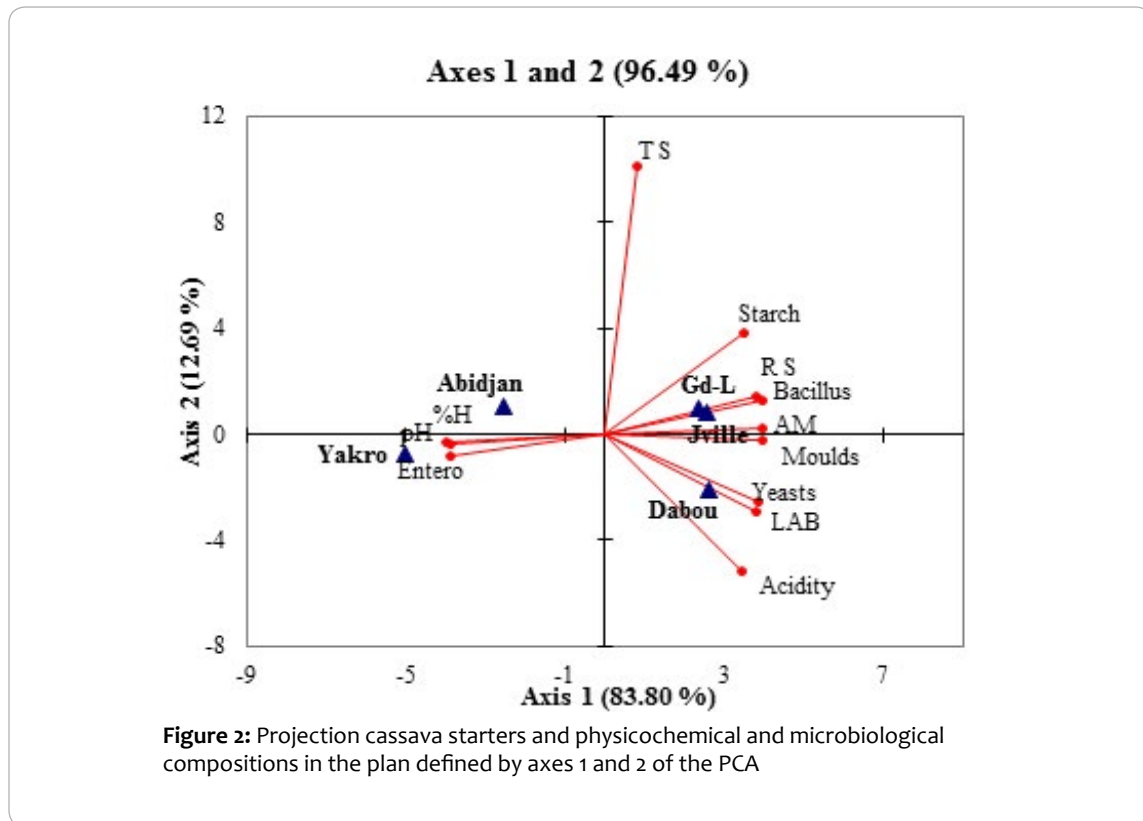


Figure 1: Grouping of cassava starters according to their physicochemical and microbiological compositions



Microbiological analysis of cassava starters showed a significant disparity in the composition of the germs studied. In general, LAB, MAG and yeasts were the most abundant germs in all starters. The strong presence of germs is a major asset in the fermentation of cassava dough for attiéké production. Indeed, **Bouatenin et al., (2016)** have previously shown that the starter provides the cassava dough with the main fermenting germs involved in its fermentation. The results of our study corroborate those of **Djéni et al., (2015)** who found higher loads of these same germs in starters from the Ebré, Adjoukrou and Alladjan ethnic groups. Except for LAB, cassava starters of the zones of Dabou, Jacquville and Grand-Lahou had similar microbial loads. This similarity may reflect the fact that producers of cassava starters in these three zones have substantially the same practices based on the shared regional and cultural space (region of Grands Ponts).

The colonization of cassava starters by germs was found to be less intense in the starters of Abidjan and Yamoussoukro production zones. Indeed, most of the producers of Abidjan involved in the study braise cassava root for starter production. Once braised, a crust appears on the cassava root which is left on the cassava root for packaging and spontaneous fermentation. However, the microbial colonisation takes place on the surface of the root (**Coulin et al., 2006; Djéni et al., 2015**). Thus, it is likely that the presence of the crust has slowed down the growth of the germs.

Regarding to boiled production, the physicochemical characterization of cassava starters showed higher values in the region of the Grands Ponts than in Yamoussoukro. The high acidity (6.75 ± 2.11 to 9.25 ± 2.46 g l⁻¹) observed in production zones of the region of Grands Ponts may be due to the relatively long spontaneous fermentation time of the cassava starter during its preparation. Indeed, starter preparation requires 36 h of spontaneous fermentation in these areas

(**Kouassi et al., 2016**) while the same process is reduced to 24 h in the Yamoussoukro production zone. This shortening of fermentation time would be at the origin of the weak growth of microorganisms as suggested by a recent study (**Tetchi et al., 2012**) showing that the growth of microorganisms during the preparation of the traditional cassava starter is proportional to the spontaneous fermentation time. According to the authors of this work, a short spontaneous fermentation time always results in starters of poor microbiological and physicochemical quality. The fermenting load is sharply reduced with a low content in total sugars, starch and a, high pH and moisture level (**Tetchi et al., 2012**). Eventhough sweet cassava varieties have the best aptitude for the starter production, the (sweet) Yavo variety used in Yamoussoukro did not give the best result.

By hierarchical classification and grouping based on the physicochemical composition and the microbial loads, three main groups of cassava starters were distinguished. The clustering of the three cassava starters from the region of Grands Ponts in the same group reflects shared know-how and procedures of local producers.

Outcomes demonstrate that cassava starters in Grand-Lahou, Jacquville and Dabou have similar physicochemical and microbiological characteristics. These cassava starters seem to have best characteristics compare with those made in the production zones of Yamoussoukro and Abidjan. This study highlights different know-how and procedures to cassava starter production in the largest attiéké production zones of Côte d'Ivoire. Cassava starters in Grand-Lahou, Jacquville and Dabou should indubitably have good trend to fermenting cassava dough for attiéké with good quality. In perspectives, others works on the link between those cassava starters and fermented cassava dough quality and also attiéké specificity would be welcome.

Conflict of interest

No conflict of interest declared

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