Use of microbial starter culture for "*Attoupkou*" (a fermented cassava pancake) production

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1 ABSTRACT:

***Attoukpou* (a traditional fermented, gelled and dehydrated cassava (*Manihot esculenta* Crantz) pancake) in Côte d'Ivoire is of insufficient, unsatisfactory and variable quality with many risks of pathogenic microorganisms due to traditional cassava starter called *magnan* used for its cassava dough fermentation. This work aims to provide the consumer with a healthy product of constant and acceptable organoleptic properties, by the control of the cassava dough fermentation. Two microbial strains of lactic bacteria and *Bacillus sp.*, previously isolated from *magnan* samples were screened for appropriate technological properties and successfully tested to replace *magnan* for cassava dough fermentation into *'attoupkou'*. Physico-chemical, microbiological and sensorial parameters of *attoupkou* samples showed that the mixed starter of *Lactobacillus plantarum* and *Bacillus sp.* at respectively 5.10^7 CFU g^{-1} and 4.10^{10} CFU g^{-1} gave an *attoupkou* with low hydrocyanic acid content and pH, best sensory quality, good aroma and taste similar to *attoupkou* obtained by the *magnan* starters. This mixed microbial starter strains could be useful for the reproducible cassava dough fermentation to improve *attoupkou* quality and to reach sustainable food security in Côte d'Ivoire.**

2 INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food crop consumed by more than a billion people worldwide and is the third major source of calories in the tropics after rice and maize (Ogueri and Helen, 2022; Faostat, 2020 ; Ospina *et al.,* 2021). It is the main food crop in Côte d'Ivoire, after yam, with an annual production estimated at around 2.45 million tonnes (Ehon, 2017). Due to its toxicity (presence of cyanogenic glucosides, linamarin and lotaustralin), its low protein content (1-5% dry matter) and its delicate digestibility, traditional processing methods including combination of peeling, retting, boiling, steaming, pounding, slicing, grating, roasting, soaking, retting, drying, pressing, fermentation and cooking have been developed both by the ingenuity of populations and by scientific research in order to improve its nutritional value (Assamoi *et al.,* 2016). The result foods products could be *attiéké*, *gari*, *foufou*, *lafun*, *chikwangue* or *'attoupkou'*, etc. *Attoukpou* is originate from Abouré people's specialty similar to *attiéké*, but different by its more compact character and its presentation in flexible pancakes. These African pancakes are not sufficiently valued despite their high consumption. They are produced at household level and therefore tend to be of varying quality and stability. Today with the increasing urbanization and the important local demand for products of best quality, these cakes have passed into the commercial stage. They are so produced and marketed by women in towns where they are consumed as snacks at affordable prices

compared to imported cakes and bisbakes by schoolchildren and children in general but also by adults. This situation requires efficient, innovative and rapid agro-food production processes. Indeed, *attoukpou* production includes a tedious and painful fermentation step of cassava dough, responsible for its quality, which carried out using a traditional cassava ferment called *magnan*, obtained after cooking orboiling and spontaneous fermentation (depending on environmental fluctuations) of fresh cassava roots for a long period of three to four days in old microbial reservoirs of jute bags (Bouatenin *et al.,* 2021). It presents many health risks by the handling of pathogens from *magnan* and exposure to wood fires and inhalation of carbon dioxide released during cooking or boiling of cassava roots. Other environmental pollution concerns waste issued from cassava roots during the *magnan* preparation. The 3-4 days of natural fermentation are too long and slow down the regularity of production, damage and limit the profitability and productivity of the sector. These traditional ferments are mostly colonized by lactic acid bacteria, *Bacillus*, yeast, mould and alteration germs and dangerous pathogenic microorganisms such as Enterobacteriaceae, *Staphyloccocus aureus* and *Bacillus cereus* which are responsible for changes of the quality and safety food (Kakou *et al.,* 2010). Although the fermentation step is a critical point like in the *gari* production (Escobar *et al.,* 2021), this fermentation process, not sufficiently mastered by the producers, variable from a woman to another and from one production to another for the same producer, results in quality end products, variable, insufficient and unsatisfactory. Fears exist regarding a probable exposure to cyanide with the use of poorly executed technological processes according to the variety of cassava used (Bouatenin *et al.,* 2013). To address these problems in order to improve *attoukpou* quality, the suppression of the *magnan* preparation step by ready-to-use microbial strains has many advantages and interests. Starter cultures are preparations of living microorganisms, whose metabolic activities are benefits in the fermentation substrate to provide a healthy product of acceptable organoleptic quality (Holzapfel, 2002). It consists of a preparation containing a large number of variable microorganisms for rapid acidification of the product and inhibit the growth of spoilage and pathogenic bacteria (Holzapfel, 2002), as well as to a product with consistent quality. Microbial starters for control the cassava dough fermentation for a*ttoukpou* production have not been reported. Therefore, this work aims to test the ability of two microbial starter strains of *Lactobacillus plantarum* and *Bacillus* sp., previously isolated from the traditional ferment called « *magnan* », to ferment cassava dough into *'attoupkou'*.

3 MATERIALS AND METHODS

3.1 Micro-organisms strains used in this study : Two strains of our Laboratory microbial collection isolated and identified during a previous study and representing predominant micro-organisms in traditional cassava starters called *magnan* (Ehon, 2015; Krabi, 2015) were used. They were screened for technological properties required for cassava dough fermentation like enzymatic, raffinose fermentation and acidification abilities, respectively.

3.2 Investigation of biochemical properties of the two potential starters strains: Production of alpha-amylase, cellulase, pectinase, phytase and tannase by each micro-organism was achieved on modified MRS agar plates containing 20 g/L cassava starch or carboxymethylcellulose (CMC) or pectin or phytic acid or tannic acid as the sole carbon source on Petri dishes. The plates were incubated at 37°C for 24 h in an anaerobic jar, and flooded with iodine. Production of the screened enzyme was evident by a clear area surrounding the colonies (Kostinek *et al.,* 2007). The screening of β-glucosidase activity was tested using 4-nitrophenyl-βD-glucopyranoside, a linamarin analogue, as substrate. The medium was prepared by adding 0.1 g of 4-nitrophenyl-

β-Dglucopyranoside (Merck, Darmstadt, Germany) to 100 mL NaH2PO4 (0.666 M; pH 6) (Merck, Darmstadt, Germany) and filter-sterilized (Millipore filter of 0.2 microns). Microbial colonies after 24 h growth at 30 °C on MRS for LAB or nutrient broth for *Bacillus* 24 h at 30 °C were picked from the plates using a sterile loop and were emulsified in trypton-salt (McFarland Turbidity Standard No. 3). Thereafter, 0.75 mL of culture was added to 0.25 mL of the test medium and was incubated at 30 °C for 12 h. After incubation, 1 mL of sodium carbonate (1 M) was added to the medium and the change of colour (from colourless to yellow) indicated a production of β-glucosidase. Fermentation of the non-digestible sugars raffinose was tested according to the ability to lactic acid fermentation from raffinose (20 g/l) as the sole carbon source at 30°C in the agar medium (MRS without glucose or nutritive medium supplemented with 0.004 g/L of bromocresol purple) in tubes. An 18 h culture of each isolate was used as the inoculums. If raffinose was fermented after the growth of the strain, the purple would turn yellow (visual evaluation). Strains were also investigated for acid production in MRS broth for LAB and nutrient broth supplemented by 5% of glucose. For this purpose, each strain was inoculated (1% of an overnight culture) into broth after autoclaving, and grown aerobically at 30 °C. The pH of the culture was determined after 24h Biomass evolution was analysed through absorbance at 600 nm. The diluted supernatant was used for the quantification of residual glucose, L-lactate and titratable acidity. Concentrations of glucose (g.l -1) and L-lactate (g.l -1) were measured by using a glucose and lactate automatic analyser YSI2700S ELECT (Yellow springs Instruments Co., Inc.) equipped with two membranes, one for glucose (2365) and another for L-lactate (2329). Total titratable acidity was determined by titrating the sample with 0.1N NaOH using phenolphthalein as indicator and the result was expressed as percentage of lactic acid.

3.3 Root samples preparation: Cylindrical fresh cassava root pieces (diameter 1-6 cm; length 2-5 cm) were cut from the flesh. These pieces were washed in water. For each experimental sample, ten cylindrical cassava pieces (totalling about 50–55 g) were soaked with 100 ml sterile milli-Q water in a 250-ml conical flask and inoculated for fermentations under laboratory conditions with single and mixed cultures.

3.4 Cassava dough fermentation process: The microbial strains were pre-incubated for 4 hours at 30 °C on cassava dough (equivalent to 10^{-1} of the whole fermentable mass) before used (Krabi, 2016). This activated microbial strain (AMS) was then used in a second step for 12 hours fermentation at 30 °C of the fermentable mass. Fermentation was conducted in a covered plastic tray (33×14×13 cm^3). All experiments were repeated in triplicate and the mean values of the trials were retained.

3.5 Preparation of *'attoupkou'* : After 12 hours of fermentation, the cassava doughs was used for *'attoupkou'* preparation as described by (Yéboué *et al.,* 2017). The fermented dough was pressed with a screw manually press to remove water. The compact mass obtained after pressing was crumbled, then sieved on a synthetic sieve to remove thick fibres and steamed for 10 min in a couscous maker.

3.6 Physico-chemical characterization of samples: pH determination was done with 10 g sample (cassava dough or *'attoupkou'*) diluted in 20 mL distilled water. The mixture was filtered with Whatman paper and used for pH measurement (Nout *et al.,* 1989). For titratable acidity, 10 g of each sample was homogenized in 90 mL of distilled water and the homogenate was filtered through Whatman paper. Then 10 mL of each filtrate was titrated with a sodium hydroxide solution (NaOH, 0.1 N) in the presence of two drops of phenolphthalein. Moisture and dry matter content were evaluated according to AOAC (1990) on five grams of sample. Ashes were assayed according to AOAC (1990) on five grams of sample previously dried in the oven and then ground. The hydrocyanic acid content was determined by titration with silver nitrate on 20 g of sample diluted in 200 mL of distilled water in a flask as described by

Mehlig (1955). Total nitrogen content was assayed on one gram of dried and crushed sample according to the Kjeldhal method (AOAC, 1990) and the total protein content (Pt) expressed as a percentage by mass was determined by the relationship below:

$$Pt\ (\%) = 6.25 \times Nt$$

The determination of the crude fibre content consisted in taking two grams of sample (me), previously dried and crushed, which were placed in a flask to which 50 mL of sulphuric acid (0.25 mol l^{-1}) were added. The resulting mixture was homogenized and then boiled under reflux refrigeration for 30 min. After this, 50 mL NaOH (0.31 mol l^{-1}) was added to the contents and boiled as before. The resulting extract was filtered with Whatman paper and the residue washed several times with hot distilled water until the alkali was completely removed. The residue was the dried for 48 hours in an oven at 105 °C. After cooling in the desiccator, the residue was weighed (m1) and then incinerated in the oven at 550°C during 3 h. After cooling, the ash obtained was weighed (m2). The crude fibre content as a percentage of sample mass was determined by the following relationship:

$$\text{Crude fiber}\ (\%) = \frac{(m1 - m2) \times 100}{me}$$

3.7 Microbiological characterization of samples

3.7.1 Preparation of stock solutions and decimal dilutions: Stock solutions were prepared under aseptic conditions by taking 10 g of each sample (fermented dough, *'attoupkou'*) which was homogenized in 90 mL of buffered peptone water, contained in a sterile Stomacher bag. Each stock suspension so formed represented a 10^{-1} dilution. Then, for dilution 10^{-2}, one (1) mL of each stock suspension was placed in a test tube containing 9 mL Tryptone-salt (Bio-Rad) and homogenized. Similarly, several successive decimal dilutions were made up to the 10^{-10} dilution.

3.8 Total Mesophilic Aerobic Flora Count (TMAF): Enumeration was performed on PCA (Plate Count Agar) according to ISO 4833-1 (2013).

3.8.1 Enumeration of total coliforms: The coliform count was performed on lactose bile agar with crystal violet and neutral red (VRBL) according to ISO 4832 (2006).

3.8.2 Enumeration of lactic acid bacteria: The enumeration of lactic acid bacteria was carried out on MRS agar (de Man, Rogosa, Sharpe) according to ISO 15214 (1998).

3.8.3 Enumerations of *Bacillus* sp. Bacteria of the *Bacillus* genus were enumerated on nutrient agar. The inoculation consisted of spreading 0.1 mL of the stock solution and its decimal dilutions on the nutrient agar pre-poured in the Petri dishes after solidification. The plates were then incubated at 30°C for 24 h. At the end of this incubation time, typical large, smooth or rough colonies were counted as *Bacillus* species.

3.8.4 Enumeration of yeasts: Yeasts enumeration was performed on Sabouraud chloramphenicol agar according to ISO 21527-1 (2008).

3.9 Sensory analysis of the *'attoupkou'* samples: The sensory analysis of the different samples of *'attoupkou'* produced was done by two tests: the ranking test and the descriptive test of the sensory attributes by a panel of trained tasters. The panel members were recruited according to their motivation, their knowledge of *'attoupkou'* and their availability. The tasting sessions took place in the morning (9:00 a.m.) in a room set up for this purpose. *'attoupkou'* samples of approximately 50 g were presented in white plates, coded with random three-digit numbers. The samples were presented in a specific order to the tasters. At the beginning of the tasting session and between each tasting, a rinse of the mouth with mineral water was performed by the panellists in order to maintain a constant sensitivity.

3.10 Ranking test of the *'attoupkou'* samples: Ranking tests were carried out according to ISO 8587 (2006). A tasting, followed by an evaluation of the *'attoupkou'* samples were carried out by 70 panellists (consisting of Biotechnology laboratory staff

and students of the University of Félix Houphouet-Boigny). The evaluation of the *'attoupkou'* samples was done by giving them marks on a structured 5-level rating scale (from very unpleasant = 1 to very pleasant = 5), expressing the general impression of their preference. Finally, the samples were ranked according to their preference (from most liked to least liked) by the various panellists.

3.11 Descriptive test of the sensory characteristics of *'attoupkou'* samples: This test was carried out according to the ISO 13299 (2016) standard and allowed the establishment of the sensory profile of the *'attoupkou'*. The ten panellists were trained to become familiar with the sensory attributes of *'attoupkou'* and the use of a rating scale to assess the intensities of these sensory attributes. Panelists were selected for their ability to evaluate sensory attributes and their availability to attend all sessions. The intensities of the attributes, on a structured intensity scale of 1 to 9 points, were assessed by the panel.

3.12 Statistical analysis: The tests were carried out in triplicate. The results of the physico-chemical parameters of the fermented dough and *'attoupkou'* as well as the sensory characteristics of *'attoupkou'* were expressed as mean ± standard deviation. A one-factor analysis of variance (ANOVA) of the means obtained was performed using SPSS. Statistics version 25.0 software and Duncan's threshold probability test ($\alpha = 0.05$) was used to determine significant differences between the means.

4 RESULTS

The two potential microbial starters produced ß-glucosidase and amylase, respectively. In addition, *Bacillus* strain produced cellulase, pectinase, tannase and phytase while the LAB strain fermented the raffinose and was more acid producer than the *Bacillus* strain (Table 1).

Table 1: Biochemical parameters of the two potential microbial starters

Strains	ßglucosidase	Amylase	Cellulase	Pectinase	Phytase	Tannase	Raffinose fermentation	Medium acidification
Lactobacillus plantarum	+	+	-	-	-	-	+	ΔpH>1 at 12 h
Bacillus sp.	+	+	+	+	+	+	-	ΔpH<1 at 12 h

The pH values of the activated microbial starter AMS (4.9 ± 0.10 - 5.0 ± 0.10) were statistically identical than that of traditional cassava starter "*magnan*" TCSM (5.0 ± 0.10) and significantly lower than the pH (6.30 ± 0.12) of natural-fermented dough and the pH (6.4 ± 0.10) of fresh dough (data not shown). After 12 hours of fermentation, AMS induced a drop of the pH dough (4.5 ± 0.10 - 4.3 ± 0.10) while dough fermented by TCSM reached a pH of 4.3 ± 0.06. Hydrocyanic acid content remains high in the natural-fermented doughs (4.10 ± 0.10 mg $100g^{-1}$), but decreases in the fermented doughs to reach a minimum value of 3.03 ± 0.05 mg $100g^{-1}$ for the dough fermented only by *Lactobacillus plantarum* strain (P325). All fermented cassava doughs showed statistically similar values of ash (0.20 – 0.30 %). Protein content was poor in the non-fermented doughs (2.11 ± 0.02 %) but was increased in the fermented doughs (2.19 ± 0.02 % to 2.50 ± 0.00 %). Dry matter was statistically different for all fermented doughs and ranging from 46.5 ± 0.10 to 50.5 ± 0.10 % (Table 2). Microbial characteristic revealed that natural-fermented dough contained the lowest concentration of lactic acid bacteria ($5.14.10^3$ CFU g^{-1}), *Bacillus* sp. ($4.55.\ 10^2$ CFU g^{-1}) and yeasts ($5.32.10^3$ CFU g^{-1}), respectively (Table 3).

Table 2: Physico-chemical parameters of the fermented doughs

Fermented doughs	pH	TA (%)	Hydrocyanic acid (mg 100g^{-1})ɨ	Ash (%)	Protein (%)	MS (%)
P203	6.30 ± 0.12^{a}	0.05 ± 0.001^{e}	4.10 ± 0.10^{b}	0.30 ± 0.10^{a}	2.11 ± 0.02^{c}	47.10 ± 0.10^{d}
P125	4.30 ± 0.10^{c}	1.08 ± 0.1^{b}	3.6 ± 0.20^{a}	0.20 ± 0.00^{a}	2.19 ± 0.02^{bc}	46.50 ± 0.10^{e}
P325	4.40±0.10^{bc}	0.98 ± 0.02^{c}	3.03 ± 0.05^{b}	0.20 ± 0.00^{a}	2.50 ± 0.00^{a}	49.80 ± 0.20^{b}
P512	4.50 ± 0.10^{b}	0.95 ± 0.10^{d}	3.10 ± 0.10^{b}	0.20 ± 0.00^{a}	2.40 ± 0.02^{a}	50.50 ± 0.10^{a}
P777	4.30 ± 0.6^{bc}	1.16 ± 0.01^{a}	3.06 ± 0.06^{b}	0.30 ± 0.10^{a}	2.35 ± 0.06^{ab}	48.36 ± 1.6^{c}

P203: negative control (natural fermented cassava dough)
P125: fermented cassava dough with *Bacillus* sp. at 4.10^{10} CFU g^{-1}
P325: fermented cassava dough with *Lactobacillus plantarum* at 5.10^{7} CFU g^{-1}
P512: fermented cassava dough with *Lactobacillus plantarum* at 5.10^{7} CFU g^{-1} and *Bacillus* sp. at 4.10^{10} CFU g^{-1}
P777: positive control (fermented cassava dough with the traditional ferment called « *magnan* ») TA: titratable acidity; MS: dry matter

Table 3: Microbiological characteristics of fermented doughs

Fermented doughs	Lactic Bacteria (CUF g^{-1})	*Bacillus* (CUF g^{-1})	Yeast (CUF g^{-1})	FMAT (CUF g^{-1})	Total coliforms (CUF g^{-1})
P203	5.14.10^{9}	4.55.10^{9}	5.32. 10^{9}	1.2.10^{11}	0
P125	1.36.10^{10}	9.09.10^{10}	1.11. 10^{10}	5.68. 10^{10}	0
P325	1.61.10^{13}	8.95.10^{9}	5.91.10^{9}	1.43.10^{11}	0
P512	2.81.10^{13}	1.34.10^{10}	6.05.10^{9}	1.66.10^{11}	0
P777	3.18.10^{13}	1.77.10^{13}	2.45.10^{13}	2.40.10^{11}	0

'attoupkou' issued from natural-fermented dough presented the highest pH value (6.6 ± 0.10) with lowest titratable acidity (0.01 ± 0.00) while the others samples pH values were statistically identical and ranged at 4.3 ± 0.10 to 4.4 ± 0.00. Hydrocyanic acid decreased in all the *'attoupkou'* samples with the lowest level (0.90 ± 0.10 mg/100g) in dough issued from *Lactobacillus plantarum* used as single starter culture "A325". The highest value of hydrocyanic acid was registered for natural-fermented dough "A203" (1.10 ± 0.10 mg/100g). All samples had protein levels greater than 1 %. Dry matter ranged from 41.40 ± 3.20 to 49.30 ± 0.10 % and ash varied from 0.30 ± 0.10 to 0.50 ± 0.30 % (Table 4).

Table 4: Physico-chemical parameters of the *'attoupkou'* samples

Samples	pHɨ	TA (%)ɨ	Hydrocyanic acid (mg 100g^{-1})ɨ	Ash (%)ɨ	Protein (%)ɨ	MS (%)ɨ
A203	6.60 ± 0.10^{a}	0.01 ± 0.00^{b}	1.10 ± 0.10^{a}	0.40 ± 0.00^{a}	1.20 ± 0.01^{d}	49.30 ± 0.10^{a}
A125	4.30 ± 0.10^{b}	0.04 ± 0.01^{a}	0.93 ± 0.06^{ab}	0.30 ± 0.10^{a}	1.31 ± 0.01^{c}	46.20 ± 0.00^{b}
A325	4.40±0.00^{b}	0.03 ± 0.01^{a}	0.90 ± 0.10^{b}	0.40 ± 0.20^{a}	1.42 ± 0.01^{b}	47.60 ± 0.40^{ab}
A512	4.30 ± 0.10^{b}	0.04 ± 0.01^{a}	1.00 ± 0.10^{ab}	0.40 ± 0.00^{a}	1.51 ± 0.01^{a}	46.50 ± 0.30^{b}
A777	4.30 ± 0.10^{b}	0.03 ± 0.01^{a}	1.00 ± 0.10^{ab}	0.50 ± 0.30^{a}	1.40 ± 0.1^{b}	41.40 ± 3.2^{c}

A203: *'attoupkou'* produced with natural-fermented cassava dough
A125: *'attoupkou'* produced with *Bacillus* sp. at 4.10^{10} CFU g^{-1}
A325: *'attoupkou'* produced with *Lactobacillus plantarum* at 5.10^{7} CFU g^{-1}
A512: *'attoupkou'* produced with *Lactobacillus plantarum* (5.10^{7} CFU g^{-1}) and *Bacillus* sp. (4.10^{10} CFU g^{-1})
A777: *'attoupkou'* produced with the traditional starter « *magnan* »)
TA: titratable acidity; ɨ MS: dry matter

Sensorial analyses indicated the average scores of the various *'attoupkou'* samples (Table 5). *'Attoupkou'* issued from the TCSM had the highest mean score (4.00) followed by *'attoupkou'* "A512" obtained with mixed starter of *Lactobacillus plantarum* and *Bacillus* sp. whom the mean score was 3.50 and followed by *'attoupkou'* "A125" (mean score 3.42) and "A325" (mean score 3.08), respectively. *'Attoupkou'* issued from natural-fermented cassava dough had the lowest mean score (3.00) and was depreciated by the panellists.

Table 5: Ranks of *'attoupkou'* samples

Samples	Average scores	Ranks
A203	3	5th
A125	3.42	3th
A325	3.08	4th
A512	3.5	2nd
A777	4	1st

Visual and touch analyses indicated that naturally fermented *'attoupkou'* samples and those produced with lactic acid bacteria starters were more fibrous compared to other samples fermented with *Bacillus sp.* or the traditional cassava starter "*magnan*" TCSM (data not shown). Furthermore, *'attoupkou'* obtained from dough fermented with mixed starter of *Lactobacillus plantarum* and *Bacillus* sp. showed the same profile as the *'attoupkou'* obtained with the traditional cassava starter "*magnan*" TCSM (Figure 1).

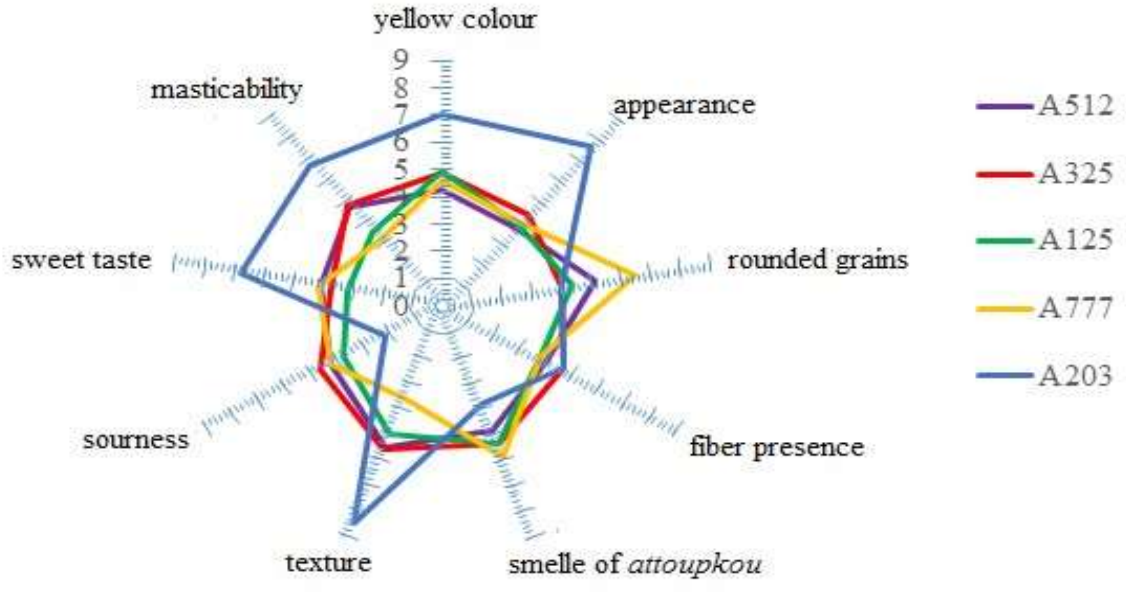

Figure 1: Sensorial profiles of *'attoupkou'* samples

4 DISCUSSION

With the development of nursery education and early childhood education centers by the state, it is difficult for households without substantial income to provide an adequate response to their children's snack needs. The trade of affordable *'attoupkou'* which is an essentially female activity, responds to solve this problem in Africa but also deserves to be supported to improve the living conditions of women producers. The use of microbial starter cultures could also be another interesting way to improve the quality of dishes in addition to fortification or supplementation by other sources which have been widely practiced in Africa. Microorganisms used as ready-to-use microbial starters in this work are adapted to cassava dough fermentation conditions by their metabolic activities, development of characteristic properties such as aroma, texture, taste, safety and nutritional value of fermented food. The observed range of the pH values (6.40 - 4.30) after 12 hours for fermented cassava doughs samples is within that observed by many researchers (Kimaryo *et al.,* 2000). In fact, the decrease of pH is essentially due to lactic acid produced by the microbial starters' strains (Ehon *et al.,* 2016; Bouatenin *et al.,* 2013). *Lactobacillus plantarum* strains are known to produce more lactic acid during cassava dough fermentation for *attiéké*, *lafun* or *foofoo* production (Coulin *et al.,* 2006; Padonou *et al.,* 2010) by using sugars from hydrolysis of starch. Strains such as *Bacillus thermoamylovorans* were also able to produce lactic acid (Ehon *et al.,* 2016). This rapid acidification of cassava dough is important to develop the food taste and safety. Hydrocyanic acid content remains high (3.03 - 4.10 mg $100g^{-1}$) in the natural-fermented dough than in the others doughs and decreased (0.90 – 1.10 mg $100g^{-1}$) in all the *'attoupkou'* samples produced after the cooking step. The *'attoupkou'* sample fermented using the single starter of *Lactobacillus plantarum* had the lowers hydrocyanic acid content, followed by *'attoupkou'* samples fermented with the single starter of *Bacillus sp.*, the mixed starter of *Lactobacillus plantarum* and *Bacillus* sp. and the traditional starter "*magnan*", respectively. Many studies showed that *Lactobacillus plantarum* and *Bacillus* sp. were able to produce β-glucosidase to hydrolysis glucosidic cyanogens. According to Tran *et al.* (2021), cooking decreased highly the HCN content which evaporates at 27 °C. In fact, hydrocyanic acid content in this study decreased to 63–70 % after cooking of fermented cassava doughs. Like roasting and dewatering in *gari* production process (Layal *et al.,* 2021), these results suggest that improvements of *'attoupkou'* production process could be achieved also by appropriate management of cooking. The total residual cyanide in the *'attoupkou'* samples is under recommended minimal content of HCN (1 to 4 mg $100g^{-1}$) (AOAC, 1990). So, *'attoupkou'* samples are considered as safe food. Elimination rates of HCN in cassava based-foods are as a function of the processes used (Njankouo *et al.,* 2019). The protein contents of all *'attoupkou'* samples ranged from 1.20% to 1.51%. These protein levels are the same with the levels showed in others cassava fermented food like *attiéké* (1 to 2% of protein in relation to the dry matter) (Ehon, 2016). According to Igbabul *et al.* (2014), fermentation increases protein content. This could be attributed to microbial growth during fermentation or hydrolysis of protein molecules into amino acids and other peptides. The increase could also be due to structural proteins of microbial cell or the result of enzymatic hydrolysis of protein inhibitors of bacteria during fermentation. For ashes, all *'attoupkou'* samples were shown content ranged of 0.30 to 0.50%, which lower than 1.4% content as recommended by Codinorm (2013) standard. Total ashes represent the inorganic matter contained in *'attoupkou'* samples, obtained after incineration. They are dependent on the raw material used and their contents can be influenced by the transformation processes (Guira, 2013). The observed ashes contents can be attributed to a possible leaching of soluble mineral elements in the fermentation medium or to the enzymatic activity of the fermenting microorganisms that causes the decomposition of food components into their absorbable forms (Igbabul *et al.,* 2014). Dry matter rates ranged

from 41.40 to 49.30 %. These values are within recommended (45-55%) Codinorm (2013) because high humidity promotes the growth of microorganisms and perishability of the *'attoupkou'* if any precautions are not taken for a good conservation. The sensory characteristics showed that *'attoupkou'* obtained by mixed starter (*Lactobacillus plantarum* and *Bacillus* sp.) "A512" was the most appreciated by members of the panel like *'attoupkou'* obtained by the traditional cassava starter "*magnan*" with good texture, good flavour and a slightly acidic taste. Results of visual and touch analyses could be attributed to the synthesis of softening enzymes by *Bacillus sp* alone or in combination with lactic acid bacteria or in the positive control could. That would explain the low quantity of fibres in the resulting *'attoupkou'* samples compared to those naturally fermented and those produced with starters of lactic acid bacteria. Importance of cassava dough softening during fermentation due to cellulolytic and pectinolytic activities of *Bacillus* strains has been reported (Ehon, 2017). *'attoupkou'* produced by natural-fermented dough was depreciated by the panel members. This result showed the importance of the fermentation of cassava dough in the organoleptic and sensory characteristics of attoukpou. Microbial starters had the potentiality to improve the aroma profile of the cassava based-food (Freire *et al.,* 2015). In others works, Ehon *et al.* (2016) and Padonou *et al.* (2010) recommended the use of a mixed starter of lactic acid bacteria and *Bacillus* sp. (or *Saccharomyces cerevisiae*) for *attiéké* and lafun production, respectively. Djoulde (2005) focused on the use of a mixed starter of *Lactobacillus plantarum* combined with *Aspergillus oryzae* for retting cassava. Although, (Kostinek *et al.* (2007)) used only *Lactobacillus plantarum* as starter to ferment cassava dough into *gari*. On general point of view in all the parameters (texture, flavour, taste, colour, masticability), *'attoupkou'* produced with mixed starter was judge acceptable and safe such as samples obtained by the cassava dough fermentation of TCSM (traditional starter "*magnan*") sample.

5 CONCLUSION

This work aims to improve working conditions, poverty reduction and empowerment of African women in order to achieve the goals of sustainable development of the United Nations. In addition to sustainable food security in Africa, it aims to establish new high-performance agro-food processing processes such as controlled fermentation for the industrialization of informal and artisanal sectors. It has showed the potential of a mixed starter of *Lactobacillus plantarum* and *Bacillus* sp. for cassava dough fermentation into the Ivorian traditional pancake *'attoupkou'*. These microbial cultures present suitable technological properties and could be further tested in their lyophilized forms for their popularization in Côte d'Ivoire.

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