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Technical Sheet of Process of Traditional Cassava Inocula Used for *attieke* Productions in South of Côte d'Ivoire

Alfred K. Kouamé[1*], Jean-Paul K. M. Bouatenin[1], Hermann W. Coulibaly[1], Charles Y. Tra Bi[1], Theodore N. Djeni[1] and Marcellin K. Dje[1]

[1]Department of Food Sciences and Technology, Laboratory of Biotechnology and Food Microbiology, University Nangui Abrogoua, Abidjan, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TND and MKD were responsible for study design and supervision of work. Authors AKK, HWC, CYTB and JPKMB were responsible for laboratory work, data analysis and manuscript preparation. All authors read and approved the final manuscript.

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ABSTRACT

Production of *attieke* in Cote d'Ivoire requires the use of inputs such as the cassava traditional inocula.Our study is a technical sheet of a process of traditional cassava inocula used for *attieke* production in south of côte d'Ivoire. Process of traditional cassava inocula for *attieke* production was: Cassava roots, peeling, pulp washing and cutting, cooking, cooling, packaging, fermentation. It contained aerobic mesophiles, total coliforms, fecal, *Bacillus*, *Staphylococcus* and moulds.

Keywords: Attieke; bacteria; cassava traditional inocula; process.

**Corresponding author: Email: kohialfred2@yahoo.fr;*

1. INTRODUCTION

Attieke is the major fermented plant food in Côte d'Ivoire. It is a steamed granular cassava (*Manihotes culenta* Crantz) meal ready to eat, couscou-like product, with slightly sour taste and whitish colour [1]. It is consumed two to three times a day with meat, fish or vegetables. The popularity of *attieke* to urban dwellers in recent years has been associated with its cheapness, lower bulk (as compared to other cassava product) and its characteristic of ready to eat food. *Attieke* is one of the few products whose fermentation is not spontaneous but involves the use of an inoculum. This inoculum is obtained after 2-3 days of spontaneous fermentation of cassava roots, thus colonized by a wide variety of microorganism which constitutes the main source of microbial activities during the cassava dough fermentation [2]. These microorganisms constitute the bulk of the inoculum which would participate in the fermentation of the cassava pulp for the production of the *attieke*. [3] Our study is a technical sheet of a process of traditional cassava inocula used for *attieke* production in south of côte d'Ivoire.

2 MATERIALS AND METHODS

2.1 Material Vegetable

The used plant material consisted of 12 months-old freshly harvested cassava roots ofthe bitter variety, IAC cultivar (Improved African Cassava).

Photo 1. Cassava roots (photo A.K Kouame)

2.2 Traditional Cassava Inocula Preparation

Cassava roots are peeled, cut into pieces, washed three times with fresh water and then ground, blanched for 10 to 20 minutes, drained, cooled in the open air for 15 minutes, then wrapped in jute bags. The whole is put to ferment for 2 to 3 days in a hot place of the kitchen.

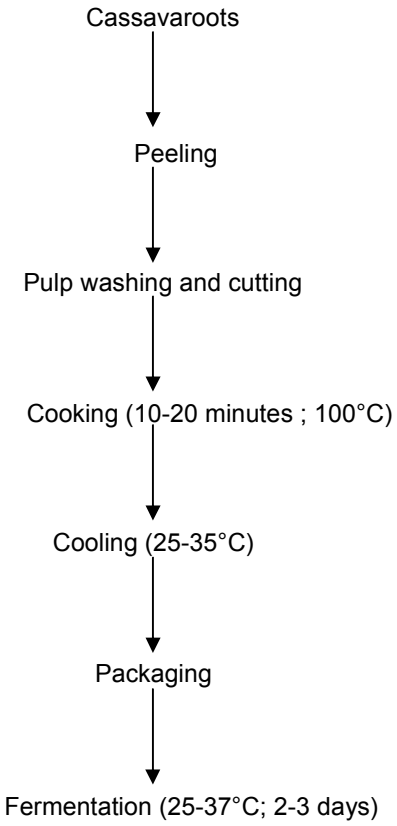

Fig. 1. Process of cassava traditional inocula production used for cassava dough fermentation during *attieke* production in southern parts of Côte d'Ivoire

Photo 2. Traditional cassava inocula (photo A.K Kouame)

2.3 Determination of pH and Total Titrable Acidity (TTA)

Thirty grams of cassava traditional inocula samples were blended with 70 ml of sterile

distilled water and filtered through a Whatman filter paper. The pH of 30 ml of the filtered solution was determined using a pH-meter (pH-meter P107, Consort, Bioblock Scientific, Illkirch, France). TTA was determined using the standard method described by [4]. Ten millilitres of filtered solution were titrated with NaOH01 N, using 1% phenolphthalein as indicator. The volume of aliquot used was recorded to determine the amount of acid in the sample. The titrable acidity was calculated as percentage of lacticacid. The determinations were carriedout in triplicates and the mean value recorded.

2.4 Enumeration and Identification of Spoilage microorganisms

Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of micro-organisms were carried out according to [1]. For all determinations, 10 g of the traditional cassava inocula samples was homogenized in a stomacher with 90 ml of sterile buffered peptone water (AES Laboratoire, Combourg, France). Tenfold serial dilutions of stomacher fluid were prepared and spread plated for determination of micro organism counts. Enumeration of coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany). The cultures were in cubatedfor 48 h at 30°C for total coliforms and 44°C for faecal coliforms. Yeasts and moulds were enumerated on plates of Sabouraud–chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich ChemieGmbH, Bangalore, India), incubated at 30°C for 4 days. Aerobic mesophiles were enumerated on plates of plate count agar (PCA Oxoid Ltd, Basingstoke, UK) and incubated at 30°C for 2 days.

2.5 Isolation of Food-borne Pathogens

2.5.1 *Staphylococcus aureus*

Staphylococcus aureus was isolated and enumerated according to the method described by [5]. A volume of 01 ml of each dilution was surface plated on Baird-Parker agar (BPA) containing egg yolk tellurite emulsion (Oxoid) and incubatedat 37°C for 24 and 48 h. The total number of colonies, colonies with different morphology to those of *Staphylococcus aureus* was counted. Five colonies from each sample were randomly selected, purified and tested for cell morphology, arrangement of the cells, Gram reaction, catalase activity, oxidase test, ability to produce acid anaerobically in a glucose-containing growth medium, coagulase activity, thermo-stable nuclease activity, acid production from mannitol and acetoin production. Only, the gram positive cocci were identified using the identification scheme sproposed by [6]. After the identification, the percentages of *Staphylococcus aureus* and the other strains were calculated. These percentages were later used to correct the results of the counts obtained from each BPA plate.

2.5.2 *Bacillus*

The quantitative estimation of spores of *B. cereus* was performed by a standard plate-counting method. Isolations were achieved from heat-treated dilutions by plating on mannitol egg yolk polymyxin B agar [7]. Presumptive colonies of *Bacillus cereus* were randomly selected based on characteristic colony feature, purified on the same medium and identified by morphological, cultural and biochemical characteristics according to the documented procedures [8].

2.5.3 *Salmonella*

The research of *Salmonella* in cassava traditional inocula, palm oil and water samples were achieved according to the procedure described in the global *Salmonella* surveillance and laboratory support project of the World Health Organization [9]. From each sample, 25 g was aseptically weighed and macerated in 225 ml of buffered peptone water (Oxoid) and incubated at 37°C for 24 h. A selective enrichment in Tetrathionate broth (Muller-Kauffmann) and Rappaport Vassiliadis soy peptone broth using 1 ml of previously incubated buffered peptone water was achieved at 37°C for 24 h, followed by a subcultivation on *Salmonella* Shigella agar incubation at 35°C for 24– 48 hours [10]. Colourless, transparent and with a black centre colonies were further identified using biochemical tests.

2.6 Statistical Analysis

Descriptive statistics for microbiological data were calculated with Excel (Microsoft, Redmond, WA, USA). All statistical analyses were implemented in STATISTICA for Windows ver. 10 (StatsoftIberica, Lisbon, Portugal).

3. RESULTS AND DISCUSSION

In Côte d'Ivoire; *attiéké* plays an important role in the population diet. It is part of the diet of many peoples. It is a typically Ivorian food, whose annual local consumption is estimated at over

450 000 tons [2]. The production of attieke necessarily requires the use of ingredient such as the cassava traditional inocula.The traditional cassava inocula used for cassava dough fermentation during *attieke* production in southern parts of Côte d'Ivoire is usually prepared by method described above (Fig. 1). Cassava traditional inocula contain several microorganisms which plays an important role in the fermentation of cassava dough for *attieke* production.The total titrable acidity and pH content of cassava traditional inocula were respectively 1.67%±0.2 and 4.94 ± 0.8.This pH was due to the fact that production of the traditional cassava ferment necessarily requires spontaneous fermentation traditional cassava inocula contained aerobic mesophiles, total coliforms, fecalcoliforms, *Bacillus, Staphylococcus* and moulds. Mean loadings of aerobic mesophiles, total coliforms, faecal coliforms, *Bacilli*, *Staphylococcus* and mould were respectively $(2.2 \pm 0.7)10^9$CFU / g; $(3.7 \pm 1.7)10^5$ CFU / g; $(1.7 \pm 0.7)10^3$ CFU / g; $(2.7 \pm 1.2)10^7$ CFU / g; $(2.2 \pm 1.5)10^5$ CFU / g and $(6.3 \pm 1.9)10^6$ CFU / g (Table 1). Similar observations were reported by [11] in their study on gari stability. Moreover, the multiplication of micro-organisms in the cassava traditional inocula samples was facilitated by the product nature (wet product), its composition and the temperature of storage, which corresponded to some micro-organisms optimal growth temperature. In fact, according to [12,13], an environment containing high sugar and moisture contents constituted a favourable medium for yeasts and moulds, Enterococci and coliforms development.

Table 1. pH, total titratable acidity and microbial population in cassava traditional inocula used in *attieke* process

Parameters	Values
pH	4.94± 0.8
Titrable acidity (%)	1.67± 0.2
Aerobic mesophiles (CFU.g^{-1})	$(2.2\pm0.7)10^9$
Moulds (CFU.g^{-1})	$(6.3\pm1.9)10^6$
Staphylococci (CFU.g^{-1})	$(2.2\pm1.5)10^5$
Bacilli (CFU.g^{-1})	$(2.7\pm1.2)10^7$
Total coliforms (CFU.g^{-1})	$(3.7\pm1.7)10^5$
Faecal coliforms (CFU.g^{-1})	$(1.7\pm0.7)10^3$
Salmonella (CFU.g^{-1})	ab

ab, absence in 25 g for Salmonella, values are expressed as mean ± standard deviation.

The presence of such micro-organisms in the cassava traditional inocula could be due to a further contamination after steaming, by the production environment, the material of production, the product handling and during the packaging [14]. These micro organisms constitute the bulk of the inoculum which would participate in the fermentation of the cassava pulp for the production of *attieke* [3].

4. CONCLUSION

Attieke is one of the few products whose fermentation is not spontaneous but involves the use of an inoculum. Traditional cassava inocula used for *attieke* productions in south of Côte d'Ivoire had an acidic pH. It contained a diversity of microorganisms which would participate in the fermentation of cassava pulp intended for the production of the *attieke* in Côte d'Ivoire.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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