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Microbiology Hazard in Inputs (Traditional Cassava Inocula, Water and Oil Palm) Used in *Attieke* Process in South of Côte d'Ivoire

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TND, CYTB and MKD were responsible for study design and supervision of work. Authors AKK, MDT 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, palm oil and water. These three inputs are involved in the entire production process. Contamination of these ingredients will result in a finished product of uncertain health quality. For the implementation of HACCP (Hazard Analysis Critical control Point) in the production of *attieke* in Cote d'Ivoire, it is therefore necessary to identify the microbiological hazards in these ingredients (cassava traditional inocula, palm oil and the water used for the production of *attieke*). The imputs contained pathogenic microorganisms. *Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus, Citrobacter freundi, Enterobacter amnigenus, Citrobacter youngae, Enterobacter aerogenes, Klebsiella pneumoniae, Enterobacter agglomerans and Klebsiella oxytoca* were the bacteria isolated, and *Rhizopus* spp*., Mucor* spp*., Thamnidium* spp*., Fusarium* spp*., Moniliella* spp*.* were the fungi isolated.The occurrence of some bacteria and fungi illustrate that cassava traditional inocula,

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water and palm oil used in *attieke* proccess collected in Côte d'Ivoire may act as a reservoir of potential pathogenic micro-organisms for human.The finished product which is the *attieke* must undergo a particular treatment in order to ensure its microbiological quality.

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

1. INTRODUCTION

Attieke is the major fermented plant food in Côte d'Ivoire. It is a steamed granular cassava (*Manihot esculenta* 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. The largest amounts of *attieke* are prepared by three ethnic groups (Adjoukrou, Alladjan and Ebrie) at the origin of *attieke* production and which supply the big city of Abidjan [2]. But, increasingly, *attieke* preparation is spreading to other countries in West Africa [3]. Also, the large export market potential of *attieke* cannot be underestimated as the size of the West African ethnic population overseas keeps increasing. It is also exported to Europe as a dehydrated product without any established specifications. Recent data on *attieke* production and consumption do not exist, but [4] estimated its consumption between 28 000 and 34 000 tons per year; in which 100 tons was daily produced only for the Abidjan city by the small-scale channels. The production of high quality *attieke* is often associated with specific locations and specific ethnic groups in Côte d'Ivoire. However, with increased commercialization production has now moved to other locations within the country not traditionally noted for major *attieke* processing. The processing of cassava into attieke needs several and hard steps. Roots are peeled, cut into pieces and then washed three times with fresh water. The milling takes place in a cooperative mill located in the village. Before milling, 5–10% (w/w) of inoculum, 10% (v/w) water and about 1 % (v/w) of palm oil are added and the pieces are ground to a fine paste, which is placed in large bowls. The mash is left to traditional cassava inocula for about 12–15 hours at ambient temperature (30–37℃). After fermentation, the mash is placed in a jute sack and pressed continuously in a hand press for an hour. The press cake is then passed through two sieves to obtain a fine powder. The grains are formed by shaking and rotating the powder in a large bowl. The grains are sun-dried on black plastic canvas or flat bowls for a time period ranging from a few minutes up to half an hour [5] After drying, fibers and dirt are removed by sprinkling the grains. The grains are poured onto the sieve up to a height of 15–20 cm forsteaming for about 20–25 hours on a cauldron filled with boiling water. *Attieke* obtained is the filled into plastic bags, sealed airtight and sold on local markets or transported in cars at ambient temperature (30–37℃) in other localities.It 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 [6]. The shelf life of *attieke* is largely determined by its preparation in terms of careful manipulations and good hygiene. Spoilage of *attieke* is caused by micro-organisms, which lead to changes in taste and color, and increase in pH [7,8] estimated the shelflife of attieke sold in Abidjan city to only 3 days before seeing appear undesirable colorations. However *attieke* is still produced following traditional methods. Very few attention is granted to production environment, staff's hygiene, production material, and inputs (water, traditional inoculum) used in the process. In most production units, water was purchased from resellers and often stored and handled under unsanitary conditions, favorable to microbial contamination. This water is used for washing the cassava chips, washing the utensils and cooking *attieke*. Most of *attieke* are processed by small scale producers, thus making quality control difficult. In other respects, the product is usually subject to various handling, storage and marketing conditions, some of which may introduce microorganisms. Indeed, foodborne diseases account for a considerable degree of morbidity and mortality and can have various origins such as chemical and parasitic; however, microbiological sources stand out for posing a great risk to public health because of the severity of the clinical symptoms and the large number of foods and microorganisms that can be involved [9,10] showed that *attieke* was most of the time susceptible to contamination by

bacteria and molds. Knowing that the food product constitutes a major part of daily diet of many Côte d'Ivoire homes and most part of West Africa. Information on this study will help to develop appropriate understanding of its spoilage and will also help to ensure its microbiological safety. In order to implement a HACCP (Hazard Analysis Critical control Point) system for production *attieke* Ivory Coast it is necessary to identify the microbiological hazards in ingredients (ferment manioc, water and oil palm) used for the production of *attieke*.

As the effect of micro-organisms on human health has been reported, the present study was performed to give information on the distribution and presence of pathogenic microorganisms in ingredients (traditional cassava inocula, water and oil palm) used for the production of *attieke* in Côte d'Ivoire and to discuss their role in the food poisoning and also the causation of many human diseases.

2. MATERIALS AND METHODS

2.1 Sample Source and Sampling

Cassava traditional inocula, palm oil and water samples (The water used during the production process *attieke*) samples used in the study were purchased from 7 towns (Abidjan, Dabou, Jacqueville, Grand-lahou, Divo, Sikensi and Adzope) in southern parts of Côte d'Ivoire. All samples were collected from sellers and transported in an icebox directly to the laboratory for microbiological analyses. Samples were processed within 4 hours.

2.2 Enumeration and Identification of Spoilage Microorganism

Preparation of stock solutions, inoculation of agar plates, cultivation and quantification of micro-organisms were carried out according to [11]. 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). Each palm oil samples and water samples (The water used during the production process *attieke*) (1 ml) was directly diluted in buffered peptoned water (BIOD-RAD). 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 incubated for 48 h at 30℃ for total coliforms and 44℃ for faecal coliforms. The eosin methylene blue agar (Becton Dickinson GmbH, Heidelberg, Germany) was used to particularly enumerate and isolate *Echerichia coli*, which grows on the medium giving a distinctive metallic green sheen colony. Yeasts and moulds were enumerated on plates of Sabouraud–chloramphenicol agar (Fluka, Bochemica 89579, Sigma-Aldrich Chemie GmbH, Bangalore, India), incubated at 30℃ for 4 days. Aerobic mesophiles were enumerated on plates of plate count agar (PCA Oxoid Ltd, Basingstoke, UK) and incubated at 30℃ for 2 days. Identification of the organisms isolated was based on cultural characteristics, morphology of cells and biochemical tests. The media and reagents were prepared as described by [12,13].

2.3 Isolation and Identification of Food-borne Pathogens

2.3.1 *Staphylococcus aureus*

Staphylococcus aureus was isolated and enumerated according to the method described by [14]. A volume of 01 ml of each dilution was surface plated on Baird-Parker agar (BPA) containing egg yolk tellurite emulsion (Oxoid) and incubated at 37℃ 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 schemes proposed by [15]. 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.

2.3.2 *Clostridium perfringens*

The method of [16] was used. The tryptone sulphite neomycine (TSN) agar (Bio-Rad, Marnes-La-Coquette, France) was used for the detection of *Cl. Perfringens* after a thermal shock of the dilutions (80℃ for 15 min and immediately

cooled). One (1) millilitre of each appropriate treated dilution was used to inoculate the tryptonesulphiteneomycine TSN agar (Bio-Rad) stored in surfusion at 45℃ in assay tubes. After the agar had solidified, all inoculated media were incubated in an upright position for 24 h at 46℃. Tubes containing between 30 and 300 colonies were counted, and five colonies were picked for confirmation in motility-nitrate medium.

2.3.3 *Bacillus cereus*

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 [17]. Presumptive colonies of *B. 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 [18].

2.3.4 *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 [19]. From each sample, 25 g was aseptically weighed and macerated in 225 ml of buffered peptone water (Oxoid) and incubated at 37℃ for 24 h. A selective enrichment in Tetrathionate broth (Mu ¨ller-Kauffmann) and Rappaport Vassiliadis soy peptone broth using 1 ml of previously incubated buffered peptone water was achieved at 37℃ for 24 h, followed by a subcultivationon Salmonella Shigella agar incubation at 35℃ for 24– 48 hours [20]. Colourless, transparent and with a black centre colonies were further identified using biochemical tests.

2.4 Isolation and Enumeration of Fungi

Yeasts and moulds were enumerated on plates of Sabouraud–chloramphenicol agar (Fluka, Biochemica 89579, Sigma-Aldrich Chemie GmbH) incubated at 30℃ for 2 days. The moulds were identified based on examination of the colonial heads, phialides, conidiophores and presence or absence of footcells or rhizoids [21].

2.5 Determination of pH and Total Titrable Acidity (TTA)

Thirty grams of cassava traditional inoculasamples were blended with 70 ml of sterile distilled water and filtered through a Whatmanfilter paper. The pH of 30 ml of the filtered solution was determined using a pH-meter (pH-meter P107, Consort, BioblockScientific, Illkirch, France). TTA was determined using the standard method described by [22]. Ten millilitres of filtered solution were titrated with NaOH 01 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 lactic acid. The determinations were carried out in triplicates and the mean value recorded.

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). Parametric tests (one-way variance analysis with Duncan's test) at 5% significance level were performed to determine whether there were significant differences between markets regarding microbiological data collected.

3. RESULTS

3.1 Cassava Traditional Inocula Used in *Attieke* Proccess

Table 1 shows some physico-chemical properties and microbiology hazard of cassava traditional inocula. pH values ranging between 4.36 (Grand-lahou localitie) and 4.94 (Sikensi localitie) while the total titrable acidity levels of the samples expressed as per cent lactic acid varied between 1.27% and 1.67%. The average aerobic mesophiles counts in the samples were comprised between $(2.2 \pm 0.7)10^9$ (Dabou localitie) and $(9.1\pm1.4)10^9$ CFU g^1 (Adzope localitie), while moulds $(7.4\pm2.2)10^6$ CFU.g^1 loads were the highest in Abidjan localitie. However Staphylococcus $(2.9 \pm 0.6)10^5$ CFU.g1 and faecal coliforms $(1.9 \pm 0.8)10^3$ CFU.g^1 loads were the highest, respectively, in Adzope and Jacqueville localitie. Divo localitie contained the highest loads of *Bacilli* spores $(2.5 \pm0.3)10^7$ CFU. g^1 and total coliforms $(4.4 \pm0.2)10^5$ CFU.$g^{1.}$

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

Parameters	Localities						
	Abidjan	**Dabou**	**Jacqueville**	**Grand-Lahou**	**Adzopé**	**Divo**	**Sikensi**
pH	4.56±0.2^{a}	4.54±0.1^{a}	4.85±0.5^{b}	4.36 ±0.1^{a}	4.71±0.9^{b}	4.55±0.2^{a}	4.94±0.4^{b}
TTA (%)	1.48 ±0.5^{a}	1.55±0.1^{a}	1.32±0.3^{b}	1.67±0.6^{a}	1.33±0.2^{b}	1.52 ±0.1a	1.27±0.2^{b}
AM (CFU.g^{-1})	(2.9± 0.3)10^{9a}	(2.2±0.7)10^{9a}	(7.4±1.2)10^{9b}	(5.3±0.9)10^{9b}	(9.1±1.4)10^{9b}	(4.2±0.6)10^{9b}	(2.8±0.2)10^{9a}
Moulds (CFU.g^{-1})	(7.4±2.2)10^{6a}	(6.9±1.7)10^{6a}	(6.2±0.9)10^{6a}	(6.7±0.5)10^{6a}	(5.8±1.2)10^{6a}	(6.1±1.4)10^{6a}	(5.4±0.7)10^{6a}
Stapylococci (CFU.g^{-1})	(2.7±0.3)10^{5a}	(2.4±0.4)10^{5a}	(2.1±0.5)10^{5a}	(1.7±0.1)10^{5a}	(2.9±0.6)10^{5a}	(1.3±0.2)10^{5a}	(1.9±0.3)10^{5a}
Bacilli (spores) (CFU.g^{-1})	(1.8±0.6)10^{7a}	(1.2±0.2)10^{7a}	(1.4±0.5)10^{7a}	(1.7±0.1)10^{7a}	(2.2±0.7)10^{7a}	(2.5±0.3)10^{7a}	(1.5±0.6)10^{7a}
Total coliforms (CFU.g^{-1})	(3.7±0.3)10^{5a}	(3.9±0.8)10^{5a}	(3.5±0.4)10^{5a}	(2.9±0.7)10^{5a}	(3.2±0.9)10^{5a}	(4.4±0.2)10^{5a}	(3.5±0.8)10^{5a}
Faecal coliforms (CFU.g^{-1})	(1.1±0.2)10^{3a}	(1.5±0.1)10^{3a}	(1.9±0.8)10^{3a}	(1.4±0.3)10^{3a}	(1.2±0.4)10^{3a}	(1.1±0.5)10^{3a}	(1.7±0.2)10^{3a}
Escherichia coli (CFU.g^{-1})	ab	ab	ab	ab	ab	ab	ab
Clostriduim perfringens (CFU.g^{-1})	ab	ab	ab	ab	ab	ab	ab
Salmonella (CFU.g^{-1})	ab	ab	ab	ab	ab	ab	ab

AM: aerobic mesophiles; ab: absence in 10 g for E. coli and Cl. perfringens and in 25 g for Salmonella, values are expressed as mean ± standard deviation. Means with different letters in the same line are significantly different (P <005)

Table 2. Microbial population in water used in *Attieke* process

Parameters	Localities						
	Abidjan	**Dabou**	**Jacqueville**	**Grand-Lahou**	**Adzopé**	**Divo**	**Sikensi**
AM (CFU.ml^{-1})	(2.6±0.1)10^{6a}	(6.7±0.7)10^{6b}	(3.4±0.8)10^{6a}	(8.2±0.3)10^{6b}	(4.5±0.9)10^{6a}	(2.9±0.1)10^{6a}	(4.2±0.3)10^{6a}
Moulds (CFU.ml^{-1})	ab	ab	ab	Ab	ab	ab	ab
Stapylococci (CFU.ml^{-1})	ab	ab	ab	Ab	ab	ab	ab
Bacilli(spores) (CFU.ml^{-1})	ab	ab	ab	Ab	ab	ab	ab
Total coliforms(CFU.ml^{-1})	(4.5±0.5)10^{3a}	(1.4±0.6)10^{4b}	(4.2±0.3)10^{3a}	(3.7±0.2)10^{3a}	(2.9±0.1)10^{3a}	(3.4±0.7)10^{3a}	(4.9±0.9)10^{3a}
Faecal coliforms(CFU.ml^{-1})	(2.1±0.8)10^{2a}	(2.5±0.4)10^{2a}	(4.7±0.6)10^{2a}	(1.9±0.3)10^{2a}	(3.5±0.5)10^{2a}	(3.1±0.2)10^{2a}	(1.7±0.2)10^{2a}
Escherichia coli(CFU.ml^{-1})	ab	ab	ab	ab	ab	ab	ab
Clostriduim perfringens (CFU.ml^{-1})	ab	ab	ab	ab	ab	ab	ab
Salmonella (CFU.ml^{-1})	ab	ab	ab	ab	ab	ab	ab

AM: aerobic mesophiles; ab: absence in 10 ml for E. coli and Cl. perfringens, moulds, Staphylococci, bacilli (spores)and in 25 gml for Salmonella, values are expressed as mean ± standard deviation. Means with different letters in the same line are significantly different (P <005)

Table 3. Microbial population in oil palm used in *attieke* process

Parameters	Localities						
	Abidjan	**Dabou**	**Jacqueville**	**Grand-Lahou**	**Adzopé**	**Divo**	**Sikensi**
AM (CFU.ml^{-1})	(5.2±0.4)10^{4a}	(3.8±0.3)10^{4a}	(2.7±0.8)10^{4a}	(9.7±0.9)10^{4b}	(3.7±0.7)10^{4a}	(2.5±0.1)10^{4a}	(3.2±0.4)10^{4a}
Moulds (CFU.ml^{-1})	(1.3±0.7)10^{3a}	(1.7±0.2)10^{3a}	(1.1±0.1)10^{3a}	(1.8±0.4)10^{3a}	(1.5±0.8)10^{3a}	(1.2±0.8)10^{3a}	(2.2±0.2)10^{3a}
Stapylococci (CFU.ml^{-1})	Ab	ab	ab	Ab	ab	ab	ab
Bacilli (spores) (CFU.ml^{-1})	Ab	ab	ab	Ab	ab	ab	ab
Total coliforms (CFU.ml^{-1})	(2.1±0.1)10^{3a}	(2.7±0.5)10^{3a}	(2.9±0.6)10^{3a}	(2.8±0.1)10^{3a}	(3.4±0.3)10^{3a}	(5.1±0.8)10^{3a}	(3.2±0.1)10^{3a}
Faecal coliforms (CFU.ml^{-1})	(5.2±0.8)10^{2a}	(2.8±0.9)10^{2a}	(3.6±0.2)10^{2a}	(4.4±0.4)10^{2a}	(4.5±0.1)10^{2a}	(2.9±0.5)10^{2a}	(3.2±0.8)10^{2a}
Escherichia coli (CFU.ml^{-1})	Ab	ab	ab	Ab	ab	ab	ab
Clostriduim perfringens	ab	ab	ab	ab	ab	ab	ab
Salmonella	ab	ab	ab	ab	ab	ab	ab

AM: aerobic mesophiles; ab: absence in 10 ml for E. coli and Cl. perfringens, moulds, Staphylococci, bacilli (spores)and in 25 gml for Salmonella, values are expressed as mean ± standard deviation. Means with different letters in the same line are significantly different (P <005)

3.2 Water Used in *Attieke* Proccess

Microbial population of water used of *attieke* process shown in Table 2. Total coliforms count values ranging between $(2.9\pm0.1)10^3$ CFU.ml^{-1} (Adzope localitie) and $(1.4\pm0.6)10^4$ CFU.ml^{-1} (Dabou localitie).The average faecal coliforms counts in the samples were comprised between $(1.7\pm0.2)10^2$ (Sikensi localitie) and $(4.7\pm0.6)10^2$ CFU ml^1 (Jacqueville localitie). All samples contain no mould*, Staphylococci, Echerichia coli, Clostridium perfringens* and *Salmonella.*

3.3 Oil Palm Used in *Attieke* Proccess

Table 3 shows microbial populations of oil palm used of *attieke* process. Aerobic mesophile count values were ranging between $(2.5\pm0.1)10^4$ CFU.ml^{-1} (Divo localitie) and $(9.7\pm0.9)10^4$ (Grand-lahou localitie). The average moulds were ranging between $(1.1\pm0.1)10^3$CFU.ml^{-1} (Jacqueville localitie) and $(2.2\pm0.2)10^3$CFU.ml^{-1} (Sikensi localitie. However total coliforms $(5.1\pm0.8)10^3$CFU.ml^{-1} and faecal coliforms $(5.2\pm0.8)10^2$ CFU.ml^{-1} loads were the highest, respectively, in Divo and Abidjan localities.

3.4 Types of Bacteria and Fungi Isolated from Cassava Traditional Inocula, Water and Palm Oil

Based on their morphological and biochemical characteristics, the bacteria strains were identified as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus* spp. *Staphylococcus aureus*, *Staphylococcus* ssp*, Citrobacter freundi, Enterobacter amnigenus, Enterobacter aerogenes, Enterobacter agglomerans, Citrobacter youngae, Klebsiella pneumoniae* and *Klebsiella oxytoca* (Tables 4, 5, 6). *Bacillus* species *(B. subtilis, B. cereus*), *Staphylococcus aureus*, and *citrobacter youngae* were the predominant bacteria in cassava traditional inocula of all the localities (Table 4). *Enterobacter* and *Citrobacter* species are the predominant bacteria in the palm oil used in the *attieke* process (Table 6). *Citrobacter* is the predominant genus of all localities in water used in *attieke* process (Table 5). The fungi isolated in samples from Abidjan and Dabou localitties were *Mucor* spp*., Rhizopus* spp*. Thamnidium* ssp*.* And *Fusarium* spp. The same fungi were isolated in samples from the other localities except Grand-lahou and Adzope localities (in cassava traditional inocula) and Jacqueville, Grand-lahou, Adzopé, Sikensi (palm oil used in attieke process) where *Moniella* spp. were isolated instead of *Rhizopus* spp. (Tables 7, 8)

4. DISCUSSION

In Côte d'Ivoire; *attieke* 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 000tons [23]. The production of *attieke* necessarily requires the use of ingredients such as the cassava traditional inocula, palm oil and water. Cassava traditional inocula contain several fermentatives microorganisms of cassava dough for attieke production.

Table 4. Types of bacteria isolated from cassava traditional inocula used in *attieke* process

Localities	Species	Mean counts (CFU.g^{-1})	Rate of isolate (%)
Abidjan	*Bacillus subilis*	$(5.4\pm0.1)10^{6a}$	30
	Bacillus cereus	$(9.1\pm0.8)10^{6a}$	50
	Bacillus ssp	$(3.6\pm0.3)10^{6a}$	20
	Staphylococcus aureus	$(1.6\pm0.7)10^{5a}$	60
	Staphylococcus ssp	$(1.1\pm0.6)10^{5a}$	40
	Klebsiella pneumoniae	$(6.3\pm0.3)10^{4a}$	17
	Klebsiella oxytoca	$(7.4\pm0.8)10^{4a}$	20
	Citrobacter youngae	$(1.5\pm0.4)10^{4a}$	40
	Enterobacter agglomerans	$(8.5\pm0.9)10^{4a}$	23
Dabou	*Bacillus subilis*	$(4.8\pm0.6)10^{6a}$	40
	Bacillus cereus	$(3.6\pm0.2)10^{6a}$	30
	Bacillus ssp	$(3.6\pm0.5)10^{6a}$	30
	Staphylococcus aureus	$(1.2\pm0.1)10^{5a}$	50
	Staphylococcus ssp	$(1.2\pm0.1)10^{5a}$	50
	Klebsiella pneumoniae	$(1.6\pm0.5)10^{5a}$	40
	Citrobacter youngae	$(1.9\pm0.9)10^{5a}$	50
	Enterobacter agglomerans	$(3.9\pm0.3)10^{4b}$	10

Localities	Species	Mean counts (CFU.g^{-1})	Rate of isolate (%)
Jacqueville	*Bacillus subilis*	(3.4±0.7)10^{6a}	20
	Bacillus cereus	(1.1±0.5)10^{7b}	60
	Bacillus ssp	(3.4±0.4)10^{6a}	20
	Staphylococcus aureus	(1.5±0.2)10^{5a}	70
	Staphylococcus ssp	(6.3±0.1)10^{4b}	30
	Klebsiella pneumoniae	(1.1±0.8).10^{5a}	32
	Klebsiella oxytoca	(4.9±0.7)10^{4b}	14
	Citrobacter youngae	(1.4±0.6)10^{5a}	40
	Enterobacter agglomerans	(4.9±0.1)10^{4b}	14
Grand-Lahou	*Bacillus subilis*	(6.8±0.5)10^{6a}	40
	Bacillus cereus	(1.1±0.1)10^{7b}	60
	Staphylococcus aureus	(8.5±0.9)10^{4a}	50
	Staphylococcus ssp	(8.5±0.4)10^{4a}	50
	Klebsiella pneumoniae	(8.7±0.2)10^{4a}	30
	Klebsiella oxytoca	(1.2±0.1)10^{5b}	40
	Citrobacter youngae	(8.7±0.3)10^{4a}	30
Adzope	*Bacillus cereus*	(1.8±0.2)10^{7a}	80
	Bacillus ssp	(4.4±0.4)10^{6b}	20
	Staphylococcus aureus	(1.7±0.1)10^{5a}	60
	Staphylococcus ssp	(1.2±0.3)10^{5a}	40
	Klebsiella pneumoniae	(6.4±0.8)10^{4a}	20
	Klebsiella oxytoca	(1.3±0.8)10^{5b}	40
	Citrobacter youngae	(3.2±0.2)10^{4a}	10
	Enterobacter agglomerans	(9.6±0.9)10^{4a}	30
Divo	*Bacillus subilis*	(1.8±0.4)10^{7a}	70
	Bacillus cereus	(7.5±0.8)10^{6b}	30
	Staphylococcus aureus	(6.5±0.1)10^{4a}	50
	Staphylococcus ssp	(6.5±0.5)10^{4a}	50
	Klebsiella pneumoniae	(2.4±0.4)10^{5a}	60
	Klebsiella oxytoca	(4.4±0.8)10^{4b}	10
	Citrobacter youngae	(1.3±0.3)10^{5a}	30
Sikensi	*Bacillus subilis*	(1.2±0.5)10^{7a}	80
	Bacillus cereus	(3.1±0.7)10^{6b}	20
	Staphylococcus aureus	(8.6±0.9)10^{4a}	45
	Staphylococcus ssp	(1.1±0.3)10^{5b}	55
	Klebsiella pneumoniae	(1.1±0.5)10^{5a}	30
	Klebsiella oxytoca	(1.1±0.1)10^{5a}	30
	Citrobacter youngae	(1.3±0.6)10^{5a}	40

Values are expressed as mean ± standard deviation. Means with different letters in the same column are significantly different (P < 005)

In the goal to implement a HACCP system for the production of *attieke* of good sanitary quality and without hazard to the consumer; it is necessary to identify the microbiological hazards in ingredients (cassava traditional inocula, palm oil, water) used in the production process to find the preventive measures of control.

The analyzes carried out on samples of traditional cassava ferment from 7 sampling areas in southern of Côte d'Ivoire has showed that ferments had an acidic pH. These results agree with those of [24], during the biochemical and microbiological characterization of the traditional cassava ferment for the production of *attieke* in Côte d'Ivoire. This pH was due to the fact that production of the traditional cassava ferment necessarily requires spontaneous fermentation. Microbiological analysis revealed an abundance of pathogenic microorganisms such as *Staphylococcus*, *Bacillus* and coliform in the cassava traditional inocula, water and as well as oil used for the production of *attieke* in Côte d'Ivoire. The presence of such micro-organisms in the cassava traditional inocula, palm oil and water samples (The water used during the production process *attieke*samples could be due to a further contamination after steaming,

Table 5. Types of bacteria isolated from water used in *attieke* process

Localities	Species	Meancounts (CFU.ml^{-1})	Rate of isolate (%)
Abidjan	*Klebsiella pneumoniae*	(9.1±0.1)10^{2a}	20
	Klebsiella oxytoca	(4.5±0.6)10^{2a}	10
	Citrobacter youngae	(9.1±0.8)10^{2a}	20
	Enterobacter aerogenus	(9.1±0.3)10^{2a}	20
	Enterobacter agglomerans	(1.4±0.7)10^{3b}	30
Dabou	*Citrobacter freundi*	(4.9±0.5)10^{3a}	35
	Citrobacter youngae	(2.1±0.4)10^{3a}	15
	Enterobacter agglomerans	(3.5±0.2)10^{3a}	25
	Enterobacter amnigenenus	(3.5±0.9)10^{3a}	25
Jacqueville	*Klebsiella pneumoniae*	(2.1±0.1)10^{3a}	50
	Klebsiella oxytoca	(8.4±0.9)10^{2b}	20
	Citrobacter youngae	(8.4±0.6)10^{2b}	20
	Enterobacter aerogenus	(4.2±0.4)10^{2b}	10
Grand-lahou	*Klebsiella pneumoniae*	(1.5±0.1)10^{3a}	40
	Klebsiella oxytoca	(3.7±0.5)10^{2b}	10
	Citrobacter freundi	(7.4±0.8)10^{2b}	20
	Citrobacter youngae	(1.1±0.3)10^{3a}	30
Adzope	*Klebsiella pneumoniae*	(4.4±0.7)10^{2a}	15
	Klebsiella oxytoca	(2.9±0.1)10^{2a}	10
	Citrobacter youngae	(5.8±0.6)10^{2b}	20
	Enterobacter agglomerans	(1.6±0.3)10^{3a}	55
Divo	*Klebsiella pneumoniae*	(1.5±0.2)10^{3a}	45
	Klebsiella oxytoca	(3.4±0.9)10^{2b}	10
	Citrobacter youngae	(6.8±0.7)10^{2b}	20
	Citrobacter freundi	(8.5±0.1)10^{2b}	25
Sikensi	*Klebsiella pneumoniae*	(2.5±0.8)10^{3a}	50
	Klebsiella oxytoca	(1.5±0.2)10^{3a}	30
	Citrobacter youngae	(4.9±0.9)10^{2b}	10
	Enterobacter aerogenes	(4.9±0.5)10^{2b}	10

Values are expressed as mean ± standard deviation. Means with different letters in the same column are significantly different (P < 005)

by the production environment, the material of production, the product handling and during the packaging. Because of this, it is essential that the food be produced of high sanitary quality. It would be useful if there could be legislation for this condition. Similar observations were reported by [25] Ofuya 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 [26,27], an environment containing high sugar and moisture contents constituted a favourable medium for yeasts and moulds, Enterococci and coliforms development.

Mould isolated from traditional cassava inocula cassava ferment as well as palm oil used in the production of *attieke* are *Mucor* spp., *Rhizopus* spp., *Fusarium* spp., *Thamnidium* spp. And *Moniella* spp. This is similar to the findings of [28, 29] who isolated some fungi from 'fufu' flour stored at different relative humidities in ambient condition. These same moulds were isolated in a study on the quality of attiéké sold in the Abidjan markets by [30]. Moreover, market studies in the lake zone of Tanzania had indicated that different moulds could have an impact on the value of the commodity [31]. In addition, some of these moulds could produce heat resistant and deadly toxins [32]. Studies on isolation of pathogenic bacteria from traditional food in this investigation indicated that some gram-negative bacteria and gram-positive bacteria were isolated and recorded in Table 3. The food bacteria of greatest importance to human pathology are the most common causes of human infection and extensively widespread in the environment using fast foods [33]. All samples of the cassava traditional inocula analysed did not contain *Escherichia coli*, *Salmonella* spp. and *Clostridium perfringens*. The absence of

Table 6. Types of bacteria isolated from oil palm used in *attieke* process

Localitie	Species	Mean counts (CFU.ml^{-1})	Rate of isolate %
Abidjan	*Klebsiella pneumoniae*	(8.4±0.8)10^{2a}	40
	Klebsiella oxytoca	(5.3±0.2)10^{2a}	25
	Citrobacter youngae	(5.3±0.6)10^{2a}	25
	Enterobacter agglomerans	(2.1±0.4)10^{2b}	10
Dabou	*Klebsiella pneumoniae*	(1.3±0.3)10^{3a}	50
	Klebsiella oxytoca	(2.7±0.9)10^{2b}	10
	Citrobacter youngae	(5.4±0.7)10^{2b}	20
	Enterobacter agglomerans	(5.4±0.5)10^{2b}	20
Jacqueville	*Klebsiella pneumoniae*	(5.8±0.4)10^{2a}	20
	Klebsiella oxytoca	(4.4±0.9)10^{2a}	15
	Citrobacteryoungae	(4.6±0.2)10^{2a}	16
	Enterobacter agglomerans	(1.4±0.5)10^{3b}	49
Grand-Lahou	*Klebsiella pneumoniae*	(3.9±0.9)10^{2a}	14
	Klebsiella oxytoca	(5.6±0.3)10^{2a}	20
	Citrobacter youngae	(1.4±0.8)10^{3b}	50
	Enterobacter agglomerans	(4.5±0.4)10^{2a}	16
Adzope	*Klebsiella pneumoniae*	(6.1±0.3)0^{2a}	18
	Klebsiella oxytoca	(6.8±0.8)10^{2a}	20
	Citrobacter youngae	(1.4±0.1)10^{3b}	40
	Enterobacter agglomerans	(7.5±0.7)10^{2a}	22
Divo	*Klebsiellapneumoniae*	(1.3±0.4)10^{3a}	25
	Klebsiella oxytoca	(1.1±0.2)10^{3a}	20
	Citrobacter youngae	(1.5±0.6)10^{3a}	30
	Enterobacter agglomerans	(7.7±0.8)10^{2b}	15
Sikensi	*Klebsiellapneumoniae*	(5.8±0.4)10^{2a}	18
	Klebsiella oxytoca	(6.4±0.1)10^{2a}	20
	Citrobacter youngae	(6.4±0.9)10^{2a}	20
	Enterobacter agglomerans	(1.3±0.2)10^{2b}	42

Values are expressed as mean ± standard deviation. Means with different letters in the same column are significantly different (P < 005)

Salmonella, Escherichia coli and *Clostridium perfringens* in cassava traditional inocula samples could be due to the low pH. In fact, the combined effect of organic acids produced during the fermentation period may possibly exert bacteriostatic effect on spoilage organisms and pathogens that might be present [34,35]. Species of bacteria isolated from traditional cassava inocula, in the palm oil as well as water used for the production of *attieke* are *Bacillus subtilis, Bacillus cereus, Bacillus* spp*. Staphylococcus aureus, Citrobacter freundi, Enterobacter amnigenus, Enterobacter aerogenes, Enterobacter agglomerans, Citrobacter youngae, Klebsiella pneumoniae and Klebsiella oxytoca*. These same species were isolated by [36] in the identification of hazards and critical control points in the production of *attieke* in Côte d'Ivoire. [37] reported that some micro-organisms are harmful and cause disease while others are benevolent neutral, or even helpful. The role of *Bacillus cereus* as an aetiological agent of food-borne disease was described by [38-40] showed that gastrointestinal disease has been reported by eating raw or inadequately cooked foods containing *bacillus* spores. *Bacillus cereus* causes food poisoning by means of enterotoxins. Because of the resistance of endospores to chemical disinfectants, autoclaving is the only reliable means of decontamination*. Bacillus subtilis* and *B. coagulans* were also isolated from traditional food samples and from fastfood samples [41]. The presence of *Staphylococcus aureus* in foods is not uncommon [42]. Human contact with cooked food invariably adds *Staphylococcus aureus* at levels 10^1 or 10^2 to many sample units [43]. Such levels are harmless but offer sufficient inoculum for growth [44]. An average prevalence of 198% *Staphylococcus aureus* was found in 10 ready-toeat consumer food types sold in Trinidad [45]. The presence of *Staphylococcus aureus* at unsatisfactory levels may be indicative of poor personal hygiene by food handlers involved in the preparation of cassava traditional inocula and/or poor temperature control. Indeed, the

extensive handling normally associated with the preparation of cassava traditional inocula lends itself to contamination by food handlers if good hygienic practices are not implemented. Unavoidable contamination usually will add coliforms to the product. The high number of coliforms is also a sign of unsanitary conditions and/or postprocessing contamination.

Table 7. Types of fungi isolated from cassava traditional inocula used in *attieke* process

Localities	Genus	Mean counts (CFU.g^{-1})	Rate of isolate (%)
Abidjan	*Thamnidium*	$(1.5\pm0.1)10^{6a}$	20
	Mucor	$(7.4\pm0.4)10^{5b}$	10
	Fusarium	$(2.9\pm0.2)10^{6a}$	40
	Rhizopus	$(2.2\pm0.1)10^{6a}$	30
Dabou	*Thamnidium*	$(4.1\pm0.5)10^{6a}$	60
	Mucor	$(6.9\pm0.8)10^{5b}$	10
	Fusarium	$(6.9\pm0.3)10^{5b}$	10
	Rhizopus	$(1.4\pm0.2)10^{6a}$	20
Jacqueville	*Thamnidium*	$(1.9\pm0.4)10^{6a}$	30
	Mucor	$(1.2\pm0.1)10^{6a}$	20
	Fusarium	$(6.2\pm0.9)10^{5b}$	10
	Rhizopus	$(2.5\pm0.7)10^{6a}$	40
Grand-Lahou	*Thamnidium*	$(1.7\pm0.1)10^{6a}$	25
	Mucor	$(6.7\pm0.2)10^{5b}$	10
	Fusarium	$(3.3\pm0.2)10^{5b}$	5
	Moniella	$(4.1\pm0.3)10^{6a}$	60
Adzope	*Thamnidium*	$(8.7\pm0.8)10^{5a}$	15
	Mucor	$(1.2\pm0.3)10^{6b}$	20
	Fusarium	$(2.9\pm0.1)10^{5a}$	5
	Moniella	$(3.5\pm0.6)10^{6b}$	60
Divo	*Thamnidium*	$(2.8\pm0.1)10^{6a}$	45
	Mucor	$(1.2\pm0.9)10^{6a}$	20
	Fusarium	$(3.1\pm0.4)10^{5b}$	5
	Rhizopus	$(1.8\pm0.2)10^{6a}$	30
Sikensi	*Thamnidium*	$(1.1\pm0.8)10^{6a}$	20
	Mucor	$(1.1\pm0.3)10^{6a}$	20
	Fusarium	$(5.4\pm0.7)10^{5b}$	10
	Moniella	$(2.7\pm0.2)10^{6a}$	50

Values are expressed as mean ± standard deviation. Means with different letters in the same column are significantly different (P < 005)

Table 8. Types of fungi isolated from palm oil used in *attieke* process

Localities	Genus	Mean counts (CFU.ml^{-1})	Rate of isolate (%)
Abidjan	*Mucor*	$(2.6\pm0.5)10^{2a}$	20
	Fusarium	$(2.6\pm0.6)10^{2a}$	20
	Rhizopus	$(7.8\pm0.2)10^{2b}$	60
Dabou	*Thamnidium*	$(5.1\pm0.1)10^{2a}$	30
	Mucor	$(4.1\pm0.8)10^{2a}$	24
	Fusarium	$(3.4\pm0.1)10^{2a}$	20
	Rhizopus	$(4.4\pm0.9)10^{2a}$	26
Jacqueville	*Thamnidium*	$(2.4\pm0.7)10^{2a}$	22
	Mucor	$(1.9\pm0.2)10^{2a}$	18
	Fusarium	$(5.5\pm0.5)10^{2a}$	50
	Moniella	$(1.1\pm0.1)10^{2a}$	10
Grand-Lahou	*Thamnidium*	$(1.1\pm0.7)10^{3a}$	60
	Mucor	$(1.8\pm0.5)10^{2b}$	10
	Fusarium	$(1.8\pm0.2)10^{2b}$	10
	Moniella	$(3.6\pm0.3)10^{2b}$	20

Localities	Genus	Mean counts (CFU.ml^{-1})	Rate of isolate (%)
Adzope	*Thamnidium*	(4.5±0.4)10^{2a}	30
	Mucor	(3.1±0.1)10^{2a}	20
	Fusarium	(6.1±0.2)10^{2b}	40
	Moniella	(1.5±0.1)10^{2a}	10
Divo	*Thamnidium*	(2.4±0.4)10^{2a}	20
	Mucor	(2.4±0.3)10^{2a}	20
	Fusarium	(6.1±0.6)10^{2b}	50
	Rhizopus	(1.2±0.1)10^{2a}	10
Sikensi	*Thamnidium*	(3.9±0.8)10^{2a}	18
	Mucor	(4.4±0.7)10^{2a}	20
	Fusarium	(2.6±0.4)10^{2a}	12
	Moniella	(1.1±0.2)10^{3b}	50

5. CONCLUSION

Ingredients used for the production of *attieke* contain pathogens. These pathogens can cause public health problems. These ingredients are contaminated by the staff, the environment and the utensils. For the implementation of HACCP, good hygiene practices and manufacturing are needful. The establishing of critical control points (CCP) in the production of traditional cassava ferment and as well as the use of water and oil are primordial for obtaining an *attieke* without danger to the consumer.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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