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Microbiological safety and quality assessment of a very appreciate traditional ready to eat plantain food, sold in retails markets

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The microbiological quality of "Dockounou", a traditional appreciate ready to eat plantain food marketed in Côte d'Ivoire was investigated. Microbial load was estimated by plate count using decimal dilution method. Culture medium was made selective depending on the species. Total aerobic mesophilic bacteria on principal component analysis (PCA) ranged from 32.10^4 to 44.10^4 cfu/g while yeast and moulds were estimated between 10 to 10^2 cfu/g. Enterococci and Lactobacilli counts ranged from 0 to 100 cfu/g and 41.10^3 to 68.10^3 cfu/g, respectively. Two thermotolerant bacteria were isolated: *Bacillus licheniformis* and *Bacillus macerans*. Baked and boiled "dockounou" were free from *Salmonella*, *E. coli*, *Staphylococcus* and *Clostridium*. The microbiological analysis showed the presence of high counts of microorganisms probably due to poor sanitary practices from empiric traditional manufacturing procedures. Hence, it is recommended to apply good hygienic and storage practices with novel technology that offer full sanitary guarantees to the consumers.

Key words: Plantain, ready to eat food, Dockounou, manufacturing process, microbiological quality.

INTRODUCTION

Plantain (*Musa* spp.) is a major food crop in the humid and sub-humid parts of Africa and valuable source of energy for millions of people in these regions (John and Marchal, 1995; Bakry et al., 2002). In fact, plantain constitutes an agriculture product with carbohydrates accounting for 22% of fruit weight and rich in vitamins A, B₆, C, minerals and dietary fibre (Chandler, 1995; Honfo et al., 2007). This vegetable resource contributes significantly to food security and provides more than 25 and

10% of the daily intake of carbohydrates and calories, respectively, for more than 70 million people in Sub-Saharan Africa (IITA, 2000).

Plantain is a highly perishable fruit, which requires processing into a more stable and convenient form. Indeed, the difficulties of plantain preservation result from its easy ripening at ambient temperature, leading to a qualitative and quantitative degradation along the distribution chain (Chia and Huggins, 2003). To face the problem of short

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Abbreviations: BEA, Bile esculin agar, TSC, tryptose sulphite cycloserine; YGC, yeast extract glucose chloramphenicol; MRS, de Man, Rogosa and Sharpemrs; VRBL, violet red bile lactose; VRBG, violet red bile glucose, RVS, Rappaport-Vassiliadis Soya, SS, *Salmonella-Shigella*.

shelf life of plantain, processing or preservation treatments are often used with the aim to decrease the post-harvest losses (Dongo et al., 2011).

The methods used for plantain processing for immediate human consumption include three main techniques: Boiling or steaming, baking or roasting and frying (Coursey, 1981). For example, in West Africa, plantains are often pounded in a mortar after boiling to form dough known variously as “fufu”, “foofoo”, “foufou” or “foutou” which is eaten with soup or a sauce of meat and vegetables (Lassoudière, 1973). Frying ripe or unripe slices of plantain in oil, usually palm or groundnut oil, is also popular in this region of Africa (Tezenas du Montcel, 1979). In Ghana, a type of pancake known as “fatale” is prepared from a mixture of pounded ripe plantain and fermented whole meal maize dough (Dei-Tutu, 1975). The pounded plantain pulp is mixed with the fermented maize dough into a paste which is seasoned with ginger, pepper, onion and salt and then fried in palm oil.

In Côte d'Ivoire, a marketed plantain-based food known as “Dockounou” is widely consumed by urban and rural populations. This traditional pancake is prepared by cooking or baking a mixture of pounded ripe plantains and cereal flour. This food is considered as a ready to eat food because it can be immediately consumed on the point of sale (Tsang, 2002). In addition, “Dockounou” is sold in markets without hygienic practices and this food is very often manipulated, coming in direct contact with the hand of customers before purchasing. Moreover, products which are not sold are very badly preserved. Therefore, a particular attention may be attached to the microbiological status of “Dockounou”. Indeed, ready to eat foods are mostly involved in food borne diseases and illnesses due to the microbial contamination during processing, preparation or conservation (Torok et al., 1997). These food borne diseases and illnesses resulting from ingestion of bacteria and toxins produced by microorganisms constitute nowadays, a major public health problem (Duff et al., 2003).

Generally, it has been estimated that six pathogens such as *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringens*, *Salmonella* spp. and *Staphylococcus aureus* account for approximately 7 million cases of food borne illnesses (Talaro, 1996). To date, there is no report on the microbiological characterization of “Dockounou”. So, the aim of this work is to investigate microbiological quality of this traditional food in order to avoid food poisoning of consumers through good hygienic and manufacturing practices.

MATERIALS AND METHODS

Samples collection and preparation

Thirty (30) different samples of boiled “Dockounou” and (30) of baked “Dockounou” were purchased from retail markets in the city of Abidjan in October 2011 after exhaustive inventory of traditional

producers. All samples were collected in sterile condition, and were transported aseptically in an icebox (4 - 6°C) to the laboratory for further analysis. The collected samples were mixed together in a sterile mortar to constitute one unit sample of boiled “Dockounou” and one unit sample of baked “Dockounou”.

Culture media

All culture media and chemical reagents used for microbiological analysis were analytical grade. Tryptone salt broth, plate count agar, Baird-Parker base agar, Bile Esculin Agar (BEA) agar, tryptose sulphite cycloserine (TSC) agar (all from AES Lab, France), yeast extract glucose chloramphenicol (YGC) agar, de Man, Rogosa and Sharpemrs agar (both from Scharlau, Spain), violet red bile lactose (VRBL) agar, violet red bile glucose (VRBG) agar, Rappaport-Vassiliadis Soya Peptone (RVS) broth, *Salmonella-Shigella* (SS) agar and Hektoen agar (all from Bio-Rad, France) were prepared and sterilized according to manufacturer's instruction.

Microbiological analysis

Enumeration of microorganisms

Before microbiological analysis, 25 g of each unit sample were homogenized with 225 ml of tryptone-salt in a Stomacher (Seward, UK) and then ten-fold serial dilutions (10^{-1} – 10^{-5}) were prepared with tryptone-salt (AFNOR, 1996). Total aerobic mesophilic bacteria counts were performed as described by AFNOR (1996). One (1) ml of each dilution was inoculated on plate count agar and the plates were incubated at 30°C for 72 h. Total psychrotrophic bacteria counts were made on plate count agar at 7°C for 10 days (Mourad and Nour-Eddine, 2006).

The enumeration of total coliform bacteria and *E. coli* were made on VRBL agar after incubation at 30°C for 24 h (coliform bacteria) and at 44°C for 24 h (*E. coli*) according to Kornacki and Johnson (2001). Enterobacteria count was performed on VRBG agar after pre-enrichment of diluted sample (37°C for 6 h) and incubation of inoculated plate at 37°C for 24 h (AFNOR, 1996). The search of *Salmonella* was carried out in three (3) steps as described by AFNOR (1996). The diluted sample (25 g into 225 ml of tryptone-salt) was incubated at 37°C for 6 h to aid in the recovery of any damaged cells. Afterward, one (1) ml of pre-enrichment broth was inoculated into 10 ml of RVS broth and the whole tube was incubated at 42°C for 24 h. Then, aliquot (100 µl) of enrichment broth were spread on *Salmonella-Shigella* (SS) agar and Hektoen agar. Both plates were incubated at 37°C for 24 h.

Staphylococcus, *Enterococcus* and *Clostridium* spp. counts were performed on Baird-parker, BEA and TSC agar, respectively after incubation at 37°C for 48 h (*Staphylococcus* and *Enterococcus*) and 46°C for 48 h (*Clostridium* spp.) as described by AFNOR (1996). Yeast and moulds were enumerated in YGC agar. The plates were incubated at 30°C for 3 days according to Hama et al. (2009). Enumeration of Lactobacilli was made according to deMan et al. (1960) and Garcia et al. (1987). The culture media used was MRS agar adjusted to pH 5.4 with sodium acetate for growth inhibition of other microorganisms. The plates were then incubated at 30 °C for 72 h under anaerobic conditions (Gaspak system). Thermotolerant bacteria were enumerated as described by Reda (2007). Three test tubes containing 5 ml of diluted sample (25 g into 225 ml of tryptone-salt) were placed in water baths set at 45, 55 and 65°C for 15 min, respectively. Then, aliquot (1 ml) of each tube was inoculated in plate count agar and plates were incubated at 30°C for 72 h.

Table 1. Microorganisms counts (cfu/g) of baked and boiled “Dockounou”.

Microbial group	Baked “Dockounou”	Boiled “Dockounou”
Total aerobic bacteria	$42 \times 10^4 \pm 2.10^4$ ^a	$34 \times 10^4 \pm 16.10^3$ ^b
Enterobacteria	0 ± 0.00 ^a	0 ± 0.00 ^a
Coliforms	0 ± 0.00 ^a	0 ± 0.00 ^a
<i>E. coli</i>	0 ± 0.00 ^a	0 ± 0.00 ^a
<i>Salmonella</i>	0 ± 0.00 ^a	0 ± 0.00 ^a
<i>Enterococcus</i>	$10^2 \pm 0.00$ ^a	0 ± 0.00 ^b
<i>Staphylococcus</i>	0 ± 0.00 ^a	0 ± 0.00 ^a
<i>Clostridium</i>	0 ± 0.00 ^a	0 ± 0.00 ^a
Yeast and moulds	10 ± 0.00 ^a	$10^2 \pm 0.00$ ^b
<i>Lactobacillus</i>	$66 \times 10^3 \pm 2.10^3$ ^a	$42 \times 10^3 \pm 9.10^2$ ^b
Psychrotrophic bacteria	0 ± 0.00 ^a	0 ± 0.00 ^a

Data represents mean \pm SD of triplicate analysis. Different lower case letters (a or b) within column indicate significant difference at $p < 0.05$.

Colonies counting

After the incubation periods, colonies were counted by using a colony counter (JP Selecta, Spain) and results were expressed as colony forming unit per gram (cfu/g) of sample.

Identification of thermotolerant bacteria

Thermotolerant bacteria colonies on Plate count agar were sub-cultured on nutrient agar and plates were incubated at 30°C for 72 h. The purified isolates were then characterized and identified using their colonial, morphological and biochemical characteristics with reference to the Bergey’s Manual of Determinative Bacteriology (Bergey and Holt, 1994).

Statistical analysis

Each sample was analyzed in triplicate and data were reported as means. Differences between means were performed by analysis of variance (one way ANOVA) using StatPlus 2008 (Analystsoft Inc) software. Statistical significance was stated at $p < 0.05$.

RESULTS AND DISCUSSION

The microbial load found in the samples is shown in Table 1. Mesophilic aerobic bacteria count was 42×10^4 and 34×10^4 cfu/g for baked “Dockounou” and boiled “Dockounou”, respectively. As regards, *Enterococcus* count, baked “Dockounou” was contaminated with 10^2 cfu/g, while boiled “Dockounou” was free of this bacteria specie. The count of yeast and moulds was 10 and 10^2 cfu/g for baked “Dockounou” and boiled “Dockounou”, respectively. The lactic acid bacteria (*Lactobacillus*) count was 66×10^3 and 42×10^3 cfu/g for baked “Dockounou” and boiled “Dockounou”, respectively. Psychrotrophic bacteria, total coliform, enterobacteria, *Salmonella*, *Staphylococcus* and *Clostridium* were not detected in baked and boiled “Dockounou”.

The counting of thermotolerant bacteria isolated from boiled and baked “Dockounou” is shown in Figure 2. Thermotolerant bacteria with 50 and 10 cfu/g at 45 and 5°C, respectively were isolated from boiled “Dockounou” while baked “Dockounou” was contaminated by 30 cfu/g at 45°C. Thermotolerant bacteria at 65°C were not detected in both baked and boiled “Dockounou”. The characteristics of the two isolated thermotolerant bacteria are shown in Table 2. The main common morphological, cultural and biochemical characteristics were: gram (+) rods, catalase positive, forms endospores, growth on nutrient agar at 45°C, glucose fermentation, motile, indole negative, citrate negative, urease negative, nitrate reduction and gelatin hydrolysis. The two isolated thermotolerant bacteria were identified as *Bacillus licheniformis* and *Bacillus macerans*.

The main steps of “Dockounou” production are: Washing, peeling, pulp crushing, mixing with cereal flour, fermentation (optional), wrapping in plantain leaves, boiling or baking (Figure 1). In the manufacturing process of “Dockounou”, the plantains were washed once or twice with potable water to eliminate all impurity. Operations such as peeling and pulp crushing were doing manually by using ordinary knife and traditional wood mortar, respectively. Cereal flour used for mixing was rice or maize flour. The time of fermentation depends on the producer appreciation and varied from 6 to 24 h. In the traditional processing, the time of boiling or baking depend on the products quantity. Nevertheless, this time could be estimated between 1 to 2 h. Each step can be source of contamination by microorganisms because of bad manipulations.

The presence of high level of total mesophilic aerobic bacteria count in foods is usually linked to their microbiological safety (Guiraud, 1998). Indeed, this bacteria group includes all mesophilic pathogens and non pathogens. Despite their high level of contamination ($30 - 50 \times 10^4$

Table 2. Morphological and biochemical characteristics of thermotolerant bacteria isolated from baked and boiled “Dockounou”.

Parameter	Isolate	
	A	B
Gram reaction	+	+
Cellular morphology	rods	rods
Catalase test	+	+
Spore forming	+	+
Growth at 45°C	+	+
Growth at 55°C	+	-
Growth at 65°C	-	-
Glucose	+	+
Gas	-	+
Motility	+	+
VP test	+	-
Indole test	-	-
Citrate test	-	-
Urease test	-	-
Nitrate reduction test	+	+
Lecithin hydrolysis test	-	-
Gelatin hydrolysis test	+	+
Probable specie	<i>Bacillus licheniformis</i>	<i>Bacillus licheniformis</i>

-, No growth; +, growth.

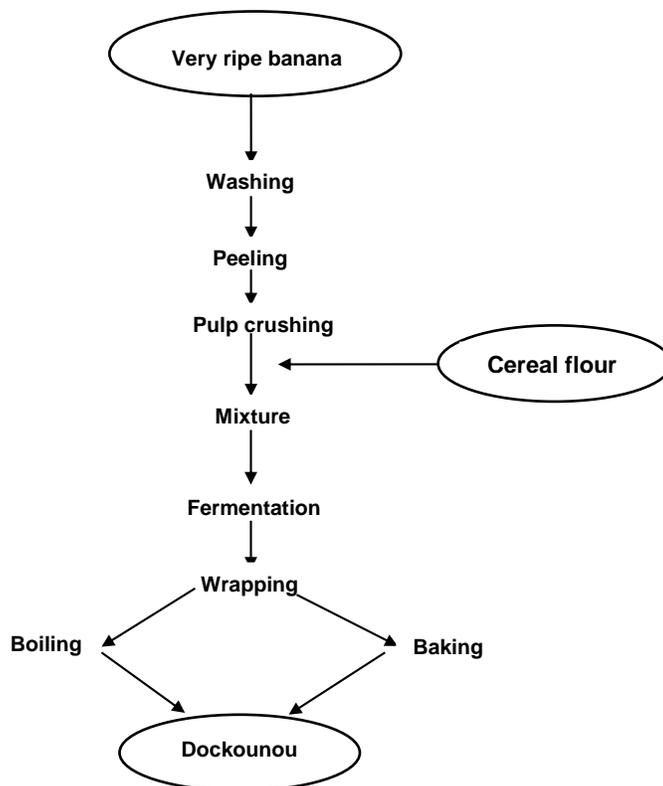


Figure 1. Manufacturing process of “Dockounou”.

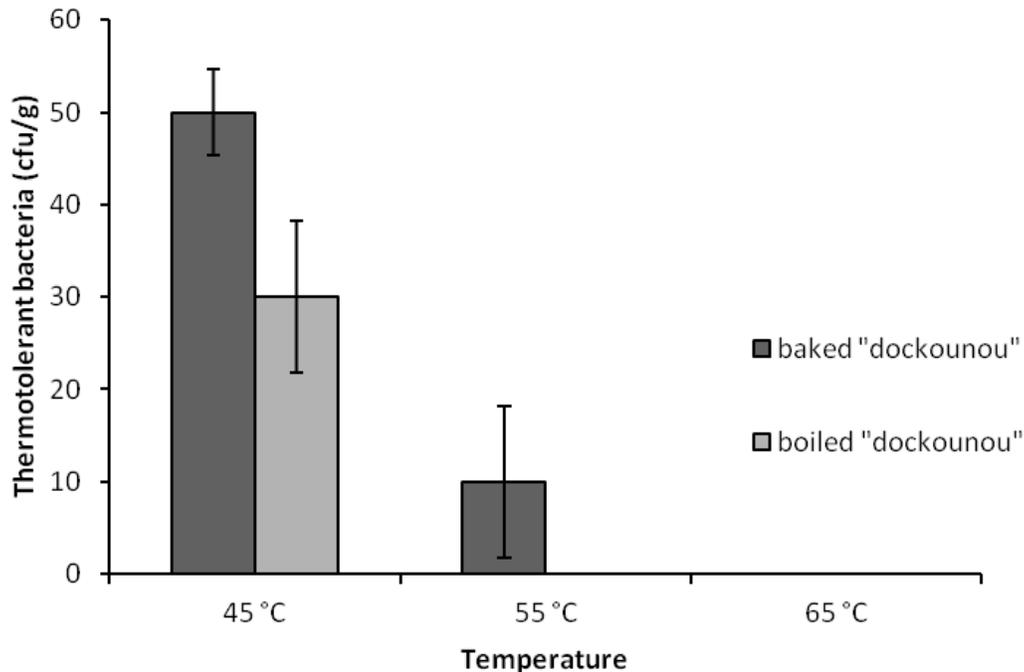


Figure 2. Thermotolerant bacteria counts isolated from baked and boiled "Dockounou".

cfu/g), boiled "Dockounou" and baked "Dockounou" samples contain total mesophilic aerobic bacteria count less than 10^6 cfu/g. In addition, the analyzed foods are exempt of pathogenic bacteria such as *Salmonella*, *Staphylococcus*, *Clostridium* and *Escherichia coli* which are usually involved in food poisoning (ICMSF, 1978; Bourgeois et al., 1996). Indeed, bacteria and toxins produced by those micro-organisms are involved in food borne diseases and illnesses.

Nevertheless, baked "Dockounou" is contaminated by bacteria which belong to the genus of *Enterococcus*. Enterococci are widely distributed in the environment (soil, surface waters, plants and vegetables), principally inhabiting the human and warm-blooded animal gastrointestinal tract (Mundt, 1986). Furthermore, it has been shown that enterococci and coliforms are considered as hygiene indicators in the industrial processing of foods (Biorollo et al., 2001). The presence of enterococci in baked "Dockounou" may be explained by the resistance of these bacteria to pasteurization temperatures and their adaptability to different substrates and growth conditions at low and high temperature, extreme pH, and salinity (Moreno et al., 2006). These properties implies that enterococci can be found either in food products manufactured from raw materials and in heat-treated food products. Contrary to boiled "Dockounou", the consumption of baked "Dockounou" may cause intra-abdominal infections following enterococci development in foods (Endtz et al., 1999). So, to avoid food borne illnesses due to enterococci, baked "Dockounou" must be prepared

with good manufacturing practices and good conditions of storage. After processing, this food may be refrigerated to inhibit the development of microorganisms.

Yeast and moulds count in baked "Dockounou" were ten (10) times lower than that of boiled "Dockounou". This result may be due to the residual moisture of boiled "Dockounou" which constitutes a favourable condition of growth of yeast and moulds in foods (Pitt and Hocking, 2009). Yeast and moulds growth on processed foods leads to textural and sensorial changes: softening, off-odours and off-flavours. The most important aspect is, however, the formation of mycotoxins. Mycotoxins are secondary fungal metabolites and are toxic to humans and animals, causing disorders like cancer, immune suppression, or endocrine disruption. Since mycotoxins are very stable and mainly resistant against heat treatment and acidic environment, they remain in the food during processing and storage, causing a serious food safety problem (Filtenborg et al., 1996). Therefore, temperature level and storage conditions as relative humidity of atmosphere must be critical control points during processing of baked and boiled "Dockounou". Lactobacilli counts were in the range of $40 - 70 \times 10^3$ cfu/g for baked and boiled "Dockounou". Lactobacilli are lactic acid bacteria which are involved in the fermentation step of "Dockounou" processing. Indeed, during the course of lactic acid fermentation, counts of lactobacilli increase (Hama et al., 2009). The growth of lactobacilli is usually linked to the production of volatile flavour compounds. The development of lactobacilli bacteria is also stimulated

by the presence of yeasts which provide soluble nitrogen compounds and factors e.g. B-vitamin (Nout, 1991). Mensah et al. (1991) believe that other substances (bacteriocin) produced by dominating lactobacilli may contribute to the disappearance of *Enterobacteriaceae* as observed in baked and boiled "Dockounou".

Bacilli are spore forming bacteria that are widely distributed in nature, and commonly associated with a variety of food products. The species isolated from baked and boiled "Dockounou" are *B. licheniformis* and *B. macerans*. These two species have been traditionally associated with spoilage of food products, in many cases with a pH as low as 3.9 (Hanlin, 1998). Additionally, there are many reports of *Bacillus* spp. surviving baking processes, a property caused by the production of exopolysaccharids (Thomson et al., 1998; Pepe et al., 2003). From the food safety point of view, *B. licheniformis* and *B. macerans* are not considered as human pathogenic bacteria contrary to *Bacillus cereus*, known for its ability to form toxins and cause food borne illnesses (Rodriguez - Lozano et al., 2010). Even if these two bacteria are not pathogenic, nutritive value of baked and boiled "Dockounou" may be decreased due to the degradation of polysaccharids, proteins and lipids by enzymes (amylases, proteases and lipases) produced by *Bacillus* spp. (Priest, 1977).

Psychrotrophic bacteria were not detected in baked and boiled "Dockounou". This bacteria group is defined as those that can grow at 7°C or below, including pathogenic strains such as *Listeria monocytogenes*, *Yersinia enterocolitica* and *B. cereus* (Griffiths, 1994). Development of psychrotrophic bacteria depends on the relative humidity of the store atmosphere. Indeed, the association of bacterial species *Pseudomonas* – *Acinetobacter* (non pathogenic psychrotrophic bacteria) can not develop in the atmosphere with relative humidity below 95% (Vasut and Robeci, 2009). The non detection of psychrotrophic bacteria in baked and boiled "Dockounou" may be advantageous for storage of these foods at refrigeration temperature.

Conclusion

"Dockounou" sold in the market, regarding the high level of total count of aerobic mesophilic microorganism, are of bad microbiological quality. Nevertheless, absence in this food of pathogen bacteria such as *Salmonella*, *Staphylococcus*, Psychrotrophic bacteria, *Clostridium*, *Escherichia coli*, Coliform and Enterobacteria show that "Dockounou" sold in the market are in satisfactory microbiologic condition and can be consumed. However, in order to obtain best quality of "Dockounou", it is necessary to apply good hygienic and storage practices with novel technology that offer full sanitary guarantees to the consumers. Moreover further study is needed for improving processing manufacturing technology of this

food. At the present, this food is manufactured by using empirical traditional procedures. Then, urgent measures to draw attention of the producers on the hygiene must be applied by the Ministry of health in order to avoid serious sanitary problems within the population

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