Microbiological Assessment of Production Stages of Fried Bean Cake (Akara) in Nigeria

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Analysis of fried bean cake (Akara), a legume-based product, was carried out to determine the microbial hazard associated with each of the five stages of its production. Various microorganisms were identified at different stages of Akara production and they include species of *Bacillus, Staphylococcus, Pseudomonas, Alcaligenes, Serratia, Aspergillus, Penicillium, Mucor, Fusarium, Rhizopus, Saccharomyces* and *Candida.* The total viable bacterial counts ranged from 4.5×10^3 cfu/g to 1.3×10^6 cfu/g, while the counts of *Staphylococcus* ranged from $1.8 \times 1\ 0^2$ cfu/g to 3.6×10^5 cfu/g and fungal counts ranged from 5.1×10^1 cfu/g to 2.6×10^4 cfu/g. The index of microbial load (10^4-10^6) was high at stages 2 and 3 of the production process, which are the milling process and addition of blended ingredients respectively, while the frying stage (stage 4) had low microbial counts. No coliforms were detected in any of the five stages of production of the fried bean cake. The results suggested that stages 2 and 3 were the Critical Control Points (CCPs), where some degree of control can be exercised over the microbial hazard of akara to prevent, eliminate, or reduce the microbial load to acceptable levels.

Keywords: Bean cake, Critical control points, Microorganisms

Introduction

Microorganisms contaminate a wide variety of foods due to their ubiquitous nature, hence the need for appropriate control measures (Skougard, 1991). Foods, because they provide nutrients for man, also are excellent environments for the growth of microorganisms, and their sources of contamination include ingredients, packaging materials, product in process, the environment and personnel, and equipment (Frazier and Westhoff, 1988; Adams and Moss, 1999; and Prescott *et al.,* 2005).

The increasing activities of foodborne pathogens have stimulated investigations aimed at ensuring food safety. Food safety can be achieved through regulation and safety control of production processes. However, studies indicate that the greatest challenge to food safety is microbiological hazard (Hobbs and Gilbert, 1981). Such hazards are further worsened

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by the complex methods of food preparation, handling and distribution (Sutherland *et al.*, 1986). Thus, to curb the menace by microbiological hazard, food Scientists and microbiologists have employed routine inspection approach which is designed to meet legislative, quality and safety standards (Sutherland *et al.*, 1986).

The technique first described in the US in 1971 relies on the analysis of those factors that influence the microbial safety of food and food production. This is done by determination of the degree of contamination, detection of point of entry and mapping out strategies at eliminating such points (Bryan, 1981; and Skougard, 1991).

The Hazard Analysis and Critical Control Point (HACCP) concept has improved on traditional practices by introducing a more systematic, rule-based approach for applying the knowledge of food microbiology to the control of microbiological quality. The same system can also be adopted with physical and chemical factors affecting food safety or acceptability. HACCP concept is mainly a preventive approach to quality assurance and as such it is a tool to control quality during processing. It can also be used to design quality into new products during their development. The concept uses a preventive method that relies on analysis of the hazards associated with a particular product and determination of Critical Control Points (CCPs). The technique is intended to extend the principle of Good Manufacturing Practice (GMP) toward zero defect manufacture (Bryan, 1981; and Adams and Moss, 1999).

This study was designed to determine the points of entry of microorganisms during the production of fried bean cake (Akara), and to identify the microbial contaminants at each stage of production.

Materials and Methods

Sample Collection

The raw materials used in the preparation of fried bean cake (Akara) were beans, onions, fresh pepper, salt and groundnut oil which were bought at Gwari market, Minna. The samples were collected and kept in sterile container, placed in an ice box and transported to the research laboratory for the preparation of fried bean cake (Akara).

Preparation of Fried Bean Cake (Akara)

200 g of beans were soaked in 100 mL of water in a clean rubber basin. After 10 min, the beans were dehulled manually with constant replacement of water to clear the coats. The decoated beans were milled with a commercial grinding machine, with fresh pepper and onions as ingredients for the preparation. The entire mixture was stirred in a clean ceramic mortar till the paste and ingredients became light when rubbed between the finger tips. A clean table spoon was used to place the mixed paste into heated groundnut oil. The frying was for 5-7 min, until the balls turned brownish in color (Aykroyd, 1973; and Williams, 1984). The flow chart for the preparation of fried bean cake (Akara) is presented in Figure 1.

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Figure 1: Flow Chart for the Preparation of Fried Bean Cake (Oyeleke, 1983; and Williams, 1984)

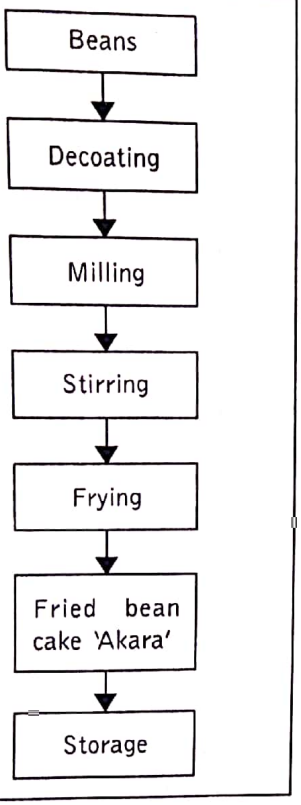
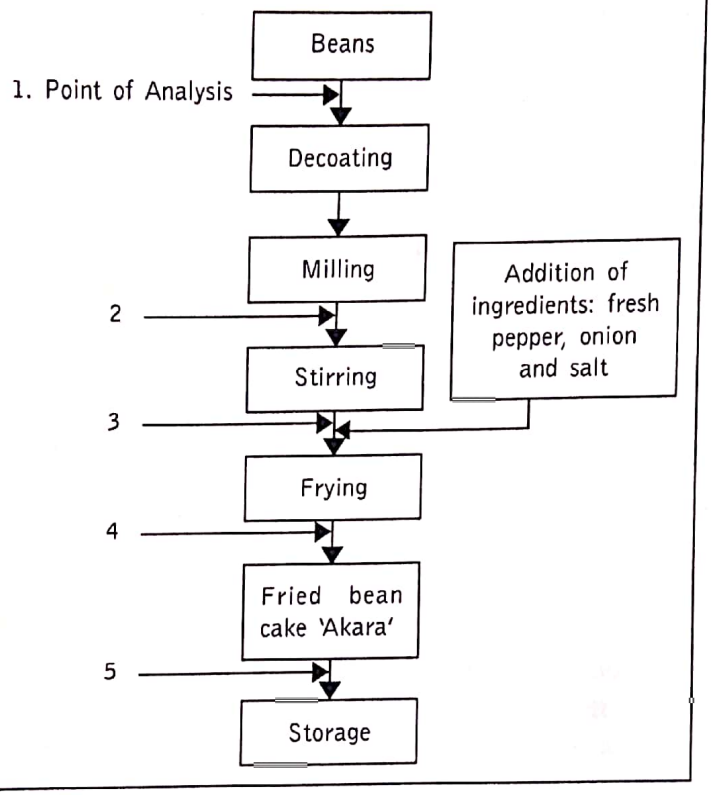


Figure 2: Flow Chart of Process Showing Points at Microbiological Analysis During the Preparation of Fried bean Cake (Akara)



Processing of Samples

The samples were taken at five different points of the preparation of fried bean cake (Akara) and processed, as presented in Figure 2. At point 1, 10 g of the sample (raw beans) was weighed and homogenized in a sterile ceramic mortar with a pestle. 90 mL of sterile water was added to the product in a sterile glass beaker and mixed properly. The sample was serially diluted and 0.1 mL of an appropriate dilution was used to inoculate Nutrient agar (NA), Mannitol Salt Agar (MSA), MacConkey Agar (MCA) and Potato Dextrose Agar (PDA) plates for the enumeration of total viable bacteria, *Staphylococcus coli* forms and fungi respectively. The NA, MSA and MCA plates were incubated at 37 °C for 24-48 h, while the PDA plates were incubated at room temperature (28 ± 2 °C) for 3-5 days. At the end of the incubation period, colonies which developed on the plates were counted, and picked and subcultured repeatedly on nutrient agar to obtain pure cultures. The pure cultures were maintained on agar slants for further characterization and identification.

At point 2, 10 g of the paste was weighed and the above procedure was repeated at all the points. At point 3, analysis was done after the addition of the blended ingredients and stirred in a sterile ceramic mortar with a pestle. At point 4, the fried bean cake (akara)

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balls were removed after 5-7 min of frying with a sterile fork. Three of the fried bean cake (Akara) balls were placed in a sterile ceramic mortar and blended together with a pestle before homogenization. At point 5, the procedure was similar to that of point 4 above. However, the difference lies in the fact that the fifth procedure was carried out 4 h after frying. This was the storage stage after the production of fried bean cake (Akara) was completed.

Characterization and Identification of Isolates

Various isolates obtained were characterized based on growth on differential and selective media and biochemical tests including Gram's reaction, production of catalase, coagulase, oxidase, urease, indole, citrate utilization, motility test and utilization of carbohydrates. The bacterial isolates were further identified based on the taxonomic schemes and descriptions by Buchanan and Gibbons (1992). The fungal isolates were characterized based on color aerial and substrate mycelia, presence of special structures, characteristic spore head and the type of spores produced. The fungal isolates were identified by comparing their characteristics with those of known taxa using the schemes of Domsch and Gams (1970) and Salako (1994).

Results

Microbial Contamination Level

The results (Table 1) revealed that the raw beans, milling process (stage 2) and the addition of blended ingredients (stage 3) contained a large number of microorganisms. However, considerably high counts were encountered at stage 3 of the production process than other stages. Coliform bacteria were not detected at any stage of the production process. Frying (stage 4) of the product caused a decrease in the counts of the total viable bacteria, *Staphylococcus* and fungi (Table 1).

Table 1: Microbial Counts at Different Stages of Production of Fried Bean Cake (Akara)

Microbial Group	Microbial Counts (cfu/g)				
	Point of Analysis				
	1	2	3	4	5
Total Viable Bacteria	2.0×10^5	1.2×10^6	1.3×10^6	4.5×10^3	6.3×10^4
Coliforms	ND	ND	ND	ND	ND
Staphylococcus	1.6×10^4	2.2×10^5	3.6×10^5	1.8×10^2	8.6×10^3
Fungi	1.0×10^3	1.0×10^4	2.6×10^4	5.1×10^1	1.4×10^2

Note: Cfu/g colony forming unit per gram; ND: Not detected; Point of analysis: Point 1: Raw beans; Point 2: Milled sample; Point 3: Addition of ingredients; Point 4: After frying; and Point 5: Storage stage.

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Identity and Occurrence of Microbial Isolates

The bacterial isolates identified in the present study include species of *Bacillus, Staphylococcus, Pseudomonas, Alcaligenes* and *Serratia.* The molds associated with different stages of production of fried bean cake (Akara) were species of *Aspergillus, Penicillium, Mucor, Fusarium* and *Rhizopus*, while the yeast isolates were identified as species of *Saccharomyces* and *Candida* (Table 2). It was observed that *Bacillus* species occurred in all the five stages of production, followed by *Staphylococcus aureus,* which occurred in 4 out of the 5 stages of production. *Staphylococcus aureus* was absent only in stage 4 of the production process, which involved frying of the paste to produce fried bean cake (Akara). *Pseudomonas aeruginosa* occurred in the first two stages, while *Alcaligenes* sp. occurred only in stage 3 (stirring of beans paste blended with ingredients). *Serratia* sp. were detected only in stage 2, in which the decoated beans were milled (Table 2).

Molds and yeasts were also encountered at different stages of production of fried bean cake (Akara). No yeast isolate was detected in stage 4. However, *Saccharomyces* species occurred in stages 1, 2 and 3, while *Candida* species occurred in stages 2, and 5. *Aspergillus* species were detected in all stages, with the exception of stage 5, while *Mucor* species were detected in stages 2 and 5, *Rhizopus* sp in stages 1 and 5, *Penicillium* sp. and *Fusarium* sp. occurred only in stages 1 and 3 respectively (Table 2).

Table 2: Identification of Microbial Isolates at Different Stages of Production of Fried Bean Cake (Akara)

Stages of Production	Event	Bacterial Isolates	Mold Isolates	Yeast Isolates
1	Beans (Main raw material)	*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus subtilis, Bacillus* sp., *Pseudomonas* sp.	*Aspergillus niger, Rhizopus* sp., *Penicillium* sp.,	*Saccharomyces* sp.
2	Milling of decoated beans	*Serratia* sp. *Bacillus cereus Staphylococcus aureus Pseudomonas* sp.	*Mucor* sp., *Aspergillus* sp.	*Saccharomyces cerevisiae, Saccharomyces* sp., *Candida tropicalis*
3	Stirring of beans paste	*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Alcaligenes* sp.,	*Fusarium* sp., *Aspergillus* sp.	*Saccharomyces cerevisiae, Saccharomyces* sp.

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Table 2 (Cont.)

Stages of Production	Event	Bacterial Isolates	Mold Isolates	Yeast Isolates
4	Frying of paste to produce fried bean cake	*Bacillus subtilis, Bacillus cereus, Bacillus* sp.	*Aspergillus* sp.	ND
5	Storage fried bean (Akara)	*Staphylococcus* of *aureus, Staphylococcus epidermidis, Bacillus* sp., *Bacillus subtilis*	*Mucor* sp., *Rhizopus* sp.,	*Candida* sp.,

Frequency of Occurrence of Bacterial Isolates at Different Stages of Production of Fried Bean Cake (Akara)

Table 3 shows the frequency of occurrence of bacterial isolates at different stages of production of fried bean cake (Akara). The results revealed that *Staphylococcus epidermidis* had the highest frequency of occurrence 3 (27.2%) at stage 1, while *Pseudomonas aeruginosa* and *Serratia* sp. had the highest occurrence 3(37.5%) each at stage 2. At stage 3, *Staphylococcus epidermidis* and *Alcaligenes* sp. had high frequency of occurrence 3 (30%). *Bacillus* species were the only bacteria prevalent at stage 4 with frequency of occurrence 3 (33.3%). It was observed at this stage (stage 4: after frying) that no other bacterial isolates were encountered. *Staphylococcus aureus* and *Staphylococcus epidermidis* had the highest frequency of occurrence 3 (37.5%) at stage 5 (Table 3).

Discussion

The results obtained in the present study indicated that all the five stages of the production of fried bean cake (Akara) harbored various microorganisms including species of *Bacillus, Staphylococcus, Pseudomonas, Alcaligenes, Serratia, Aspergillus, Penicillium, Mucor, Fusarium, Rhizopus, Saccharomyces* and *Candida*. These findings are similar to those of the earlier reports for the occurrence of these microbial populations in a wide variety of many different kinds of food, where they cause food infection, poisoning and intoxication (Frazier and Westhoff, 1988; Ashi, 1992; Nester *et al.*, 2004; Prescott *et al.*, 2005; and Talaro, 2005). The isolation of these microbes from the production stages of fried bean cake (Akara) is worrisome because these organisms are potential pathogens of man capable of causing a variety of diseases.

The microbial load was higher at stages 2 (milling process) and 3 (addition of blended ingredients) than other stages of production. The index of the microbial load (10^4-10^6) is high and indicates dense population of microbes in the production process. This could be due to the fact that the milling machine or equipment used and the ingredients added contained some microbial contaminants. This is in line with the reports of Frazier and

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Table 3: Frequency of Occurrence of Bacterial Isolates at Different Stages of Production of Fried Bean Cake

Stages of Production	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus* sp.	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*	*Alcaligenes* sp.	*Serratia* sp.
1	1(9.0)	2(18.1)	2(18.1)	2(18.1)	2(27.2)	1(9.0)	ND	ND
2	ND	1(12.5)	ND	1(12.5)	ND	3(37.5)	ND	3(37.5)
3	1(10)	2(20)	ND	1(10)	3(30)	ND	3(30)	ND
4	2(33.3)	2(33.3)	2(33.3)	ND	ND	ND	ND	ND
5	1(12.5)	ND	1(12.5)	3(37.5)	3(37.5)	ND	ND	ND

Note: ND – Not Detected; Stages of production: 1 – Raw beans; 2 – Milled sample; 3 – Addition of ingredients; 4 – After frying; and 5 – Storage stage.

Westhoff (1988), Adams and Moss (1999) and Prescott *et al.* (2005) that foods which provide nutrients for man also are also excellent environments for the growth of microorganisms, and their sources of contamination include ingredients, packaging materials, product in process, the environment, personnel and equipment. The two stages should be carefully monitored during the production of fried bean cake (Akara). Thus, stages 2 and 3 are the CCPs, which according to Adams and Moss (1999) are locations, step, or procedure, at which some degree of control can be exercised over a microbial hazard, that is, the hazard can be either prevented, eliminated, or reduced to acceptable levels. Loss of control at a CCP would result in unacceptable risk to the consumer or product. The counts of *Staphylococcus* species were also higher at stages 2 and 3 than other stages. This could be due to the fact that these bacteria are widely distributed in the environment and they occur on the skin and nostrils of humans (Obuekwe and Ogbimi, 1989; Talaro and Talaro, 1995; and Prescott *et al.,* 1999). It is therefore possible that these organisms can be transferred from these sources to the product during processing. Mims *et al.* (2005) reported that up to 50% of *Staphylococcus aureus* strains produce enterotoxin, and food is contaminated by human carriers and the bacteria grow at room temperature. Ijah and Antai (1991) also noted that the temperature of the environment favors the proliferation of *S. aureus.*

Stage 4 (frying of paste to produce 'Akara') of the production process did not harbor much microbial load probably because of the heat involved in frying. Frazier and Westhoff (1988) and Nester *et al.* (2004) reported that heat destroys or kills some microorganisms in food. It is worthy to note that while heat plays a key role in the production process, the frying was unable to eliminate the *Bacillus* species in stage 4, which validates the fact that members of the genera *Bacillus* are particularly troublesome because their heat-resistant endospores survive cooking and in

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some cases, canning. *Bacillus coagulans* and *Bacillus stearothermophilus* spoil some canned foods (Nester *et al.*, 2004).

Aspergillus species also survived the heating process (stage 4). These organisms are widely distributed in the environment and produce spores which are heat resistant and allow them to proliferate at that stage. Adelberg *et al.* (1995), Nester *et al.* (2004) and Makun (2007) reported the presence of conidiospore and that *Aspergillus flavus* infects peanuts and other grains producing aflatoxins in foods, a potent carcinogen.

The fact that some organisms are absent in some stages of the production process suggests that the activities at those stages did not favor their growth; for example, the frying process (stage 4) eliminated *Saccharomyces* and *Candida* species identified earlier in stages 1, 2 and 3. This supports the reports of Nester *et al.* (2004) and prescott *et al.* (2005) that certain strains of *Saccharomyces* thrive best or ferment best at temperatures ranging from 6 °C to 28 °C. It is therefore observed that the temperature used in frying is above the optimum for their growth and hence kills or destroys these organisms at that stage. *Coli* forms were not detected at any stage of production, which means that the raw materials or samples used in this study were not contaminated by fecal matter.

Conclusion

The study has indicated that the different stages of production of fried bean cake (Akara) harbor microorganisms such as *Staphylococcus aureus, Bacillus* sp., *Pseudomonas* sp., *Aspergillus* sp. and *Candida* sp. The microbial load was higher during the milling process (stage 2) and addition of blended ingredients (stage 3) than other stages. However, the microbial load was low after frying (stage 4). Only *Aspergillus* sp. and *Bacillus* sp. were able to survive the heating effect.

The study has revealed the Critical Control Points (CCPs) of the production of fried bean cake (Akara) at stages 2 and 3 (milling process and addition of blended ingredients respectively), where some degree of control can be exercised over the microbial hazard in order to prevent, eliminate or reduce the microbial load to acceptable levels. The isolation of these organisms is a serious public health problem and it has been found associated with environmental health risk to the public.

Recommendations

Based on the results of this study, it is recommended that:

- Vigorous washing of the beans with clean water should be undertaken to reduce the level of contamination.
- Aseptic techniques are essential in the kitchen or where the production is taking place.
- Contamination of fried bean cake (Akara) by fingers can be easily remedied by hand washing and proper hygiene, and contamination by flies or other insects can be stopped by covering the 'Akara'.

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- Clean utensils should be used in the production of akara to reduce the level of contamination.

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