

MICROBIOLOGICAL ASSAY OF INGREDIENTS, CONTACT SURFACES AND STAGES IN AKARA PROCESSING AT THREE LOCATIONS IN MAIDUGURI, NIGERIA

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ABSTRACT

Sample at various stages and contact surfaces were obtained during akara processing from three locations in Maiduguri metropolis. The locations were Hausari, Mairi and Wulari. Samples of the ingredients and swabs of contact surfaces were taken. Total aerobic plate count, coliform count, taphylococcal count and yeast/mould count, were determined. Microorganism were solated, identified and their percentage frequency of occurrence on plated samples of the ingredients and contact surfaces were also determined. The total aerobic mesophilic count of ingredients from the three locations range from 3.10 to 8.40 cfu/g, while coliform count ranged from 1.10 to 9.00 cfu/g, staphylococcal count ranged from 1.20 to 3.29 cfu/g and yeast/mould count ranged from 1.60 to 4.80 cfu/g. The total aerobic plate count of contact surfaces ranged from 4.20 to 7.90 cfu/cm², coliform from 1.00 to 7.80 cfu/cm², staphylococcal count from 2.10 to 5.90 cfu/cm² and yeast/mould count from 1.20 to 4.80 cfu/cm². The predominant microorganisms isolated from this study were *Klebsiella pneumoniae*, *Bacillus subtilis*, *candida tropicalis* and *Aspergillus niger*. They occurred at all the various stages and on contact surfaces during akara processing at percentage frequency of occurrence ranging from 5 to 50%. The presence of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus feacalis* and *klebsiella pneumoniae* is not a healthy development. These microorganisms are pathogenic and therefore their presence during akara processing could cause a serious health problem. The need for advising akara producers and vendors of the necessity to adopt strict hygienic practice, at various stages of processing is essential.

Key words: Akara, Contamination, microbiological.

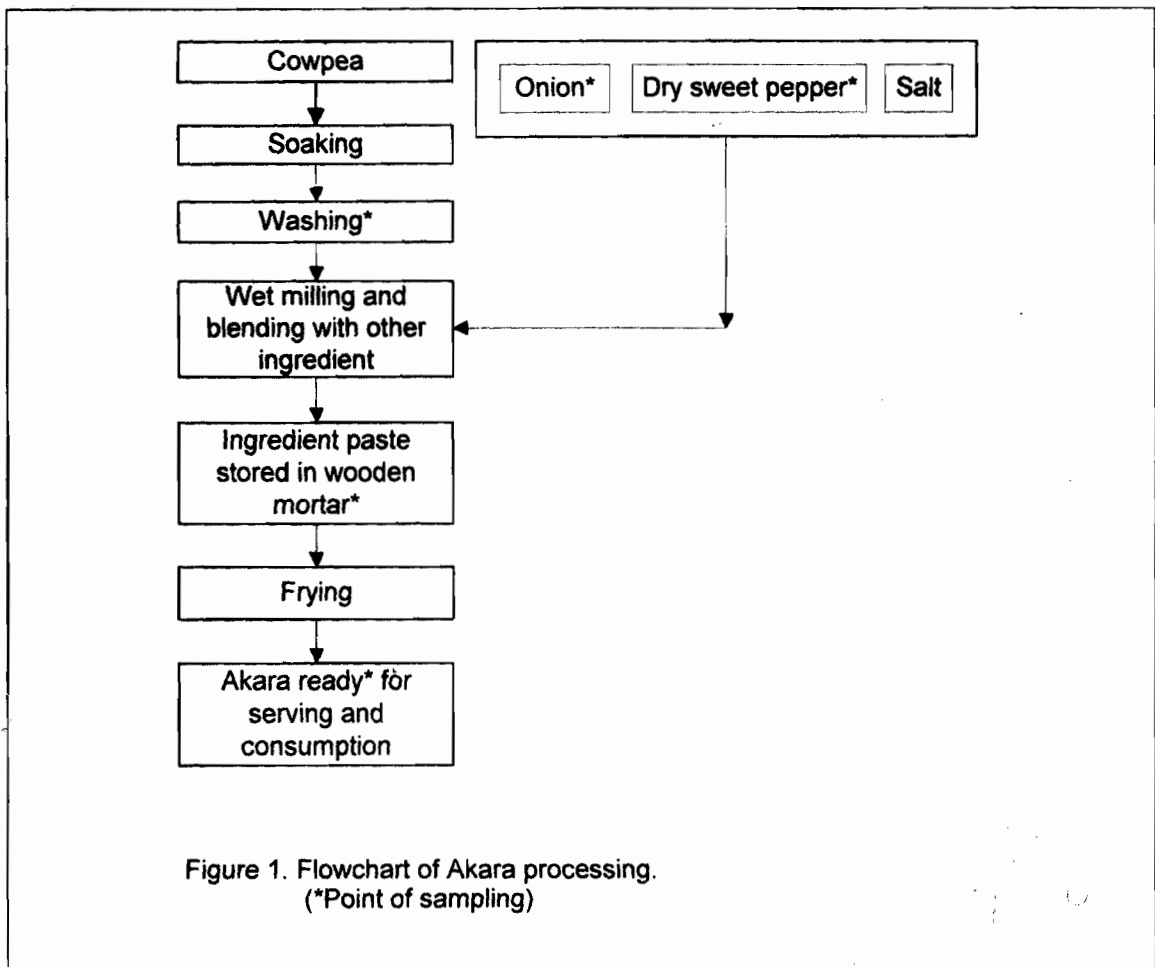
INTRODUCTION

Careful microbiological analysis of ingredients, products and process steps is needed to determine those components or areas that must be maintained under very strict sanitary control to ensure that the end product meets the microbiological specifications and safety (Bauman, 1974).

Akara is a deep fat fried product made from whipped cowpea paste and is one of the most common cowpea-based food products in West Africa (Reber *et al.*, 1983, Ngoddy *et al.*, 1986). It is traditionally prepared at home and by street vendors in the market place. There is

neither proper sanitary practice during processing nor satisfactory packaging material for the finished product. There is the possibility of the product being contaminated constituting a risk to public health. Such a product may become microbiologically contaminated from its raw materials, handlers, equipment and packaging materials (Jay, 1987).

Akara, like any food requires inspection at different stages; from raw materials to finished product to make sure that required legislative quality or safety standards are met (Sutherland *et al.*, 1986). The objectives of this study were to determine the microbial load, isolate and



identify microorganisms associated with the ingredients at each stage of *akara* processing.

MATERIALS AND METHODS

Figure 1 shows the various stages of *akara* processing indicating sampling points. A total of 72 samples were randomly collected at each stage of *akara* processing from 9 *akara* vendors from three locations (Hausari, Mairi and Wulari) in Maiduguri metropolis. Samples were collected in sterile polythene bags, stored in a portable cooler containing ice blocks and immediately transported to laboratory for microbiological analysis (Badau *et al.*, 1999). Random swabs of the contact surfaces of milling machine, wooden mortar and personnel hand were obtained before *akara* processing. The swabs of the contact surfaces were stored on ice portable cooler and immediately transported to laboratory for microbiological analysis.

Microbiological Analysis

Ten grammes of each sample were homogenized in a sterile blender (Kenwood England. A707A, Mc D₃ 19328) containing 90ml of distilled water to give a dilution of 10⁻¹. For each of the homogenized sample, 1 ml of it was aseptically withdrawn and added to 9ml of sterile distilled water in a McCartney bottle, to provide a dilution of 10⁻², which was serially diluted up to 10⁻¹⁰.

The diluted sample (1ml) was inoculated in duplicates into nutrient agar plates, MacConkey agar, mannitol salt and potato dextrose/ Czapek dox agar for total bacterial count, coliform count, staphylococcal count and fungal count, respectively. The plates were incubated aerobically for 24 to 48 h at 37°C for total viable counts, 48 h at 37°C for coliform count, 48 h at 37°C for staphylococcal count and 3 to 6 days at room temperature (30°C) for

fungal counts (Owhe-Ureghe *et al.*, 1993, Aminu *et al.*, 1993; Lee and Lim, 1985, Badau *et al.*, 1999; Bulgarelli *et al.*, 1988). Swab counts of contact surfaces were determined as reported by Collins and Lyne (1970). Colonies of bacteria and fungi appearing on incubated plate were counted and expressed as either colony forming units per gramme (cfu/g) or colony-forming units per square centimeter (cfu/cm²). Representative colonies were isolated and sub cultured to obtained pure cultures.

The cultures were identified based on morphological, cultural and biochemical characteristics (Cowan and Steel, 1961; Collins and Lyne, 1970; Gilman, 1957). The percentage frequency of microorganisms was calculated based on percentage occurrence on plated samples (Lee and Lim, 1985; Owhe-Ureghe *et al.*, 1993; Badau *et al.*, 1999).

Statistical Analysis

Results of the total aerobic plate count, coliform count, staphylococcal count and fungal count were statistically analyzed by analysis of variance (ANOVA). Means were compared by student t-test as described by Gomez and Gomez (1983) and Mead *et al.*, (1993).

RESULTS AND DISCUSSION

The log₁₀ mean total aerobic bacterial, coliform, staphylococcal and yeast/mould counts of *akara* at various stages are shown in Figure 2. In Hausari, the mean bacterial plate count at various stages of *akara* processing ranged from 3.00 cfu/g for onion to 8.30 cfu/g for water coliform count ranged from 2.00 cfu/g for sweet pepper to 8.13 cfu/g for water. Staphylococcal count was recorded highest in fresh onion and lowest in water. On the other hand, yeast/mould account was recorded highest in ingredient paste and fresh onion, while water recorded the lowest.

The microbial population of *akara* at various stages of production obtained from Mairi showed that mean total aerobic plate count ranged from 3.10 to 8.40 cfu/g; coliform count from 1.10 to 8.50 cfu/g; staphylococcal

count from 1.2 to 3.00 cfu/g and yeast/mould count from 1.60 to 4.40 cfu/g.

Wulari ward had mean total aerobic plate count ranging from 3.80 cfu/g for onion to 8.30 cfu/g for water, coliform count ranged from 1.40 cfu/g for sweet pepper to 9.00 cfu/g for water, staphylococcal count from 1.30 cfu/g for fresh onion to 2.90 cfu/g for ingredients paste and yeast/mould count ranged from 1.6 cfu/g for fresh onion to 4.80 cfu/g for ingredients paste and yeast/mould ranged from 1.60 cfu/g for fresh onion to 4.80 cfu/g for ingredients paste. There was non-detectable count obtained in fresh *akara* removed from hot oil.

The microbial population of contact surfaces during *akara* processing is shown in Figure 3. The contact surfaces of *akara* vendors from Hausari showed that milling machine and personnel hand had highest total aerobic plate counts than wooden mortar, while personnel hand recorded the highest coliform count and wooden mortar the least. The staphylococcal count of personnel hand was higher than that of the milling machine and wooden mortar. Yeast/mould count of contact surfaces for Hausari *akara* vendors had higher counts on milling machine and wooden mortar than personnel hand.

In Mairi, the mean total aerobic plate count of contact surfaces ranged from 3.90 cfu/cm² for wooden mortar to 8.20 cfu/cm² for milling machine. Coliform count ranged from 1.20 cfu/cm² for wooden mortar to 7.80 cfu/cm² for milling machine. Staphylococcal count ranged from 2.10 cfu/cm² for wooden mortar to 5.90 cfu/cm² for personnel hand. Yeast/mould count of the contact surfaces ranged from 1.30 for personnel hand to 4.80 for milling machine.

Akara vendors from Wulari recorded the highest total aerobic plate count on the surface of their milling machine, while wooden mortar had the least. For coliform count, personnel hand recorded the highest, followed by milling machine and wooden mortar had the least. Personnel hand recorded the highest staphylococcal count, while the milling machine had the least. Wulari *akara* vendor recorded the highest yeast/mould count on

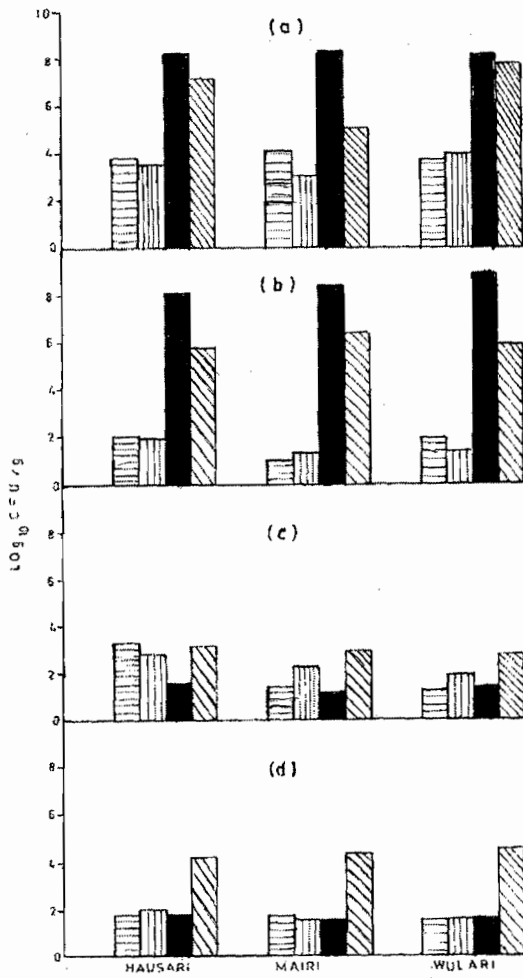






Figure 2 (a) Total aerobic plate count, (b) Coliform count, (c) Staphylococcal count and (d) Yeast/mould count at various stages of akara processing, Fresh Onion , Dry sweet pepper , Water  and Ingredients paste  from three locations.

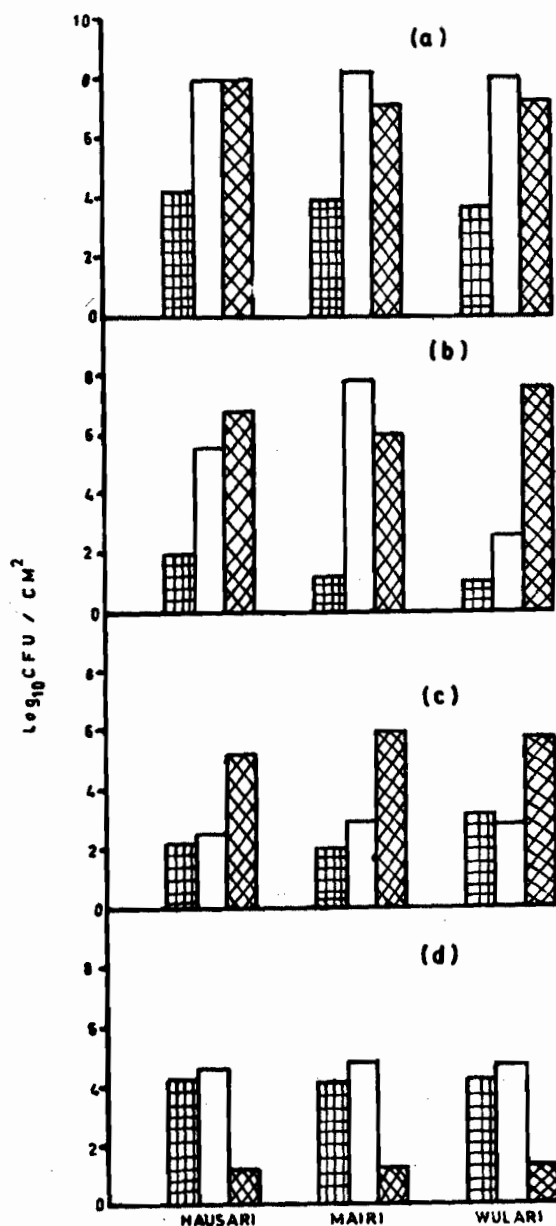





Figure 3 (a) Total aerobic plate count, (b) Coliform count, (c) Staphylococcal count and (d) Yeast/mould count of contact surfaces during Akara processing, Wooden mortar ; Milling machine  and personnels' hand  from three locations.

milling machine and personnel hand the least.

The distribution of microorganisms and

and personnel hand have been found to be important source of contamination. These

Table 1: Distribution of Microorganisms and their percentage frequency of occurrence at various stages of akara processing.

Microorganism	Ingredients				Contact surface		
	Fresh onion	Sweet pepper	Water	Ingredient paste	Wooden mortar	Milling machines	Personnel hand
<i>Staphylococcus aureus</i>	15	20	-	10	20	15	90
<i>Klebsiella pneumoniae</i>	20	15	50	20	40	20	30
<i>Enterobacter sakazakii</i>	-	20	40	15	20	30	20
<i>Escherichia coli</i>	10	-	60	30	50	20	30
<i>Corynebacterium sp.</i>	-	15	-	35	-	40	-
<i>Bacillus subtilis</i>	30	20	10	15	10	20	10
<i>Lactobacillus fermentum</i>	-	-	20	25	18	10	50
<i>Streptococcus fae calis</i>	-	-	15	15	-	-	-
<i>Pseudomonas sp.</i>	-	-	30	15	10	10	5
<i>Candida tropicalis</i>	5	10	15	10	15	10	10
<i>Aspergillus niger</i>	10	10	5	10	10	5	5
<i>Saccharomyces cerevisiae</i>	5	-	10	15	5	15	5
<i>Geotrichum scandium</i>	-	5	5	5	-	5	5
<i>Lactobacillus delbruekii</i>	-	-	-	10	-	10	-
<i>Enterobacter sp.</i>	-	-	-	5	10	10	-

their percentage frequency of occurrence are presented in Table 1. The most predominant microorganism in fresh onion was *Bacillus subtilis*, while *Enterobacter sakazakii*, *Bacillus subtilis* and *staphylococcus aureus* were most predominant in sweet pepper and *Escherichia coli* in water. All the microorganisms encountered in this study were isolated in the ingredient paste at varying degree of occurrence (5 to 35%) with *Corynebacterium spp* occurring most frequency (35%). Wooden mortar used for storing the paste prior to frying had *Echerichia coli* as the most predominant organism, milling machine had *Corynebacterium sp.*, and personnel hand had *staphylococcus aureus* as the most frequently occurred organism.

The sensitive ingredients were cowpea, sweet pepper, onion and water. Among the ingredients, water had the highest ($P < 0.01$) \log_{10} means total aerobic and coliform counts, followed by ingredient paste. Milling machine

findings agree with the report of Jay (1987). The ingredient paste had the highest ($P < 0.01$) staphylococcal count while the highest yeast/mould count was recorded on milling machine surface.

Determination of total aerobic plate count indicates contaminated raw materials, unsatisfactory sanitation, or unsuitable time/temperature condition during production, storage, or combination of these. Coliform count, which includes *Escherichia coli* and other genera of the *Enterobacteriaceae*, are indicator organisms of faecal contamination. Staphylococcal count indicates contamination from the skin, mouth or nose of workers handling the food, but inadequately cleaned equipment may also be a source of contamination. The presence of large number of staphylococci is, in general, a good indication that sanitation and temperature control have been inadequate (Jay, 1987). Yeast/mould count is very important in

determining the level of mould/yeast contamination, which connotes the likely presence of aflatoxin producing fungi (Nkama, 1987).

It has been observed that the most sensitive ingredient in *akara* processing was water. This was used in washing, milling and preparation of the ingredients paste. Ingredients paste was another sensitive ingredient. Sources of microorganisms could have been from the contact surfaces, cowpea, onion and sweet pepper. Nkama *et al.* (1994) have isolated *Aspergillus fumigatus*, *Rhizopus arrhizus*, *Bacillus brevis* and *Bacillus alvei* from sweet pepper ("tattashe") and therefore, if dry pepper is not sterilized, it could add to microbial load of the ingredients paste. Other researchers have also isolated various bacteria, mould and yeast spp. from *akara* ingredients pastes sampled from local markets in Nigeria (Bulgarelli *et al.*, 1988). Surprisingly, earlier reports did not encounter *staphylococcus aureus* in the relevant studies. This could have been due to delay in time of sampling the ingredients paste with the other organisms out growing *staphylococcus aureus* which is less competitive (Jay, 1987).

The ingredient paste contained all the microorganisms isolated at varying percentage frequency of occurrence (5 to 35%). Milling machine had all the organisms except *Streptococcus faecalis*. Generally, it has been observed that initial micro flora of the ingredients and those found on contact surfaces were the source of microbial contamination of *akara*.

CONCLUSION

Akara, which is widely consumed cowpea based food as a break fast meal all over West Africa and other part of the world, could constitute a microbiological hazard if adequate hygiene practices are not adopted at various stages of it's preparation. This study had identified critical points and sensitive ingredients that needed to be handled properly by Maiduguri *akara* vendors for the safety of consumers. The sensitive ingredients are water and dry sweet pepper. Coliform and

staphylococcus spp. were identified and these are well known indicator organisms of faecal contamination and general indicator that sanitation has been inadequate.

The presence of *staphylococcus aureus* and other pathogenic microorganisms encountered in this study is a cause for concern from public point of view. Although there was no detectable counts in fresh *akara* removed immediately from hot oil, there is a need to screen the product for microbial toxins, since *staphylococcus aureus* and other pathogenic microorganisms capable of producing toxin have been isolated at various stages.

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