Akara Ogbomoso: Microbiological Examination and Identification of Hazards and Critical Control Points

A. Lateef,* T.E. Davies, A. Adelekan, I.A. Adelere, A.A. Adedeji and A.H. Fadahunsi

Microbiology Unit, Department of Pure and Applied Biology, Ladoke Akintola University of Technology, PMB 4000, Ogbomoso, Nigeria

Akara Ogbomoso was examined toward the establishment of hazard analysis and critical control point (HACCP). The *akara* was produced in residential buildings with the attendant consequence of contamination. There was ample growth of aerobes, coliforms, staphylococci, *Shigella* and yeast/mold from the samples, water and cowpea pastes. Microbial contaminations occur through the processing, which can be corrected through education by adopting good hygienic and manufacturing practices. The critical control points were identified as frying, storage and refrying. It may be heated in the microwave for 10 s before consumption. *Akara*, prepared in the laboratory through the implementation of HACCP was not contaminated. Several bacterial isolates, namely; *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus vulgaris*, *Bacillus cereus*, *Streptococcus pyogenes*, *Bacillus* sp. and *Shigella* sp., showed multiple resistance to antibiotics ranging from two to nine. Seven strains were not resistant to the antibiotics, while five were resistant to one type of antibiotic.

Key Words: Akara Ogbomoso, microbiology, hazards, control points, drug resistance, public health

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are highly nutritious legumes providing much needed protein, fiber and B-vitamins to the diet and other nutrients such as calcium, magnesium, iron, potassium and zinc (Aykroyd et al., 1982; Uzogara and Ofuya, 1992). Most of the world's cowpea production, about 80%, is produced in West Africa (FAO, 2000), where cowpeas are eaten on a daily basis in foods like soups, *moin-moin* (steamed cowpea paste) and *akara* (fried cowpea paste). The consumption of cowpea is especially important in Nigeria since malnutrition is a major problem in this area of the world. With the increasing prices of animal protein, legumes continue to provide a cheaper source of protein than meats (Akinyele and Onigbinde, 1988).

Akara is a fried nugget-type product that is popular in many West African and South American countries. This savory finger food is made from whipped cowpea paste seasoned with chopped fresh pepper (either hot or mild), chopped fresh onion and salt (Hung et al., 1995), which is fried in palm oil or vegetable oil at about 193 °C. Its consumption cuts across different social and economic strata. It is often consumed alone, or along a number of starch-based foods such as *garri* (fried fermented grated cassava that is soaked in water), solid or liquid *ogi* (boiled fermented milled corn), bread and *dun-du* (fried pieces of yam). Its popularity is spreading to other parts of the world too. In particular, a number of investigations have been carried out in the US to determine its acceptability (McWatters et al., 1990, 1997; Patterson et al., 2002). The major limitation in the production of *akara*, the time-consuming production process has been simplified through improved process, by using cowpea flour (Prinyawiwatkul et al., 1996). Investigations have also been carried out on the suitability of the partial substitution of maize and soya bean in the production of *akara* (Falade et al., 2003; Giami et al., 2003).

There are two different types of *akara* depending on the method of production, moisture content and the extent of drying. The first type, the conventional *akara* is the most popular, which is produced by a single deep frying in palm or vegetable oil and contains a lot of moisture which varies considerably (38.9–49%; Patterson et al., 2002; Giami et al., 2003). It cannot be stored for more than 8–10 h at ambient temperature,

**To whom correspondence should be sent (e-mail: agbaje72@yahoo.com).*
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while the second type which is low in moisture content (usually ground as thick paste without water) and often deep-fried two times in hot vegetable oil, can be stored for 8–10 weeks without spoilage by microorganisms. While whipping is an important step in the preparation of conventional *akara* to form foam which impacts the texture of *akara*, the preparation of the second type does not involve whipping in any form. Also, unlike in the first type of *akara*, seasoning ingredients such as onion and pepper are not used in the preparation of the second type of *akara*.

Akara Ogbomoso represents the second group, whose name was derived from the main center of production (Falade et al., 2003). *Akara* Ogbomoso differs from the conventional *akara* in terms of process, product characteristics and shelf stability (Falade et al., 2003). Its production had been an exclusive preserve of Ogbomoso sub-tribe of 'Yoruba' race, although its preparation can now be found in other towns and cities in 'Yoruba' land, Southwest Nigeria. Because of its long shelf-life, the popularity and consumption of *akara* Ogbomoso have increased tremendously and it can now be found in several other cities in Southwestern Nigeria.

In view of the problem that may be associated with the consumption of *akara* Ogbomoso, particularly if basic hygienic considerations are not observed during its production, this study, which is first of its kind to the best of our knowledge, was designed to examine the samples of *akara* Ogbomoso for microorganisms from both the production and selling points within Ogbomoso metropolis. The bacterial isolates were then subjected to antibiotic sensitivity testing, with the view of establishing the public health implications of their occurrence in *akara* Ogbomoso. In addition, the environment, raw materials and the procedure for preparing *akara* Ogbomoso were monitored to identify sources of hazards and possible critical control points by applying the hazard analysis and critical control point (HACCP) strategy. HACCP strategy identifies hazards associated with different stages of preparation and handling, assesses the relative risk and identifies points where control measures would be effective (Bryan, 1988; Ehiri et al., 2001; Oranusi et al., 2003; Obadina et al., 2008). It has been used in various studies in recent times to improve the safety of a number of Nigerian indigenous foods and snacks (Oranusi et al., 2003; Adegoke et al., 2008; Obadina et al., 2008; Sobukola et al., 2009).

MATERIALS AND METHODS

Sampling

Samples of *akara* Ogbomoso were collected from five designated production (A, B, C, D and E) and selling points of the *akara* (S1–S5), respectively, within Ogbomoso metropolis, Southwest Nigeria. Sampling was done twice a month for a period of 5 months cutting across both dry and rain seasons. The samples were collected as prepared and offered for sales (six to seven pieces packed in transparent nylon bag), taken to the laboratory under cold condition and analyzed within 4 h of collection. A control experiment was carried by preparing *akara* Ogbomoso in the laboratory and having the samples analyzed. In all, a total of 105 *akara* samples were analyzed in this study (10 samples from each of the 10 production and selling points and 5 additional samples from the laboratory-prepared *akara*). In addition, samples of cowpea paste and water used in the preparation of *akara* were obtained from the five production points in sterile plastic containers and used for microbiological analysis.

Methods

Physico-chemical Analysis

The *akara* samples were analyzed for a number of attributes, which include the diameter, thickness (using caliper), color, weight per pack, yield per kilogram of beans and texture. Similarly, the moisture and lipid contents of *akara* samples were determined. Moisture content was determined using 5 g samples dried overnight in a vacuum oven at 70 °C (AOAC, 1995). The weight loss was then calculated to determine moisture content. Dried samples remaining from moisture analysis were used for fat analysis that was determined by overnight solvent extraction with petroleum ether.

Microbiological Analysis

About 1 g was weighed aseptically from the ground *akara* sample/cowpea seeds (cowpea paste) and then suspended in sterile distilled water for serial dilution. In the case of water, 1 mL of water was used. Appropriate dilutions of 10th, 100th and 1000th fold were done and 0.2 mL of solvent was used to inoculate the agar using the pour plate method. Agars that were used for enumeration and isolation of microorganisms are: yeast extract agar for aerobic bacteria, *Salmonella-Shigella* agar for *Salmonella* and *Shigella*, mannitol salt agar for *Staphylococcus*, potato dextrose agar (PDA) for yeasts and molds and MacConkey agar for coliforms. All the plates were incubated at 37 °C for 24–48 h, except the PDA plates that were incubated at room temperature (30 ± 2 °C). At the end of incubation, the number of distinct colonies was counted and used to calculate the microbial load in each case. Thereafter, colonies were purified to obtain pure cultures and then stored on agar slants at 4 °C. Distinct bacterial colonies were screened and identified by means of taxonomic schemes and descriptions (Buchanan and

Gibbons, 1974), which was complemented with the API identification kit (API System, Biomerieux, France).

Preparation of Akara *Ogbomoso*

The preparation of *akara* Ogbomoso was carried out in the laboratory following the method used by the producers, but under stringent hygienic conditions, by incorporating the various control measures/good manufacturing practice (GMP) for the identified sources of hazards. About 500 g of blackeye beans sourced from a local market in Ogbomoso was soaked in 2.5 L of tap water for 5 min. This was followed by removing the outer coat and then draining to remove water. The beans were then blended without addition of water using corn milling machine. After blending, the thick paste was molded into cylindrical forms (Figure 1) after the addition of salt, without whipping in any form. There was no addition of seasoning agent such as onion, although pepper may seldom be added. The schematic diagram of the process is shown in Figure 2. Some samples of deep-fried *akara* were heated in microwave in the range of 5–30 s and then stored in low-density polythene (50 μm) at ambient temperature (30 ± 2 °C) for up to 10 weeks.

Antibiotic Sensitivity Test

Three selected strains of each bacterial isolate were tested for their sensitivity to antibiotics by means of M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, NCCLS (1997) using Mueller Hinton agar. Antibiotics discs (Fondoz Laboratories) containing: Augmentin (Aug), 30 μg; Amoxycillin (Amx), 25 μg; Ofloxacin (Ofl), 5 μg; Tetracycline (Tet), 30 μg; Ceftriazone (Cro), 30 μg; Nitrofurantoin (Nit), 30 μg; Gentamycin (Gen), 10 μg; Cotrimoxazole (Cot), 25 μg; Ciprofloxacin (Cpx), 10 μg; Pefloxacin (Pfx), 5 μg were used for Gram-negative isolates. Cloxacillin (Cxc), 30 μg; Gentamicin (Gen), 10 μg; Cotrimoxazole (Cot), 50 μg; Erythromycin (Ery), 10 μg; Clindamycin (Cld), 10 μg; Cephaplexin (Cpx), 30 μg; Floxapen (Flx), 30 μg; Ciprofloxacin (Cpx), 5 μg; Augmentin (Aug), 30 μg; and Ofloxacin (Ofl), 5 μg were used for Gram-positive isolates. The plates were incubated at 37 °C for 24 h. After the incubation, zones of inhibition were examined and interpreted accordingly (Chortyk et al., 1993) considering the appropriate breakpoints (Andrews, 2005). Earlier, the potencies of all the antibiotics used in the study were confirmed using susceptible *Escherichia coli* strains.

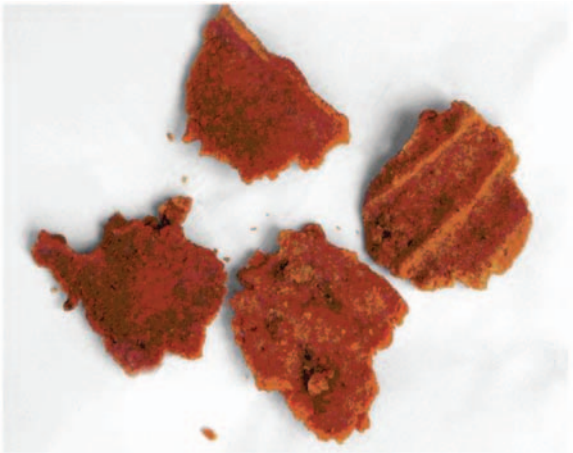

Figure 1. Samples of *akara* Ogbomoso.

Identification of Hazards

Identification of hazards was carried out through visitations to the 10 production and selling points that were designated for the study. Production of the *akara* was observed among the producers, while the sellers were also monitored with regard to the display of *akara* for sales. Several other parameters were noticed, among which are: the hygienic condition of the production environment, personal hygiene of the producers and sources of water used for the production, packaging and storage of *akara* by the producers and the sellers. Samples of water used in the production of *akara* and cowpea pastes were obtained from the producers and used for microbiological analysis as stated above. All these were done to identify the likely sources of contamination in the production and marketing chains of *akara* Ogbomoso.

RESULTS AND DISCUSSIONS

The descriptive analysis of the production points is shown in Table 1. It can be seen that in all cases that were investigated, *akara* Ogbomoso was produced in residential buildings and not in designated areas for food processing or cottage industrial outlets. In this wise, it was a regular occurrence for the producers to rear different forms of animals within the household where production takes place. The involvement of children in the production process differs from one producer to another. In most cases, underground water was used in the production, which may be a deep well or borehole. The hygienic nature of the production outlets, which encompasses the existence of toilets, nature of kitchens used for production, level of sanitation and personal hygiene was just about average. While it was discovered that all the producers learned the act from family members, none of them have had a formal training on the preparation of food. They were also involved in both production and selling of *akara* Ogbomoso. Several inadequacies such as storage of water in contamination-prone manner, closeness of kitchen to refuse

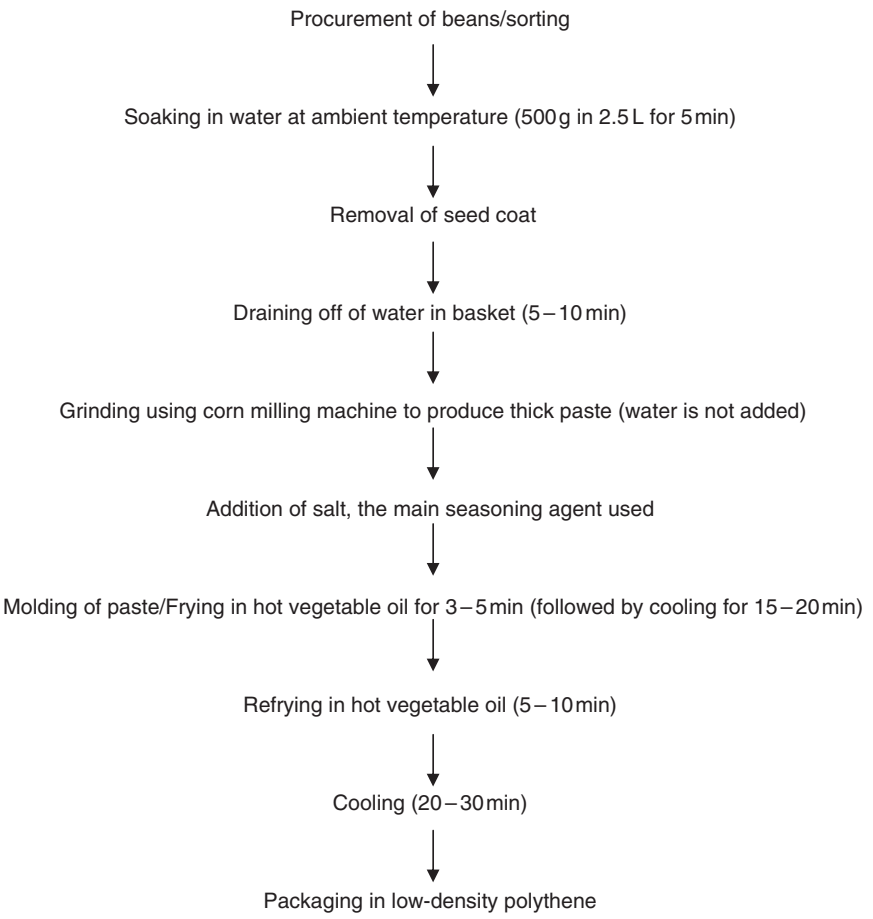

Figure 2. Flow chart for the production of *akara* Ogbomoso.

dump and toilets and improper storage of utensils were identified from one production point to another. In all cases, the selling points are usually motor parks or garages, where patronage of the buyers is ensured. These parks are characterized with low level of sanitation, with a lot of human activities. Usually, *akara* packed in nylon bags is displayed in the open for the buyers to purchase.

The microbial loads of samples of *akara* obtained from both production and selling points are as presented in Tables 2 and 3, respectively. No growth was obtained on MacConkey agar on all samples obtained from one of the producer (E), while samples obtained from three producers (A, B and C) did not produce growth on *Salmonella-Shigella* agar. Only one sample each obtained from producers D and E produced growth on the *Salmonella-Shigella* agar. In situations where growths were obtained, the number of samples that produced growth varied, from 1 to 10 and microbial indices of range 10^1–10^5.

With regard to the samples obtained from the selling points, the loads of microbial indices varied considerably (10^2–10^5). None of the samples from two selling points (S1 and S2) had growth on *Salmonella-Shigella* agar. A comparison of microbial indices indicates that generally more total microbial cells were encountered in samples from the production points. However, with regard to some samples and microorganisms, counts were higher in the samples from selling points, with an important increase in all cases for the final concentration of the molds and yeasts. It was also discovered that more samples from the selling points had microbial growth than those obtained at production points. However, no strain of *Salmonella* was isolated in this study.

The results of microbial attributes of water and cowpea pastes obtained from the production points are shown in Table 4. Generally, all the samples were contaminated, although the level of contamination varied from one producer to another. The microbial loads of pastes were generally higher than that of water samples. In all, water sample from producer A had the highest microbial load of 3.228×10^5 cfu/mL for the microbial indices investigated, while water sample from producer E, had the least cumulative load of 4.23×10^4 cfu/mL. The level of contamination in water samples is A>C>D>B>E. However, the cowpea paste obtained from producer B had the highest cumulative load of 8.07×10^5 cfu/g, while the least value of 2.985×10^5 cfu/g was obtained in cowpea paste of

Table 1. The descriptive analysis of the production points.

Parameters/production points	A	B	C	D	E
Type of building/house	16-Room bungalow	Traditional mud house	Traditional mud house	Bungalow	2-Storey mud house
Type of family	Nuclear	Extended	Extended	Extended	Extended
Rearing of domestic animals	Yes; goats	Yes; goats, cats and hens	Yes; dogs and hens	No	Yes; goats and hens
Involvement of children in production	Yes	Yes	Yes	No	No
Source of water	Tap and deep well (with cover)	Borehole and rain water	Borehole	Deep well with cover	Deep well with cover
Presence of toilets	Yes; pit latrine	No	Yes; pit latrine	No	Yes; pit latrine
Presence of wash basin	Yes	Yes	No	Yes	No
Level of personal hygiene[1]	Good	Fair	Fair	Fair	Fair
Type of kitchen	Semi-closed kitchen	Open outlet kitchen covered with iron sheets	Open outlet kitchen covered with iron sheets	Open outlet kitchen covered with iron sheets	Open outlet kitchen covered with palm leaves
Level of environmental sanitation[1]	Good	Fair	Fair	Fair	Fair
Any formal training on food preparation?	No	No	No	No	No
Acquisition of knowledge about production of *akara* Ogbomoso	Taught by the mother	Taught by the mother	Taught by a friend	Taught by the mother	Taught by the mother in-law
Involvement in marketing chain	Yes, produces and sells	Yes, produces and sells	Yes, produces and sells	Yes, produces and sells	Yes, produces and sells
Other remarks	Water stored in an open cemented metallic drum	Water stored in open rusty metallic drum, and refuse dump is close to the kitchen	The latrine is very close to the kitchen	Usually production utensils (basket, turning stick and frying spoon) are not washed	Production utensils usually kept on the kitchen's roof

[1]Level of personal hygiene/environmental sanitation: very good, good, fair or poor based on repeated observation.

Table 2. The average microbial load of *akara* samples obtained from production points.

	Microbial load (cfu/g $\times 10^4$) of samples[1]				
	A	B	C	D	E
Aerobic plate count	40.711 (10)	11.454 (10)	9.258 (10)	113.436 (10)	15.246 (10)
Coliforms	25 (2)	0.07 (2)	0.089 (10)	0.902 (10)	–
Salmonella-Shigella	–	–	–	0.005 (1)	0.02 (1)
Staphylococci	0.24 (6)	1.524 (10)	0.304 (10)	0.005 (2)	0.37 (6)
Mold-yeast	3.29 (10)	3.91 (6)	49.566 (10)	4.408 (4)	2.508 (6)

Number in parenthesis indicates the number of positive samples.
[1]Microbial load is an average 10 readings and has uniform index of 10^4.

Table 3. The average microbial loads of *akara* samples obtained from selling points.

	Microbial load (cfu/g $\times 10^4$) of samples after a month[1]				
	S1	S2	S3	S4	S5
Aerobic plate count	8.434 (8)	9.33 (10)	6.7 (10)	41.36 (10)	9.62 (10)
Coliforms	0.166 (6)	0.626 (10)	0.1802 (10)	0.1252 (8)	0.034 (8)
Salmonella-Shigella	–	0.306 (2)	–	0.0636 (2)	0.01 (1)
Staphylococci	0.114 (6)	0.342 (8)	0.2052 (8)	0.7886 (8)	0.184 (8)
Mold-yeast	62.07 (10)	26.382 (10)	96.066 (10)	14.792 (10)	80.744 (8)

Number in parenthesis indicates the number of positive samples.
[1]Microbial load is an average 10 readings and has uniform index of 10^4.

Table 4. The microbial loads and pH of water and cowpea paste samples obtained from production points.

Samples/microbial load (cfu/mL $\times 10^5$)[1]	A	B	C	D	E
Water samples					
Aerobic plate count	1.5	0.07	0.81	0.98	0.14
Coliform count	0.12	0.5	1.08	0.84	0.015
Salmonella-Shigella count	0.028	0.2	0.005	0.005	0.008
Staphylococcal count	0.082	0.005	0.48	0.48	0.04
Mold-yeast count	1.5	1.5	0.025	0.025	0.22
pH	6.9	6.7	6.8	7.5	7.6
Cowpea paste					
Aerobic plate count	1.5	1.0	0.81	0.595	0.78
Coliform count	0.056	3.0	1.38	1.12	0.86
Salmonella-Shigella count	0.096	1.0	1.32	1.43	0.115
Staphylococcal count	0.09	3.0	0.99	1.08	1.16
Mold-yeast count	1.5	0.07	0.26	0.09	0.07
pH	5.8	5.6	5.3	6.4	6.2

[1]Microbial load is an average reading of duplicate taken in a month and has uniform index of 10^5.

producer E. In this case, the level of contamination is B>C>D>A>E. The pH of the water samples varied from 6.7 to 7.6, while the pH of the cowpea pastes varied from 5.3 to 6.4.

The results of microbial analysis of laboratory-prepared *akara* and the ingredients are as shown in Table 5. The tap water used is of high quality, having only aerobic plate and mold-yeast counts of 1 and 90 cfu/mL, respectively. It did not contain coliform, *Salmonella*/*Shigella* or *Staphylococcus*. On the other hand, the cowpea paste had aerobic plate, coliform, staphylococcal and mold-yeast counts of 300, 3900, 900 and 1500 cfu/g, respectively. There was no growth on *Salmonella-Shigella* agar. However, with regard to the microbial indices used in this study, no growth was obtained in samples of freshly laboratory-prepared *akara* and the microwaved *akara*. Mold/yeast count of only 2 cfu/g was obtained in microwaved *akara* that was stored for a period of 10 weeks.

The physico-chemical attributes of *akara* Ogbomoso are shown in Table 6. It is a coarse, tinted brown product with a diameter of about 4.5 cm and average thickness of 1.35 mm. Usually, six or seven pieces are packed in nylon bag, with an average weight of 28.5 g. About 700 g of *akara* can be obtained from 1 kg of beans. The moisture and lipid contents are about 8.5% and 11.5%, respectively.

Table 7 shows the details of processing steps, sources of hazards and control measures in the production of *akara* Ogbomoso. It was observed that both microbial and chemical hazards can be encountered during various stages of production. The chemical hazards are encountered during procurement of beans in the form of residual pesticides, water (through improper storage in rusty metallic containers) and heavy-metal contamination from the grinding machines. The microbial hazards in the form of vegetative pathogens and spores can be encountered through the use of non-potable or contaminated water, grinding, use of bare hands, low level of sanitation and personal hygiene and poor manufacturing practices such as cooling in uncontrolled environment, poor storage conditions and lack of use of basic equipment. These hazards can be abated using a number of control measures that bother on the use of high quality raw materials, maintenance of high level of hygiene, adoption of GMPs and use of basic equipment. All these control measures can be achieved through education and enlightenment.

The resistance patterns of some of the bacterial isolates to different antibiotics are as shown in Table 8. Seven bacterial strains did not show resistance to any of the antibiotics that were evaluated. These were strains of *Staphylococcus aureus*, *E. coli*, *Streptococcus pyogenes* and *Shigella* sp. obtained from diverse samples, including cowpea paste and samples of *akara* from both production and selling points. Five bacterial isolates showed resistance to only one type of antibiotic; namely cloxacillin, floxapen and pefloxacin. These were strains of *S. aureus* and *Staphylococcus epidermidis*. A total of 38 other bacterial isolates showed multiple-drug resistance, with the number of antibiotics ranging from two to nine. These were strains of *E. coli*, *S. aureus*, *S. epidermidis*, *Citrobacter freundii*, *Serratia marcescens*, *Proteus*

Table 5. The microbial attributes of ingredients and *akara* Ogbomoso produced in the laboratory.

Samples/microbial load (cfu/mL × 10^3)[1]	Tap water (pH 7.6)	Cowpea paste (pH 6.2)	Freshly prepared *akara*	Microwave-fried *akara*[2]	Microwaved *akara* stored for 10 weeks
Aerobic plate count	0.001	0.3	–	–	–
Coliform count	–	3.9	–	–	–
Salmonella-Shigella count	–	–	–	–	–
Staphylococcal count	–	0.9	–	–	–
Mold-yeast count	0.09	1.5	–	–	0.002

[1]Microbial load is an average reading of duplicate and has uniform index 10^3.
[2]Microwave refrying for 10 s before serving; (–) no growth.

Table 6. The physico-chemical attributes of *akara* Ogbomoso.

Parameters	Attributes of *akara* Ogbomoso[1]
Color	Tinted brown
Diameter (cm)	4.50±0.05
Thickness (mm)	1.35±0.06
Weight per pack[2] (g)	28.5±1.5
Texture	Coarse hard texture
Moisture content (%)	8.5±1.2
Lipid content (%)	11.5±1.6
Yield per kg of beans (kg)	0.70±0.2

[1]Each numerical value is an average of five readings.
[2]Each pack contains six to seven pieces of *akara*.

vulgaris, *Bacillus cereus*, *S. pyogenes*, *Bacillus* sp. and *Shigella* sp.

Akara Ogbomoso differs from the conventional *akara* in terms of process, product characteristics and shelf stability (Falade et al., 2003). It has a low moisture content which extends its shelf-life to more than 10 weeks, while its low fat content is advantageous in reducing a number of health problems that are associated with the consumption of fats. From these points of view, *akara* Ogbomoso would meet the need of every lover of *akara*, for its long shelf-life and low fat content. It has been reported that despite the growing number of low-fat foods in the market, more and more Americans are overweight (Binkley et al., 2000). Obesity leads to a decrease in life span, because risks of developing certain diseases such as coronary heart disease, diabetes and hypertension are increased (Thompson et al., 1998). Therefore, making *akara* that is lower in fat than the traditional West African version could provide a product that would be more suitable for consumers. *Akara* Ogbomoso seems to have a tremendous application in this regard having a fat content generally below 15%, unlike the traditional/conventional *akara* that has fat content of more than 30%. It has been found that the higher the initial moisture content of the food, the more fat the food will absorb during frying (Gamble and Rice, 1988), because the frying process involves migration of oil into the food and water into the oil simultaneously (Singh, 1995). Therefore, lowering the moisture content of the paste initially could reduce the final fat content of *akara*, because it would decrease the amount of water available to migrate. Obviously, this is the situation in the product of *akara* Ogbomoso (Figure 1), since the cowpea is ground as a thick paste, without using water. Because of the long shelf-life of *akara* Ogbomoso, it can easily fit in to the convenience food that can be purchased by the consumer and then microwaved for about 10 s before consumption. It can also be a snack fit for marketing by eateries or snacks outlets, which can easily microwave the *akara* for the consumers. This will extend the popularity of *akara* Ogbomoso among elite and foreign consumers.

The descriptive analysis of the production points is a pointer to the environmental condition of the processing areas. Results obtained in this study have shown that all the producers processed *akara* within residential apartments, with the attendant consequence of contamination. Practices, such as rearing of animals, involvement of teenagers/children in the production of *akara*, use of unwholesome water, lack of basic production tools, low level of hygiene, poor means of storage and lack of formal training on food preparation can contribute to the contamination of the product during preparation and storage. These practices among others were witnessed during the course of this investigation. It has been established that incidence of bacteria in food can occur as a result of post-processing contamination by the producers, water, utensils and animals in the environment (Umoh et al., 1999). Studies have shown that many of the street food vendors in Nigeria neither have formal training on the preparation of food, nor medical health certificate to indicate that they have carried out the recommended physical and medical examinations required for them to be licensed as food vendors (Omemu and Aderoju, 2008). Even, some of the food safety knowledge of the vendors could not be translated into practice due to the lack of basic facilities, such as water and toilets. It is therefore important that training on hygiene and sanitation, provision of basic amenities

Table 7. Processing steps, sources of hazard and control measures in the production of *akara* Ogbomoso.

Processing steps	Sources of hazard	Hazard	Control measures
Procurement of beans/sorting	Beans	Chemicals (pesticides, heavy metals) and stones	Use of high quality beans/quality assurance and inspection
Soaking	Water, metallic container, soil and sewage	Pathogenic organisms and metals	Use of potable water
Removal of seed coat	Hands	Pathogenic organisms	Personal hygiene
Grinding	Milling machine	Heavy metals, pathogenic organisms	Personal hygiene/GMP
Molding of cakes	Hand	Pathogenic organisms	Use of molds, personal hygiene/GMP
Cooling	Air and baskets	Vegetative pathogenic organisms and spores	Cooling under basket covered with muslin cloth; regular cleaning of baskets
Packaging	Hand (picking) and mouth (air-blowing to open nylon)	Pathogenic organisms	Picking using forceps or wearing of gloves; good personal hygiene
Storage	Vegetative cells and spores	Pathogenic organisms	GMP, storage in freezing bags at low temperature; microwave heated for 10 s to be ready for consumption
Hawking/selling	Hand (through repackaging) and air	Pathogenic organisms and particulates	Good personal hygiene; discouragement of repackaging

such as potable water and public toilets and the establishment of code of ethics for street food industry should be pursued vigorously to ensure safety of street foods including *akara* Ogbomoso. These can be better achieved if cottage industries revolving around production of street foods can be established, whereby basic amenities are provided, only certified processors are allowed to work and effective monitoring is put in place to ensure compliance with good hygienic and manufacturing practices. Our interactions with the producers and sellers of *akara* Ogbomoso showed that this would be accepted by them.

The level of contamination in the samples as determined by the loads of different microbial indices showed that about 58.9% and 75.6% of *akara* samples from production and selling points, respectively, were contaminated. Generally, the index of microbial load was high (10^4–10^5) for a number of samples. The lapses observed in the processing areas and during the process were responsible for these levels of contamination. Although, higher loads were obtained from a number of samples from production points than those from selling points, more samples from the selling points were contaminated. The reduction in the microbial load of samples from selling points might be due to the nature of *akara* Ogbomoso, having a very low moisture content that may not favor the growth/survival of bacterial species over a long period of time. In contrast, there was upsurge in the incidence of yeasts/mold in the samples from selling points, which might be as a result of their capabilities to thrive in substrates of low a_w. Similarly, more samples from selling points were contaminated with coliforms and *Shigella*. The high level of contamination of samples from selling points might be attributed to post-processing contamination by the producers during packaging using bare hands, mouth-blowing of the polythene bags to open them and repackaging and storage at ambient temperature (28–35 °C) that promotes the proliferation of microorganisms.

Two important ingredients in the production of *akara* Ogbomoso are water and cowpea, as such; microbial examinations of water used in the production and cowpea pastes were undertaken. All the samples (both water and pastes) were contaminated. The sources and storing conditions of water used were likely sources of contamination. Several authors have reported high level of contamination of water in the country (Ehiri et al., 2001; Fawole et al., 2002; Lateef et al., 2005). Besides, cowpea itself would contribute to the microbial load observed in the pastes, as a result of natural and soil microflora that are associated with it. Other factors that might have contributed to the level of contamination include carry-over effects as regard utensils, containers and milling machines. The results obtained in this study are within the range reported by previous workers. In a study carried out to determine the microbiological quality of cowpea paste to prepare Nigerian *akara*, Bulgarelli et al. (1988) reported total aerobic microbial populations of 10^6–10^8/g, which increased after 12-h incubation at 30–32 °C to ca. 10^9/g. Initial coliform populations were 10^2–10^4/g and decreased slightly between 6 and 12 h; while the yeast/mold populations remained constant at 10^4–10^5/g. The average initial pH of 6.0 declined to 5.1 and 4.5 after 6 and 12 h incubation, respectively.

By incorporating necessary corrective measures in the production process, *akara* Ogbomoso that was prepared in the laboratory was not contaminated. Such corrective measures are listed in Table 7. The use of high quality water, high level of personal hygiene, use of molds instead of hands, cooling in a container covered with muslin cloth and packaging using forceps to pick

Table 8. The antibiogram profile of bacteria isolated from water, paste and *akara* Ogbomoso.

Number of antibiotics	Resistance patterns[1]	Isolates	Sources of the isolate[2]
		S. aureus	E
		S. aureus	Paste E
		E. coli	S5
0	Nil	*S. pyogenes*	S5
		Shigella sp.	S1
		S. aureus	E
		S. aureus	A
	Cxc	*S. epidermidis*; *S. aureus*	S3; C
1	Flx	*S. aureus*	A
	Flx	*S. aureus*	B
	Pfx	*S. aureus*	S2
2	Cpx Flx	*S. aureus*	Paste C
	Cot Flx	*S. aureus*	C
	Cxc Flx	*S. epidermidis*	Paste E
	Cro Gen	*Shigella* sp.	Water A
3	Cpx Cxc Flx	*S. aureus*; *S. pyogenes*	Water C; B
	Nit Aug Cro	*E. coli*; *P. vulgaris*	S4; S3
	Nit Aug Cro	*E. coli*	S2
	Cxc Cot Flx	*S. aureus*	S3
	Cxc Aug Flx	*S. aureus*	E
	Aug Cro Tet	*S. marcescens*	D
	Aug Cro Amx	*S. marcescens*	D
	Aug Cro Cot	*S. aureus*	C
4	Cpx Cot Cxc Flx	*Bacillus* sp.	B
	Aug Cot Cxc Flx	*S. pyogenes*	Paste C
	Tet Amx Aug Cro	*E. coli*	Paste C
	Nit Aug Cot Cro	*E. coli*	S2
	Cot Cld Cxc Flx	*P. vulgaris*; *S. aureus*	S3; B
5	Cpx Cot Cld Cxc Flx	*B. cereus*; *S. aureus*	S2; Water B
	Aug Cro Cot Amx Nit	*S. marcescens*	D
6	Aug Cpx Cot Cld Cxc Flx	*S. aureus*	Paste C
	Aug Cro Cot Amx Nit Pfx	*C. freundii*	B
	Aug Cro Cot Amx Nit Tet	*E. coli*	A
	Aug Cro Cot Nit Tet Pfx	*E. coli*	D
7	Aug Ery Gen Cpx Cld Cxc Flx	*S. pyogenes*	S5
	Cip Gen Cpx Cot Cld Cxc Flx	*S. aureus*	S5
	Aug Cro Cot Amx Nit Tet Pfx	*E. coli*	D
8	Cpx Cot Cld Cxc Flx Aug Ery Gen	*Bacillus* sp.	S1
	Aug Ery Gen Cpx Cot Cld Cxc Flx	*S. epidermidis*	S5
	Aug Cro Nit Gen Cot Ofl Amx Tet	*C. freundii*	S4
9	Aug Ery Cip Gen Cpx Cot Cld Cxc Flx	*S. aureus*	S4
	Aug Ofl Ery Gen Cpx Cot Cld Cxc Flx	*S. aureus*	S1
	Aug Ery Cip Gen Cpx Cot Cld Cxc Flx	*S. aureus*	S1
	Aug Ofl Ery Gen Cpx Cot Cld Cxc Flx	*S. aureus*	S4
	Aug Ery Cip Gen Cpx Cot Cld Cxc Flx	*S. epidermidis*	S5

[1]Augmentin (Aug), Amoxycillin (Amx), Ofloxacin (Ofl), Tetracycline (Tet), Ceftriazone (Cro), Nitrofurantoin (Nit), Gentamycin (Gen), Cotrimoxazole (Cot), Ciprofloxacin (Cpx), Pefloxacin (Pfx), Cloxacillin (Cxc), Gentamicin (Gen), Cotrimoxazole (Cot), Erythromycin (Ery), Clindamycin (Cld), Cephaplexin (Cpx), Floxapen (Flx), Ciprofloxacin (Cpx), Augmentin (Aug) and Ofloxacin (Ofl).
[2]A–E, *akara* samples from production points; S1–S5, *akara* samples from selling points; water, water samples from production point; paste, cowpea pastes from production point.

akara were some of the measures undertook. In certain cases, freshly prepared *akara* were further heated in the microwave oven and stored. At the end of 10 weeks, the samples were devoid of contamination, except for a yeast load of 2 cfu/g. The major critical points are frying, storage and refrying.

The strains of bacteria that were isolated from the samples include *E. coli*, *S. aureus*, *S. epidermidis*, *C. freundii*, *S. marcescens*, *P. vulgaris*, *B. cereus*, *S. pyogenes*, *Bacillus* sp. and *Shigella* sp. In a recent study on the ready-to-eat foods, *E. coli*, *S. aureus* and *Bacillus* sp. were isolated from hawked *akara* (Adegoke et al., 2008).

Similarly, there has been report of the isolation of *Enterobacter*, *Klebsiella* and *Lactobacillus* from cowpea pastes used in making Nigerian *akara* (Bulgarelli et al., 1988). Some workers have also reported the isolation of *S. aureus*, *E. coli*, *Streptococcus faecalis*, *Bacillus subtilis* and *Klebsiella pneumoniae* from contact surfaces and ingredients during the production of *akara* (Badau et al., 2001). The variation in the type of bacterial species isolated from *akara* by different authors is attributable to the microflora associated with the cowpea, water, processing equipment and handlers, which may vary widely. With the two levels of frying, it would not have been possible for any of these isolates to survive in *akara* Ogbomoso. However, during the stages of cooling, which is usually done in an open space, varying degrees of handling and re-packaging, including those by children of low hygiene and inadvertent inoculation of the polythene bags by mouth-blowing to open them, this level of contamination is inevitable. Similarly, fungi were isolated from the various samples, but these are mainly *Candida* yeasts and *Aspergillus niger*. Various authors have previously isolated these fungal isolates from cowpea pastes and *akara* samples (Bulgarelli et al., 1988; Badau et al., 2001). In any case, the isolation of these organisms portends a great danger for public health. These are organisms that can cause varying degrees of infections and poisoning in humans.

Although, some few bacterial strains notably *S. aureus*, *E. coli*, *S. pyogenes* and *Shigella* sp. did not show resistance to the antibiotics used in this study, the multiple-drug resistance shown by 38 isolates of *S. aureus*, *E. coli*, *S. pyogenes*, *S. marcescens*, *C. freundii*, *S. epidermidis*, *B. cereus*, *P. vulgaris*, *Bacillus* sp. and *Shigella* sp. is a worrisome development. This in view of the potential pathogenicity of the isolates and the concern of public health, since major epidemics throughout the world are increasingly found associated with resistant pathogens (Levy, 2001; Canton et al., 2003). The resistances of a number of these isolates have been reported in literature.

Staphylococci are Gram-positive facultative anaerobic bacteria. They are widespread among mammalians where they belong to the healthy microflora of skin and mucosa. However, staphylococci are also common human and animal pathogens. The coagulase-positive species *S. aureus* are the species with the broadest pathogenic potential. In contrast to *S. aureus*, members of the heterogeneous group of coagulase-negative staphylococci (CNS) are regarded as less pathogenic bacteria. CNS indeed represent a substantial part of the saprophytic microflora in humans and they rarely cause disease in immunocompetent outpatients. In recent decades, however, CNS have emerged as nosocomial pathogens in immunocompromised individuals. Specifically, *S. epidermidis* is a common cause of line-associated septicemia and other polymer-related infections. Nosocomial isolates of both *S. aureus* and CNS are characterized by increasing resistance toward antibiotics which is a great challenge for the management of hospital-acquired infections (Ziebuhr, 2001). It is noteworthy that in this study, about 45% of multi-drug resistance occurred among the staphylococci.

Gündogan et al. (2006) reported high prevalence of resistance of strains of *E. coli* and *S. marcescens* isolated from samples of raw minced calf meat, chicken carcasses and meatballs (ready-to-eat meat) to a number of antibiotics, including nalidixic acid, tetracycline, ampicillin, kanamycin, chloramphenicol and erythromycin. *S. marcescens* is recognized as an opportunistic pathogen and strains of it are now resistant to commonly used antibiotics (Hejazi and Falkiner, 1997). However, up to 1950, the species was thought to be a harmless saprophytic organism (Anía, 2008). It has also been reported that most *C. freundii* isolates showed resistance to anti-pseudomonal penicillins, first-, second- and third-generation cephalosporins, gentamicin, tobramycin and aztreonam (Wang et al., 2000). *C. freundii* often causes severe infections in immunocompromised, aged and debilitated patients and its infections have been reported to account for 1.5–2% of total nosocomial infections (Schaberg et al., 1991). In the same vein, *Proteus* species are highly resistant to antibiotics; therefore infections caused by *Proteus* species are difficult to cure (Yah, 2007). Their plasmids are responsible for spreading antibiotic resistance genes in a microbial population. A large number of *Proteus* species have varied multi-drug resistant markers that are encoded on transferable plasmids. Similarly, it has been reported that *Shigella* sp. isolated in Cordoba, Argentina, were resistant to multiple antibiotics, particularly traditional agents such as ampicillin, chloramphenicol and trimethoprim and all the isolates harbored at least one plasmid (range 1–12) and the plasmid profiles differing in every case (Brito-Alayon, 1994). Shigellosis is a global health problem. However, developing countries, where there is poor hygiene and unsafe water supplies, are especially affected (Yismaw et al., 2008). A result obtained from a study in Ethiopia has demonstrated continued sensitivity of *Shigella* to gentamicin and ciprofloxacin and widespread resistance to tetracycline, ampicillin and cotrimoxazole (Yismaw et al., 2008).

We have reported high levels of multiple-drug resistance in a number of studies carried out on bacterial isolates such as *E. coli*, *S. aureus*, *S. marcescens*, *P. vulgaris*, *S. pyogenes*, *B. cereus*, *Klebsiella* sp., *Micrococcus* sp., *Pseudomonas aeruginosa*, *Enterobacter* sp., *Streptococcus equi*, *S. epidermidis* and *B. subtilis* from diverse environmental samples (Adewoye and Lateef, 2004; Lateef, 2004; Lateef et al., 2004, 2005, 2006). These studies among other things showed steady rise in the resistance of various isolates to different antibiotics. Since there is continuous interaction among different components of the environment, it is not surprising that a high level of antibacterial resistance is

obtained in this study. A number of practices that contribute to the emergence, development and spread of resistance in bacteria are rampant in the country. These include: poor prescription without sensitivity testing, self-prescription by patients, availability of drugs on the counter, proliferation of fake or sub-standard drugs and indiscriminate disposal of untreated wastes (pharmaceutical, medical and industrial). In addition, many antibiotics persist in the environment (Zuccato et al., 2000) and this enhances resistance in bacteria that are exposed to sub-optimal dose of antibiotics.

In conclusion, it has been shown that contamination of *akara* Ogbomoso is largely due to post-processing operations. The contamination can be abated through the use of high quality water, high level of personal hygiene, use of molds instead of hands, cooling in a container covered with muslin cloth and packaging using forceps to pick *akara*. It is also recommended that refrying in the microwave can be undertaken before consumption. These actions shall ensure the safety of *akara* Ogbomoso.

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