Nigerian Food Journal
Official Journal of Nigerian Institute of Food Science and Techonology www.nifst.org
NIFOJ Vol. 30 No. 1, pages 59 – 66, 2012

Development of Hazard Analysis Critical Control Points (HACCP) and Enhancement of Microbial Safety Quality during Production of Fermented Legume Based Condiments in Nigeria

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ABSTRACT

Critical control points during production of *iru* and *okpehe*, two fermented condiments, were identified in four processors in Nigeria. Physical objects such as stone and metallic objects were mixed in with the seeds; contamination resulted from wet-dehulling by foot pressing and water used in washing. The pH variation during fermentation ranged from 6.9 – 8.5 for both unfermented cotyledon and fermented condiments. Total aerobic viable count (TAVC) ranged from 1.2 – 6.2 Log_{10}CFU/g. Coliforms, enterococci, staphylococci and *B. cereus* were pathogens detected during processing and post-processing of condiments. This indicates that the processing of condiment using traditional method is subject to microbial contamination. Dry-dehulling, cooking under pressure and inoculation of starter cultures reduced contamination and enhanced safety quality. Training of processors about Hazard Analysis Critical Control Points (HACCP), processing, environmental sanitation and personal hygiene were suggested as strategies to improve the safety of these traditional fermented condiments.

Keywords: HACCP, contamination, fermentation, condiments, processing, environmental sanitation.

Introduction

Leguminous oil seeds are produced in large quantities in many West African countries. They are fermented to produce highly priced condiments, used as seasoning for soup and sauces as well as a source of plant proteins consumed to supplement dietary intake of people. In Nigeria, *iru* or *dawadawa* is a fermented locust bean (*Parkia biglobosa*) cotyledon consumed in South and Northern Nigeria (Odunfa, 1985). *Okpehe* is fermented *Prosopis africana* seeds popularly consumed in the eastern region of Nigeria (Oguntoyinbo *et al.* 2007).

Traditional methods are employed during the production of condiments in West Africa (Odunfa 1985; Sanni 1993). Food processing in unhygienic environment using rudimentary equipment without consideration for Good Manufacturing Practice (GMP) may subject product to contamination with grievous health hazards. Although efforts have been made to characterize microbial diversity of fermented condiments in West Africa, dominant bacteria such as *Bacillus subtilis* has been identified (Oguntoyinbo *et al.* 2010; Ouaba *et al.* 2003). However, consumer preference for safe and quality food products is increasing and the need to develop strategy to improve safety of condiment is essential for product competitiveness with standardized bouillon cubes as well as guarantee product acceptability and consistency. Hazard critical control points (HACCP) is a systemic approach for identification of hazard and prevention strategy for the improvement of food safety without necessary reliance on end product testing (Cormier *et al.* 2007). Therefore, implementation of HACCP that can

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identify hazards, risk assessment and procedure to ameliorate risk, prevent and control hazards will be useful to improve the safety of condiments in West Africa. This is in agreement with the WHO/FAO requirement for implementation of HACCP. The support and backing of WHO, FAO and national government have been identified as essential perquisite for success of implementation (Williams *et al.* 2003; WHO 1995).

Challenges of implementation of HACCP in small scale food production have been critically reviewed (Panisello *et al.*, 2001). Although limitation of resources and poor implementation of policies militate against the development of HACCP in small scale industries in developing countries, importance of HACCP is gradually being recognized during traditional fermented food production in West Africa; for instance, Obadina *et al.* (2008; 2010) identified HACCP during wet cassava fermentation and suggested strategies for improvement in the hygiene quality. These strategies can also be adopted for enhancement of safety of other traditional fermented food especially fermented legumes in West Africa.

In this study, significance of implementation of GMP and good hygiene practice (GHP) were evaluated at production sites of fermented condiment production in Nigeria. Impact of usage of starter cultures during fermentation and improvement of hygiene practices on product quality were determined.

Materials and Methods

Determination of production sites

Different producers of fermented *Parkia biglobosa* and *Prosopis africana* seeds were identified in Makurdi, Nsukka, Ibadan and Ado-Ekiti, Nigeria. Processors were selected based on their location, track record of consistent production, production environmental conditions as well as interest in participation. Twenty local producers were identified, five from each production town listed above. Site analysis showed that all producers lacked basic facilities to support GMP and GHP with poor adherence to environmental hygiene.

Traditional production of iru and okpehe

Production of *okpehe* and *iru* were observed to be similar in all production sites except for the usage of different leguminous seeds, *Prosopis africana* seeds (East and middle belt of Nigeria) for *okpehe* and *Parkia biglobosa* seeds for *iru* production (South-West Nigeria). Five hundred grams of each seed were soaked in water overnight at room temperature before boiling at 100°C for 12 h using firewood. During this process the seeds were moistened, soft seeds were wet-dehulled manually by foot pressing. The dehulled cotyledons were washed in water before second boiling at 100°C for 5 – 10 h. Hot cotyledons were spread on calabash trays already lined with pawpaw (*Carica papaya*) leaves. The trays were stacked together, wrapped with jute bags and kept for 48 – 72 h as a fermenting mash. During this period natural fermentation took place. The final product is a dark coloured, pungent smelling *iru* or *okpehe* respectively.

Hazard analysis

Hazard analysis was conducted on fermented legume seeds from four manufacturers (one from each of the identified towns) in the study sites and critical control points were identified. The hazard analysis consisted evaluation of raw materials, water and its sources, environment, fermentation vessel, personal hygiene and other sources of contamination during processing as well as handling during packaging of final products. Detailed schematic flow diagrams for *iru* and *okpehe* production were developed based on the methods employed by processors as observed during traditional production and different control points identified (Figure 1). Potential sources of hazards, microbial contamination of raw materials, equipment, human and animal contamination were identified. The dominant microorganisms, the ability for survival, destruction and increase in population were also determined.

Collection of samples

Samples were obtained at different stages of processing and analyzed for microbial safety assessment; fermentation time by different processors were observed, pH changes were determined using

digital HI222 pH meter (Hanna Instruments, Woonsocket, RI, USA). Approximately 200 g of unfermented cotyledon, freshly fermented condiments and packaged condiments in leaf and polyethylene bags were collected and analyzed.

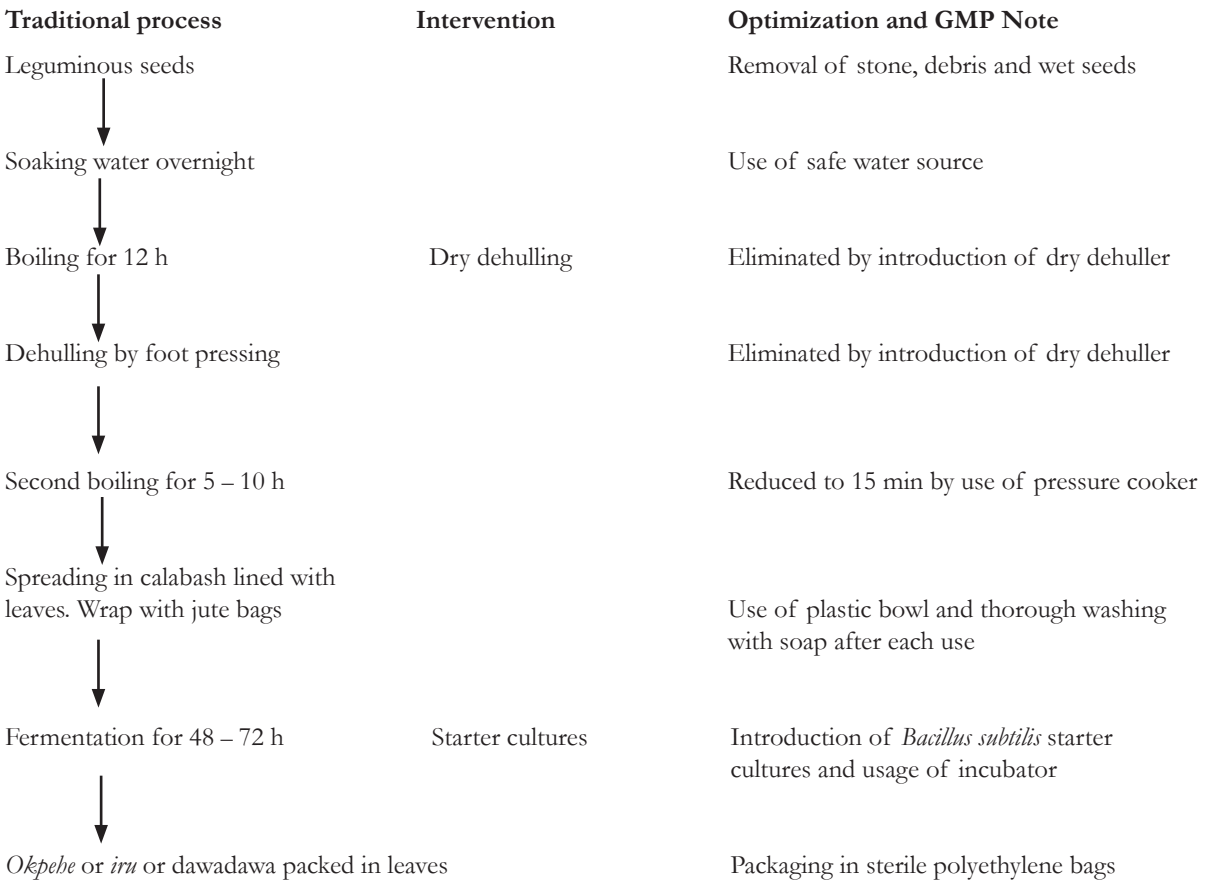

Fig. 1: Flow diagram and identified CCP generated during taditional *iru* or *okpehe* production in Nigeria

Laboratory scale production of condiment following Good Manufacturing Practice and Plant hygiene

The traditional method of production was optimized in laboratory scale fermentation. Laborious and unhygienic foot pressing for dehulling to obtain cotyledon from legume seeds, was replaced with dry dehulled using dry dehulling, by Institute of Food Research Oshodi, Lagos, Nigeria. The cotyledons (500 g) were boiled in 1.5 l of water for 15 min at 121°C using Kohn pressure cooker (Switzerland). Soft cotyledons were inoculated with *B. subtilis* ULAG 712 (obtained from Department of Microbiology, culture collection) and incubated at 37°C for 24 h. Fermentation was monitored by determination of pH using digital pH meter, microbial enumeration and determination of ammonia-like pungent aroma production as well as colour change.

Microbial enumeration and identification

Fermented and unfermented cotyledons were analyzed for microbial composition. Serial dilution of 10 g samples in quarter strength Ringers solution (Oxoid Hampshire, United Kingdom) was

used for microbial enumeration with the following media: Plate count agar (Oxoid) for total aerobic viable count (TAVC), MacConkey agar, for gram negative bacteria and coliforms, Trypton Soya agar for *Bacillus* (samples were preheated at 80 °C for 10 min before inoculation) and Kanamycin Aesculin Azide agar (Oxoid) for enterococci. Portions (0.1 ml) of appropriate dilution were spread plated in triplicate. Counts on agar media were obtained after incubation for 24 h at 30 °C. Representative isolates were identified using 16 SrRNA gene sequencing as previously described (Oguntoyinbo *et al.*, 2010).

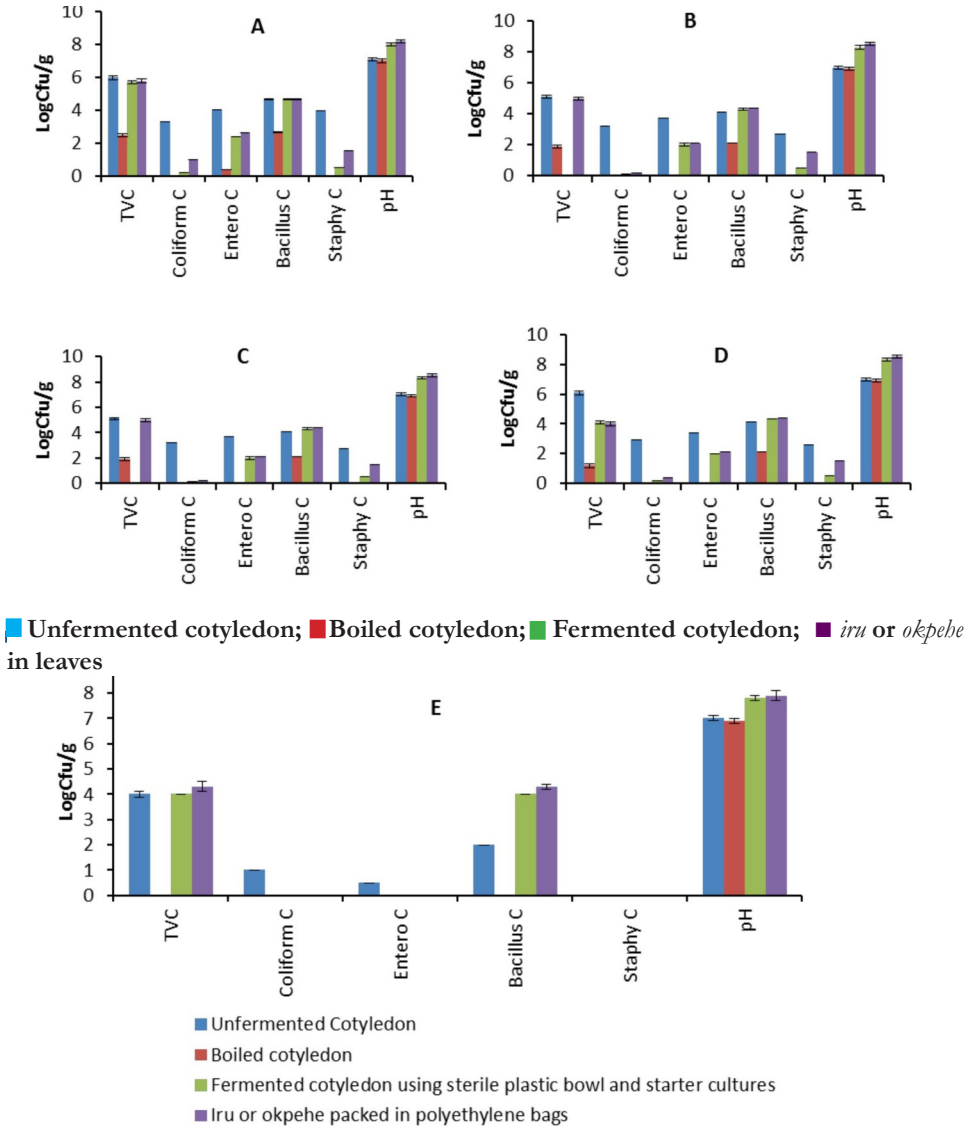

Fig. 2: Bacterial counts and pH changes during traditional and controlled *iru* or *okpehe* production in Nigeria.

A & B *iru* samples obtained from Ibadan and Ado-Ekiti, C & D *okpehe* samples from Markudi and Nsukka respectively. Error bars represent standard deviation of mean of triple replicate determination.

Statistical analysis

Analysis of variance test (ANOVA) and Duncan's multiple range test (Duncan 1955) were used to evaluate the mean differences, which were deemed significant at $p < 0.05$ to compare means coliform and enterococci in water from different sources used for processing as faecal indicator.

Results

Okpehe processors in Markurdi and Nsukka produced condiments at home, and interference with domestic activities and animals were observed. Water used in processing was obtained from pipe borne, stream and well water stored in metal drums and plastic bowls. Two to three personnel were involved with processing with no food hygiene and handling training. Fermented products were packed inside leaves or rolled into balls while some portions were sun dried before sales. The flow diagram deduced from the manufacturer is shown in Figure 1.

Iru processors in Ibadan and Ado-Ekiti live in communal housing units shared with neighbours. Animal and pests were noticed around processing sites. Well water supply was the main source of water used for processing. Processors were assisted with two personnel with limited knowledge of food hygiene and handling. Finished products were set on calabash trays or wrapped in leaves and sold in open market. The flow diagram deduced from the manufacturers and *okpehe* processing is shown in Figure 1.

The bacterial count at different stages of both *iru* and *okpehe* processing is shown in Figure 2. The pH of the samples obtained during processing ranged from 6.9 – 8.5 for both unfermented and fermented condiments. Total aerobic viable count (TAVC) ranged from 1.2 to 6.2 Log10CFU/g, coliform count from 0.1 to 3.3 Log10CFU/g, Enterococci count 0.4 – 4.0 Log10CFU/g, Bacilli count 2.0 to 4.6 Log10CFU/g and Staphylococci 0.5 to 3.9 Log10CFU/g. Bacilli count was generally high in all the samples except cotyledons cooked at 121°C for 15 min. Also, *Staphylococcus* counts were higher in cotyledons that were wet dehulled using foot pressing. Enterococci were the only Lactic acid bacteria detected among the samples. The pathogens were further identified using genomic sequencing of 16S rRNA gene as *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecium*, *E. faecalis* and *Micrococcus luteus*. Faecal contamination must have affected some of the water used during processing. High counts of coliforms and enterococci were observed in stream and well water samples (Table 1).

Table 1: Bacterial population profile of different water sources used during traditional iru and okpehe production in Nigeria

Organism	Stream water	Well water	Chlorinated pipe-borne water	Spring water
Faecal coliform	$21.56^{a} \pm 1.2$	$7.80^{b} \pm 2.4$	$2.52^{c} \pm 1.2$	$3.61^{c} \pm 0.8$
Enterococci	$81.1^{a} \pm 2.7$	$19^{a} \pm 2.8$	$2.7^{b} \pm 3.5$	$4.0^{b} \pm 1.6$

Mean values with the same superscript alphabet are not significantly different ($p < 0.05$)

The identified hazards during condiment production are shown in (Table 2). Results showed that leguminous seeds can be sources of hazard which can be physical, chemical and biological. Soaking, dehulling and packaging are other sources of contamination due to chemical and microbiological hazards originating from water and food handlers. Dehulling and fermentation were the critical control points (CCP) which must be controlled and optimized to prevent microbial contamination (Table 3). Use of foot pressing might have introduced pathogenic and spoilage endospore forming bacteria into the cotyledon yield. These organisms cannot be eliminated by cooking and can readily make the product unsafe. This dehullling process takes longer time with low

cotyledon yield. The introduction of dry dehulling brought about the elimination of the first boiling step and prevented contamination. Use of starter cultures of *B. subtilis* enhanced colonization of the fermentation, prevented spoilage and pathogenic bacteria from proliferation.

Table 2: Identified hazards during processing of legume oil seeds for condiment production by fermentation

Stage	Hazard	Source	Control
Seed	Chemical		
	Oligosaccharide	Seeds	Supplier
	Pesticides	Farm/storage	QA/inspection
	Heavy metals	Water	SQA
	Chloride	Water	SQA
	Stone	Farm and processing	SQA
	Microbiology	Processor, seeds	SQA, Inspection
	Pathogen, *E. coli*, *S. aureus*, *B. cereus*		
Soaking	Vegetative pathogen	Handlers, environment	GMP
	Stone	Handling	GHP
	Metal	Tank and water source	GMP
Dehulling	Spore forming pathogen	Food handler, environment	GMP
	Stone	Environment	GMP
Packaging	Vegetative and spore forming pathogen	Food handler, environment	GMP

Table 3: Identified intervention summary for HACCP implementation

Processing	Hazard	Control measure	Critical limits	Monitoring	Corrective action
Dehulling	Contamination with spoilage microorganism and pathogens	Use of dry dehuller	pH 6.5, moisture <20%	Quality of seeds evaluation, processing timing, Microbiological assessment	Cooperative utilization and acquisition of dry dehuller
Fermentation	Contamination by spoilage Enterococci strains and pathogenic B. cereus	Use of *B. subtilis* starter cultures with inhibitory properties against pathogen	Reduce fermentation time less than 24 h to achieve pH 7.8 – 8, good hygiene	pH, mild ammonical pungent smell and colour change	Training on starter cultures handling and GMP

Discussion

Endospore forming bacteria identified as bacilli predominated tested unfermented cotyledon and fermented condiment. The two stages of cooking used during traditional fermentation did not eliminate this bacterium. This organism must have originated from seeds, dehulling process, handling and fermentation calabash. Increase in the population of bacilli and enterococci during 24 – 48 h of fermentation showed that this stage of processing constitutes a CCP. Toxigenic potential of some bacilli and spoilage potential of enterococci strains isolated during fermentation of cotyledon for condiment and other traditional fermented foods in Africa during production has been previously well documented (Oguntoyinbo and Oni, 2004).

Detection of coliforms, enterococci and staphylococci in condiments showed usage of contaminated sources of water for production and possible post-processing contamination by food handlers, and cross contamination from domestic animals or pests in the processing environment. Increase in pH was noted during the fermentation. This is in agreement with previous studies during *kinema* production in India and *soumbala* production in Burkina Faso (Sarkar and Tamang 1995; Ouaba *et al.,* 2008). Alkaline fermentation with pungent ammonia-like aroma associated with fermentation of legume based foods is very common in West Africa.

Legume seeds meant for condiment production should be stored in a dry and pest-free environment. They should not be mouldy and should be free of stone. Water to be used for soaking, washing and cooking should originate from safe sources, colourless, odourless and neutral. Dry dehulling using a simple and cheap dry dehuller is recommended; this will result in water reduction, less contamination and saves time. Fermentation period of 24 h using starter cultures of *B. subtilis* has been previously recommended (Oguntoyinbo *et al*., 2007) and the use of starter cultures with known functional properties that can impact desirable properties during traditional fermentation has been previously suggested (Holzapfel, 2002). Moreover, pathogenic and spoilage microorganisms observed during fermentation may make product unacceptable and unattractive. Cooking cotyledon under pressure before fermentation created a bactericidal effect on all pathogens including *B. cereus*. Plastic bowls can be used as fermentation vessel instead of calabash; they are cheap and can be thoroughly cleaned with soap after every fermentation process. Prevention of post-fermentation contamination during processing is essential to product safety. This will require good hygiene practices by food handlers. Post-fermentation contamination can be prevented by wearing gloves and packaging fermented condiments into polypropylene bags instead of leaves.

Conclusion

Results from this study indicated that contamination of condiments might have originated from dirty environment used for production, foot pressing during dehulling, contaminated water and poor personal hygiene of food handlers. Critical control points during processing are dehulling methods and controlled fermentation by monitoring pH changes. Training of processors about hazards during and after production and essentials of CCP are desirable. Education about environmental sanitation, thorough washing of hands and utensils with soap or detergent before and after use and application of dry dehulling method and use of starter cultures for fermentation will contribute significantly to product safety.

Acknowledgement

Study leave granted during the period of this study by University of Lagos is gratefully appreciated.

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