

Full Length Research Paper

Microbial profile and critical control points during processing of 'robo' snack from melon seed (*Citrullus lunatus* thumb) in Abeokuta, Nigeria

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Accepted 23 May, 2006

A study was carried out to determine the level of microbial contamination and establish the critical control points associated with the processing of a locally produced 'Robo' snack from melon seeds in the Abeokuta Metropolis. Samples were collected at different points of processing from randomly selected local producers and subjected to microbiological hazard analysis. The results were used to evaluate the relevant critical control points especially in relation to raw materials and human contaminations, process requirements and contacting of ingredients with equipment. The observed contaminants common to all samples and irrespective of the producers were the *Staphylococcus aureus*, *Salmonella* spp., *Bacillus* spp. and *Aspergillus fumigatus*. The major operations directly implicated in addition to the quality of the various raw ingredients used were the roasting using earthen wares, grinding in local mill, hand mixing of ingredients, kneading and moulding manually, deep fat frying, surface fat draining, open-air cooling, and holding/packaging in polyethylene films during sales and distribution. The product was, however, classified under category III with respect to risk and the significance of monitoring and evaluation of quality using the hazard analysis critical control point (HACCP) principles and adoption by the local producers of 'robo' are highlighted and discussed in relation to existing practices and major constrains.

Key words: Critical control points, 'robo', melon seeds, HACCP.

INTRODUCTION

There are varieties of indigenous snacks consumed in parts of Nigeria among which include 'Robo', 'Kulikuli', 'fura' (Sobukola, 2003). The technologies employed in the processing, distribution and storage of such foods are based on the long established knowledge of traditional processing of such foods. Robo is produced from the seeds of watermelon fruit (*Citrullus lunatus*) and is widely consumed in the western part of Nigeria, including Abeokuta. The recipes for Robo production and processing procedures have recently been reported (Sobukola et al., 2006).

The bulk of such local snacks sold along the streets in Nigeria as in most developing countries are characterized by low level of hygiene, which generally starts from the farm where the raw materials were obtained through processing stages to the consumers (Aroyeun and Adegoke, 2000). Traders and cottage processors tend to adopt measures which attract the least cost in relation to expected earnings from the local varieties of foods being processed. It is conceivable that even if it were possible to carry out necessary analysis at every stage of processing, the facilities to do so are presently too expensive for the local processors. This situation, however, poses a dilemma to the society.

The occurrence of food poisoning outbreaks worldwide has considerably increased public awareness about food safety (Anifantaki et al., 2002) and the need for better

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Table 1. Mean bacterial counts (cfu/g) and fungal counts (propagules/g) of raw ingredients used for the preparation of Robo snack.

Days of Sampling	Bacterial counts			Fungal counts		
	A	B	C	A	B	C
1	1.4×10^2	1.2×10^2	2.7×10^2	8.1×10^3	4.2×10^3	3.2×10^2
2	1.5×10^2	1.1×10^2	1.2×10^2	7.9×10^3	5.0×10^3	2.8×10^2
3	1.2×10^2	1.2×10^2	2.4×10^2	9.4×10^3	5.2×10^3	3.1×10^2

Legend: A – Melon seeds; B – Dried ground pepper; and C – Onions

control of processing conditions to ensure a safe and high quality product (Awonorin and Rotimi, 1992). However, in the developing countries, such as Nigeria, estimates of food – borne diseases are very difficult to perform due to lack of reliable epidemiological data (Adegoke et al., 1994). Therefore, in the absence of epidemiological data, hazard analysis critical control point (HACCP) evaluations can be used to draw attention to important food safety measures (Michanie et al., 1987; Bryan et al., 1992). The HACCP concept is a systematic approach based on hazard identification, assessment and control but places premium on both process and raw material control than the testing of final products (Savage, 1995). But it is often necessary to carry out individual analysis of the critical control point (CCP) required for a given production process rather than applying a generalized procedure for all types of foods. Hence, the objectives of this research were to identify the critical control points most appropriate to the safety of product during processing of Robo snack and identify the hazards and microbiological status of Robo produced commercially and recommend measures to improve its quality.

MATERIALS AND METHOD

Samples

Samples for the analysis were collected from three processors at Lafenwa area of Abeokuta. The method adopted involves the monitoring of the Robo preparation, identification of possible sources and modes of contamination, collection of samples during production and testing for microbiological hazards, categorizing risks and documenting a flow chart indicating relevant critical control points (Peri, 1993). The traditional method of Robo production has been reported (Sobukola et al., 2006).

Hazard analysis

The following parameters were considered in conducting hazard analysis at sampling point: Observation (at least thrice) of unit operations and storage practice in order to identify the main sources of microbial contamination, collecting of food samples before and/or after each critical control points identified along preparation line and testing samples collected on each of three occasions using spatulas (previously washed, rinsed with potable water and followed by immersion in 95% alcohol with flaming after

each immersion) as reported by Peri (1993). All samples (carefully labeled) were then placed into an insulated plastic box containing ice flakes and held there until arrival and processing in the laboratory. Samples were either analyzed immediately or kept chilled in the refrigerator at $5 \pm 1^\circ\text{C}$ until the following day for analysis.

Laboratory procedures

Enumeration of aerobic mesophilic organisms (bacteria and moulds): For enumeration, plate count agar (PCA) and potato dextrose agar (PDA) were used for bacterial and fungal counts, respectively. Approximately 10 ± 1.0 g samples were mixed with peptone water (1:10) to prepare the initial homogenate. This was homogenized for about 3 min using hand agitator followed by making serial dilutions with 1 ml of the initial homogenate being added to 9 ml of sterile peptone water (oxoid). Twenty microlitres (20 μl) of the initial dilution (10^{-1}) and through each of the dilutions up to 10^{-6} were added, respectively, to PCA and PDA using the drop plate technique (Samson et al., 1981). The PCA agar plates were incubated in air at 35°C for 18 – 24 h after which plates were read. The PDA was also incubated in air at room temperature $26 \pm 2^\circ\text{C}$ for 5 – 7 days after which fungal isolates were examined macro- and micromorphologically (Cowan, 1981). All colonies that grew on PCA and PDA were expressed in cfu/g and propagules/g, respectively.

Characterization of isolates using biochemical methods: For the identification of bacterial isolates, Gram staining procedure, production of extra cellular enzymes (e.g. catalase, coagulases, oxidase and urease) and fermentation of simple sugars were carried out (Codex, 1993). Wet mounts using lactophenol cotton blue followed by microscopic characteristics of fungal elements were used for the presumptive identification of fungi (Cowan, 1981).

RESULTS AND DISCUSSION

In this study, raw sensitive ingredients such as melon seeds, dried ground pepper and onions were found to be grossly contaminated with a variety of microorganisms of public health importance (Tables 1 to 3) since they are commonly marketed without sterilization or consideration for safety (McKee, 1995; Adams and Moss, 1995). Because of these, the supply of ingredients can be considered as critical control point (Bryan et al., 1992). In addition to the raw ingredients, final products were also found to be contaminated (Tables 2 and 3).

The isolation of *Bacillus* spp, *Staphylococcus aureus*,

Table 2: Mean aerobic bacterial count cfu/g and fungal count (propagules/g) obtained during preparation of Robo snack

Days of sampling	A	B	C	D	E	F
Aerobic Bacterial counts						
1.	4.3×10^4	6.2×10^4	7.4×10^4	7.6×10^4	2.7×10^1	3.2×10^1
2.	4.9×10^4	6.0×10^4	7.1×10^4	7.1×10^4	2.5×10^1	3.1×10^1
3.	4.5×10^4	5.9×10^4	7.2×10^4	7.3×10^4	2.6×10^1	3.0×10^1
Fungal count						
1	4.1×10^1	5.4×10^2	5.4×10^2	5.5×10^2	5.4×10^2	5.4×10^2
2	4.2×10^1	5.1×10^2	5.2×10^2	6.0×10^2	6.0×10^2	5.9×10^2
3	3.8×10^1	4.7×10^2	4.6×10^2	4.9×10^2	4.4×10^2	4.5×10^2

Legend: A – Grinding; B – Mixing; C – Kneading; D – Moulding; E – Surface fat draining / cooling; and F – Products

Table 3. Microorganisms isolated during preparation of Robo snack for sale at Lafenwa market, Abeokuta.

Organism	A	B	C	D	E	F	G	H	I
<i>Bacillus</i> spp	+	+	+	+	+	+	+	-	+
<i>Staphylococcus aureus</i>	+	+	+	+	-	+	+	+	+
<i>Salmonella</i> cells	+	+	+	+	-	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	+	+	+	+	+	+	+

Legend: A- dehusking; B – Roasting; C – Grinding; D – Mixing; E – Kneading; F - Moulding G- Deep fat frying H- Surface fat draining/cooling; and I – Robo - Absent; + Present

Salmonella spp. and *Aspergillus fumigatus* along the line of Robo preparation is of public health importance, more so as there were no reports available on the microbiological status of Robo in the literature. The use of contaminated raw ingredients, unclean utensils and poor environment in which robo is often prepared, coupled with the holding period and with little or no attention being paid to good manufacturing practice by Robo processors can independently or collectively be responsible for the contaminations. Furthermore, as long as the Robo is prepared manually and with no consideration for sanitary practices, hazards in the forms of microbial contamination and ultimately food-borne illness will occur when consuming such products.

Some of the organisms might have been picked up during grinding, mixing, kneading and moulding. Increase in microbial count after grinding can be an indication of unhygienic condition of the locally fabricated machine in which the grinding zones harbor certain materials with water activity (a_w) conducive enough for survival of the organisms (Anifantaki et al., 2002; Adams and Moss, 1995). However, isolation of *Staphylococcus aureus* as well as *Salmonella* may be due to cross contamination of using unhygienic utensils or due to contamination from the skin, mouth, or nose of the food handlers which can be introduced directly into foods by process line workers, with lesions caused by *S. aureus* on hands and arms coming into contact with the food, or by coughing and sneezing (Holah and Thorpe, 1990). Although, there was no human contact during surface fat draining/cooling, but the product may be contaminated from the poor

environment or from the containing basket.

It is interesting to note that high temperatures are commonly involved in the preparation of robo ($160 \pm 5^\circ\text{C}$ for 15 – 20 min) which is sufficient to eliminate most of the microbes isolated in this study. Unfortunately, post-processing contamination by processors, holding period and poor environment all of which affect Robo are forms of hazards/risk (Bryan et al., 1992) which should be controlled.

Microorganisms isolated from samples in this study have been earlier found in foods, environment and other places. *Bacillus* species have been implicated in cases of food poisoning (Goepfert et al., 1972) and *Bacillus cereus* are frequent inhabitants of places such as soils, clays, dust, sediments, natural water, vegetation and many foods (Bergdoll, 1980; Jay, 1986). The shelf stability, nutritional and sensory characteristics of such products would tend to decrease as microbial proliferation remains high.

In conclusion, microbial contamination of samples examined in this study which posed health hazards can be attributed to initial contamination of raw ingredients, cross contamination from raw foods via processors hands, equipment or utensils that are poorly sanitized/cleaned and contamination during storage/holding periods. Since street vending is an important economic activity in this part of the world, post-process contamination must be avoided. Furthermore, operations such as reheating before sales/consumption or refrigeration if possible should be carried out. Education and training, especially that which create awareness among producti-

on and processing personnel, and all forms of hygiene practices, including equipment design and good sanitary provisions should be encouraged.

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