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Critical control points in the fermentation of *oso* (fermented seeds of *Cathormion altissimum*)

[1]Jolaoso A.A.*, [2]Popoola T.O.S

[1]*Moshood Abiola Polytechnic Ojere, Abeokuta*
[2]*Federal University of Agriculture, Abeokuta.*
**Corresponding author email: jolaazeezat@yahoo.co.u*

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ABSTRACT

The traditional fermentation of *Oso*, a locally fermented food from the seeds of *Cathormion altissimum* is mainly carried out by local uneducated processors living in suburbs with poor sanitary conditions.There is the need to develop a better understanding of the microbiological problems by applying the hazards analysis and critical control point (HACCP) strategy. Microbial counts in ten processing sites were analysed and two of the sites were selected for critical control analysis. Data obtained were subjected to Analysis of Variance (ANOVA) and means separated by Duncan multiple range test. Microbiological results revealed aerobic mesophilic count of 4.72 x 10^5cfu/g to 5.34 x10^5cfu/g. Aerobic count on all the locations were significantly different ($p<0.05$). *E.coli, Klebsiella* and *Enterobacter* were isolated from the processing materials in some of the locations, an indication of poor hygiene by processors.

Keywords: Critical control points, aerobic counts, sanitary, *Oso*.

INTRODUCTION

Majority of fermented foods in Africa are produced at household level in a poor hygiene state (Gadaga *et al*, 2008). The problem of food-borne pathogenic disease cannot be ruled out because of the poor hygiene level of the processors, the equipment used, raw materials and contaminated water used (Okorie and Olasupo, 2013). Contaminated water may be the potential source of food contamination; therefore the use of potable water is essential in food preparation to reduce food contamination (WHO, 2015). By identifying the critical control points (CCP), industries and enforcement authorities have more information and are better equipped on methods for control measures, education and enlightenment of processors. There have been few documented reports on the prevalence of coliforms and indicator bacteria isolates from indigenous fermented condiments in Nigeria. However, high coliforms and indicator organisms had been observed in fermented condiments like *Iru, Ogiri, Okpehe* and *Ugba* (Ogunshe and Olasugba, 2008). In view of these, the objective of this research is to identify the critical control points in the process of preparation to reduce the problem of food-borne pathogen.

MATERIALS AND METHODS

Collection of samples

The evaluation of the critical control points was carried out using the method described by Edema and Fawole (2006). Products from ten traditional processing sites were studied while only two of the processing sites were monitored in order to identify critical control points (CCP).

Samples were collected at different stages of processing for microbiological analysis. All the samples were aseptically collected in triplicate and taken to the laboratory in sterile ice bags.

Isolation and enumeration of microflora

Replicate portions of ten-fold dilutions of samples in sterile peptone water were made for all samples collected. The preparations were homogenized and 0.1 ml each of appropriate dilutions was plated using the pour–plate method. Enumerations of the total viable counts/aerobic mesophiles were carried out using plate count agar (Oxoid, UK) and sabouraud dextrose agar (SDA) (Oxoid, UK) for fungi counts. Coliform were isolated using Mac-Conkey broth and Eosin methylene blue. *Bacillus cereus* was isolated on mannitol/egg yolk/ polymyxin agar (MYP). MYP was prepared using peptone, meat extract, D-mannitol, sodium chloride, phenol red, agar-agar, egg yolk (Edema and Fawole, 2006). *Salmonella* were isolated using *Salmonella-Shigella* agar. The plates were incubated at 37^{o}C for 24 hrs except SDA plates which were incubated at 28^{o}C for 24hrs.

Characterisation of isolates from Oso and the processing materials collected at the monitored location.

All colonies of incubated plates were picked at intervals, purified by repeated subculturing before being examined for Gram reaction, microscopically. Cell morphology (using 24-hour old culture), motility, pigmentation and sporulation were observed. Biochemical tests included catalase, oxidase, nitrate reduction, patterns of sugar utilization as well as urea and starch hydrolysis were carried out.

Identification of isolates

Morphological and biochemical tests were used for preliminary or initial identification of isolates. The identities of which were confirmed by Analytical Profile Index (API).

Statistical Analysis

Data obtained from all the analysis were subjected to statistical analysis (ANOVA and Duncan's test) using SPSS 21 for windows.

RESULTS AND DISCUSSION

The microbial counts discovered in the fermented samples from different locations were shown in Table 1. The aerobic counts ranged from 4.72 x 10^5 cfu/g to 5.34 x 10^5 cfu/g. The aerobic counts in all other locations were significantly different ($P < 0.05$) except in two locations which recorded low aerobic counts that were not significantly different ($P > 0.05$). The highest aerobic count was 5.41 x 10^5cfu/g in location (ii).Fungal count ranged from 4.84 x 10^5 cfu/g to 5.39 x 10^5 cfu/g. Two locations (vii) and (ii) recorded counts that were not significantly different ($P > 0.05$) while other locations recorded fungal counts that were significantly different ($P < 0.05$). The highest fungal count was recorded as 5.39 x 10^5 cfu/g.

Coliform count ranged from 4.20 x 10^5cfu/g to 5.38 x 10^5 cfu/g. The coliform counts were not significantly different in locations (i) and (iii) which recorded low coliform counts. Other coliform counts in other samples were significantly different ($P < 0.05$). *Bacillus cereus* counts were not detected from samples in two locations (iv) and (v). The *Bacillus cereus* count from the other locations ranged from 4.00 x 10^5 cfu/g to 5.08 x 10^5 cfu/g.

Table 1: Microbial counts of *Oso* from ten different locations (10^5CFU/G)

Location	pH	AMC	Fungi	Coliform	B.cereus	Salmonella
i	7.9^{b}	5.08^{c}	N.D	4.20^{a}	4.08^{b}	4.00^{a}
ii	7.81^{a}	5.41^{h}	4.88^{b}	4.79^{d}	4.26^{d}	4.00^{a}
iii	8.00^{c}	5.29^{c}	5.27^{g}	4.23^{b}	4.00^{a}	4.26^{c}
iv	8.1^{f}	5.34^{g}	5.26^{g}	4.85^{e}	N.D	5.33^{g}
v	8.2^{i}	5.3^{f}	5.32^{h}	5.13^{g}	N.D	5.19^{f}
vi	8.05^{e}	4.74^{b}	5.15^{e}	5.38^{j}	5.08^{h}	5.18^{e}
vii	8.12^{h}	5.31^{f}	4.84^{a}	4.32^{c}	4.18^{c}	N.D
viii	8.11^{g}	5.27^{d}	5.18^{f}	5.03^{f}	4.38^{e}	4.23^{b}
ix	8.03^{d}	4.72^{a}	5.04^{d}	5.30^{i}	4.96^{f}	4.30^{d}
x	7.92^{bc}	5.3^{f}	5.39^{i}	5.21^{h}	5.05^{g}	5.44^{h}

Values are mean values of replicate samples; AMC-Aerobic mesophilic count; N.D-Not detected

. The counts were significantly different ($P < 0.05$). *Salmonella* counts ranged from 4.00 x 10^5 cfu/g to 5.44 x 10^5 cfu/g. The highest *Salmonella* counts were significantly different ($P<0.05$) to other counts. *Salmonella* counts were not detected in location vii.

Table 2: Morphology and biochemical characteristics of isolates at some stages in fermentation

CODE	GR	SP	CA	ID	CO	MO	IN	OX	CI	UR	HS	MR	VP	G	L	S	M
L1	GNB	-	+	+	-	-	+	-	+	+	-	-	+	A	A	A	A
L2	GNB	-	+	+	-	-	-	-	+	+	-	-	+	A	A	A	A
L3	GNB	-	+	+	-	+	-	-	+	+	-	-	+	A	A	-	A
L4	GNB	-	+	+	-	+	-	+	+	-	-	+	-	-	-	-	-
L5	GPB	-	+	+	+	-	-	-	-	-	-	-	+	A	A	A	A
L6	GPB	+	+	+	-	+	+	-	-	-	-	-	+	A	-	-	-
L7	GPB	-	+	+	-	-	-	-	-	-	-	-	+	A	-	-	-

Codes L1-L7 are isolates : L1-*Escherichia coli*, L2-*Klebsiella sp*, L3-*Enterobacter sp*, L4-*Pseudomonas sp*, L5-*Staphylococcus aureus*, L6-*Bacillus subtilis*, L7-*Staphylococcus epidermidis.*

Table3: Microbial counts in processing materials from locations i and iv**.** (10^5cfu/g)

Samples		Aerobic mesophiles	Fungi	Coliforms	B. cereus	Salmonella
Raw seeds	i	4.47^{d}	3.69^{c}	N.D	3.30^{a}	N.D
	iv	4.39^{c}	3.60^{b}	N.D	3.30^{a}	N.D
Water	i	5.60^{h}	3.60^{b}	4.08^{a}	N.D	4.97^{c}
	iv	5.12^{g}	3.47^{a}	4.59^{d}	N.D	4.60^{a}
Calabash	i	4.30^{b}	4.30^{d}	4.08^{a}	N.D	N.D
	iv	4.00^{a}	4.73^{c}	4.15^{b}	N.D	N.D
Leaves	i	5.05^{c}	4.90^{g}	4.28^{c}	4.85^{c}	4.78^{b}
	iv	5.08^{f}	4.79^{f}	4.83^{c}	4.78^{b}	5.19^{d}

Values are mean values of replicate samples.N.D-Not detected.

Table 4: Micro-organisms isolated at critical control points identified during fermentation process

ISOLATES	STAGES	I (WASHING)		II (WASHING)		III (SEEDS)		IV (PACKAGING)	
	LOCATION	i	iv	i	iv	i	iv	i	iv
B.subtilis		+	+	+	+	+	+	+	+
S.aureus		+	+	+	+	+	+	+	+
E.coli		-	-	-	-	-	-	+	+
Enterobacter		-	-	-	-	+	+	-	-
Klebsiella sp.		-	-	-	-	+	+	+	+
S.epidermidis		+	+	+	-	-	-	+	+
Pseudomonas sp.		-	-	-	-	-	-	+	+

+ = Present, - =absent

The morphology and biochemical characteristics of micro-organisms from *Oso* collected during some processing stages were shown in Table 2. The organisms isolated were identified as *E.coli*, *Klebsiella sp*, *Enterobacter sp*, *Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermidis*.

E.coli is a common faecal indicator which indicates direct or indirect contamination when present in food.This suggests a general lack of cleanliness in handling and improper storage (Edema and Fawole, 2006).

Washing was another process step through which vegetative pathogens could get into the seeds through the water used for washing. Also, heavy metals from the water could get into the seeds. Good manufacturing practice is the control measure highlighted. Another process step is fermentation of seeds in calabash lined with plantain leaves where vegetative pathogens from the leaves and stones could get into the *Oso* seeds. Control measures suggested in this process step include good hygienic practices (GHP) by handlers and good manufacturing practice (GMP). The last process step involves packaging with leaves or nylon where the sources of contaminant could be the packaging materials. Good hygienic practice and good manufacturing practice are the suggested control measures.

The critical control points during the fermentation of *Oso* identified were boiling, fermentation and packaging. They were the three main points where control measure can be introduced to improve the production processes. During boiling, pathogenic organisms were observed as the hazard while the use of clean water and adequate boiling temperature were observed as control measures. During fermentation, the use of clean water and clean utensils were also observed as control measures while the growth or survival of pathogenic organisms was the hazard identified. At the point of packaging, the survival of pathogenic organisms were identified as the hazards while the use of clean water and clean packaging materials were the critical limits. Inspection of packaging material and replacement of leaves with nylon to minimize contamination were the monitoring procedures suggested.

Seeds *

↓

Washing*

↓

Boiling (5 hours) **CCP

↓

Washing*

↓

Transfer of seeds into calabash lined and covered with banana leaves then, fermentation**CCP

↓

Packaging in leaves/nylons **CCP

Figure 1: HACCP control chart during the processing of *oso*

*stages where microbial analysis were performed;
**CCP---Critical control points

Table 5: **C**ritical control points during fermentation and monitoring procedures

Process steps	Hazards	Control measures	Critical limits	Monitoring procedure
Boiling	survival of pathogenic organism	use of clean water, adequate boiling temperature	clean water	inspection of water
Fermentation	survival of pathogenic organism	use of clean utensils, use of clean water	clean utensils	Inspection of water, educate processors

The presence of *Salmonella* from some of the locations rendered the *Oso* unacceptable. According to the Public Health Laboratory Service (PHLS, 2000) guidelines, ready-to-eat food should be free from *Salmonella* and it is termed unacceptable if detected at all and unfit for consumption. The already prepared *Oso* in this study were not reheated but were left for many hours before consumption and transported from one place to another. Handling of the condiments after preparation is a critical control point. Ingestion of foods held within 20 – 30^{o}C can be an effective medium for food borne illness (Adegoke *et al.*, 2008). Therefore, it is recommended that reheating of these ready-to-eat condiments/ foods be put in practice in order to kill vegetative cells that might emerge from spores or newly-acquired bacteria contaminants (Bryan *et al*, 1992). The processing of the *Oso* in this study was done by illiterate women who used bare unwashed hands with no knowledge of good sanitary practices. The water that was used for processing was fetched from nearby streams and rivers while the surroundings were considered unhygienic with garbage littering the environment. The source of water is quite worrisome, since most of the enteric organisms were likely to come from water source. The presence of coliform group *E.coli, Klebsiella sp* and *Enterobacter sp* is a worrisome trend, since the indicator of sanitary quality in food and water are coliform and *Enterobacter* (Jay, 1993). The presence of these organisms indicates faecal contaminants and since food-borne diseases are intestinal diseases, the existence of pollution is taken to indicate the possibility that the aetiological agents of these diseases may be present. The presence of these organisms at various stages is in line with the reports that indicator organisms were observed in fermented condiments and that the organisms are able to withstand alkaline conditions in fermentation (Ogunshe and Olasugba, 2008). The presence of *Staphylococcus aureus* in the condiments could be attributed to contact and poor hygiene since it is a normal flora of the skin and nasal passage (Edema and Fawole, 2006). However, implementing HACCP can reduce food borne illnesses by preventing the introduction of pathogen to food during and after processing, eating food as soon as practicable and introduction of health education and surveillance system (Adegoke *et al*, 2008).

CONCLUSION

Boiling, fermentation and packaging were identified as critical control points during the production of *Oso*. Improvement in personal hygiene should be ensured in all the processing sites by education and enlightenments of the local processors.

RECOMMENDATIONS

Process steps should be monitored in all local fermentation process and control measure like the hazards and critical control points (HACCP) should be encouraged. Good hygiene and good manufacturing pactices should be encouraged.

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