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Post Fermentation Quality Changes in Bobozi Produced from Cassava (*Manihot Esculenta* Crantz) and the Effects of Sodium Metabisulphite Soaking in Combination with Refrigeration

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Abstract: Changes in the microbiological, physico-chemical and organoleptic quality of bobozi (African snack) produced from cassava (*Manihot esculenta* Crantz) during processing and the effects of sodium metabisulphite or in combination with refrigeration at 10^{o}C were investigated results shows that the bioload increased from 0.3 x 10^{1}cfu/g to 1.04 x 10^{5} at the 96th hours of fermentation and thereafter decreased gradually. Post fermentation soaking in 5% sodium metabisulphite decongest and reduced the bioload to 0.9 x 10^{1} cfu/g. Extended storage of 48hrs fermented samples indicates slightly high count which peaked at 1.11 x 10^{4} at 21st day of storage for samples stored at ambient temperature (30±2^{o}C) whereas the bioload of refrigerated (10^{o}C) samples were stable all through the 28 days of storage. Six bacteria genera (*Bacillus, Streptococcus, Staphylococcus, Leuconostoc, Lactobacillus*, and *Corynebacterium, E Coli, Klebsiella* and *Salmonella*) and few fungi genera(*Candida, Geotrichum, Aspergillus* and *Penicillum* dominated the preboiling and fermentation phase while (3) three bacteria genera (*Bacillus, Streptococcus* and *Lactobacillus*) and three (3) fungi group (*Geotrichum, Aspergillus*, and *Penicillum*) dominated the post fermentation and extended storage phase. However, *E coli, Klebsialla and Salmonella)* were eliminated after boiling and were not detected after 24hour of fermentation. The P^{H} decreased from 4.58±0.01 to 3.75±0.02 at the end of the fermentation period. Although slight increase as to 4.31±0.02 was recorded after soaking in Sodium metabisulphite. Further decreased to 3.61±0.01 was recorded in samples held at 30±2^{o}C whereas it was fairly stable in refrigerated samples all through the storage period. However, reverse pattern of changes was observed and recorded in the titratable acidity. Steady increase from 31.20±0.5 to 46.6±0.4 was recorded for the moisture content at the end of the fermentation period. Slight increase was recorded in the post fermented sample held at 30±2^{o}C. Nevertheless, sample stored at 10^{o}C were fairly stable. The hydrocyanic acid decreased through out the processing and storage period. Overall sensory acceptability scores shows that refrigerated samples were highly acceptable even though freshly prepared samples were preferred.

Key words: Post fermentation, Bobozi, Cassava, sodium metabisulphite

Introduction

Bobozi (tapioca snack), a common urban and rural fast food in the rain forest belt of West Africa is produced by the fermentation of sliced and parboiled cassava root tubers (*Manihot esculenta* Crantz). It ranked third in popularity amongst the various types of food items derived from cassava. It is gaining wide acceptability, being consuming by several millions of people from different ethnic sub-groups and socio-economic classes. Furthermore, the ease of consumption in homes, in transit, offices and as post meal relaxation snacks with coconut, palm kernel nut, and other convenient additives makes it a local snack of choice which often attracts curious consumers.

Production technology vary with locality, customs and belief, with the result that available market bobozi differs in quality, safety and shelf stability. Post fermentation shelf stability vary between 24-72 hours and the production process is expensive, cumbersome and laborious. Hence methods of extending the shelf life without altering the product quality is desirable. In addition despite the popularity of this relish snack scientific information on the processing, microbiological, physico-chemical and sensory quality is hardly available. To meet the increasing demand of the teaming population of the West African sub region and especially Nigeria and the wider acceptability being created by curious consumers, this study was designed to investigate the changes associated with the microbiological, physico-chemical, and sensory quality during processing and fermentation and the combined effects of refrigeration hygienic handling and sodium metabisnlphile soaking on the post processed and fermented bobozi.

Materials and Methods

Source of cassava and processing: The cassava root tubers (*Manihot esculenta* Crantz) was obtained from

the open market (Santana market), Benin City, Nigeria. The cassava tubers were processed into bobozi according to the traditional method with some modifications. Briefly, they were peeled, washed and sliced into various sizes of 1-2cm x 10-12cm, washed and rinsed severally with clean tap water, placed in a clean 1 litre beaker and boiled over a bunsen flame for 10 minutes and thereafter allowed to cool to ambient temperature 30.0±2^{o}C). Five batches were produced and allowed to ferment at 24, 48, 72, 96, and 120 hours at ambient (30.0±2^{o}C) laboratory temperatures. At the end of the fermentation, the selected sample was rinsed severally with sterile clean water (previously sterilized) and thereafter soaked in 5% of sodium metabisulphite for 25-30, minutes following this, it was rinsed again several times with clean sterile water and thereafter packaged into high density polythene (HDPE) bags (approx 500g/pack) and sealed with the aid of a hand sealing machine (super master, Japan) adopting standard safety and hygienic precautions. The packaged samples were divided into two batches. A batch was kept in the refrigerator at temperature of 10^{o}C while the 2nd batch was left on the laboratory bench at 30.0±2^{o}C (Fig. 1).

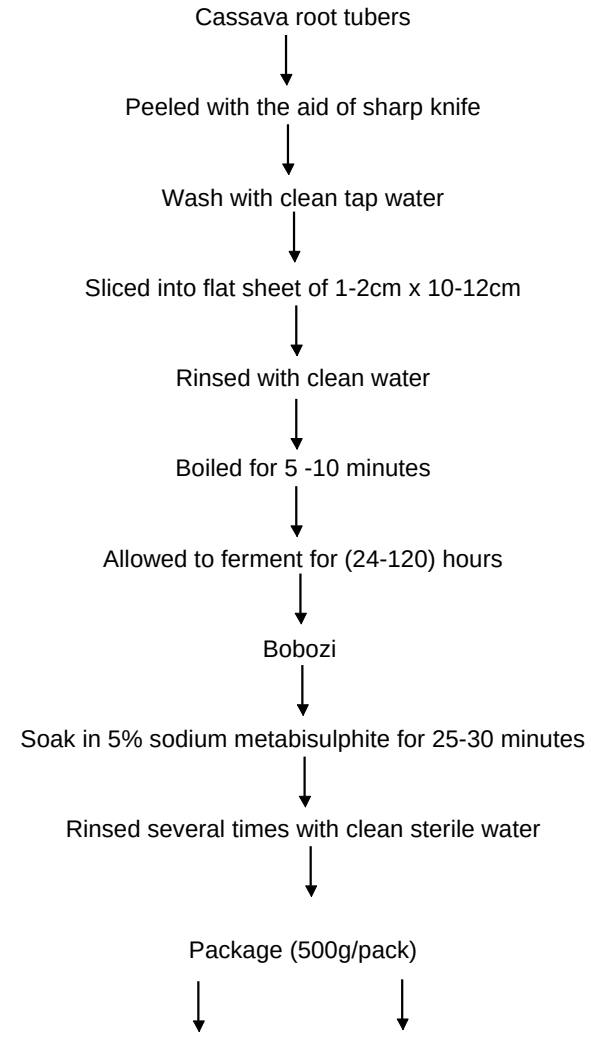

Fig. 1: Traditional Method of Processing bobozi with some modification

Analysis

Microbiological: The various groups and types of microorganisms associated with bobozi were analyzed, enumerated and quantified according to the methods described by Harrigan and McCance (1976). Weighed 25g of bobozi was aseptically removed from each samples and homogenized in 225ml of 0.1% (w/v) sterile peptone water for 3 minutes in a Colworth stomacher (A.J Seward, & Co. London). Thereafter ten fold serial dilution were prepared by transferring 1ml of the homogenate into 0.1% (w/v) sterile peptone water as diluent. Further serial dilutions were carried out. Following this, 1ml of appropriate dilutions were aseptically plated using the pour plate technique for total viable aerobic bacteria count on nutrient agar(Oxoid) and total viable fungi count on potato dextrose agar(LABM), supplemented with chloramphenicol. The various media used were prepared and incubated according to the manufacturers instructions. At the end of the incubation periods, the colonies were enumerated and expressed as colony forming unit per gram (cfu/g) according to Vanderzannt and Splittstoesser (1992). Isolation, characterization and identification of the associated microorganisms were carried out for qualitative determination using colonial, morphological and biochemical characteristics (Harrigan and McCance, 1976). The fungal isolates were identified based on examination of the colonial heads, phialides, conidiophores and presence or absence of foot cells or rhizoids (Samson and Reenen- Hoekstra, 1988).

Physico-chemical

P^{H}: The P^{H} was determined by homogenizing 10 grams of the various samples in 20ml of distilled water and using a refenced glass electrode P^{H} meter (JENWAY, 3020, England).

Titatable acidity (TA%): This was determined by titrating 0.1N sodium hydroxide against 10ml of supernatant of homogenized sample, using phenolphthalein indicator as previous described by AOAC(1980).

Hydrocyanic acid (mg/kg): This was determined by the alkaline titration method previous described by AOAC (1980).

Sensory evaluation: Attributes such as appearance, taste aroma, texture and mouth feel which determine the

organoleptic quality of bobozi were evaluated. Overall acceptability score of freshly prepared samples and stored samples after 20 days was carried out. Using a nine point hedonic scale (1=9…9=1), Watts *et al.* (1989), a ten member panel was used to assess the various quality attributes for overall acceptability.

Statistical analysis: The various data obtained were subjected to statistical analysis – mean, standard deviation and analysis of variance (ANOVA). The significant value was determine by the t-distribution test using appropriate computer software.

Results

Finding of the effects of sodium metabisulphite soaking in combination with refrigeration on the post fermentation quality changes in bobozi produce from cassava (*Manihot esculenta* Crantz) are shown in Tables 1-5 respectively. Boiling for 5-10 minutes decongested and reduced the bioload from 3.7×10^1 cfu/g to 0.3×10^1 cfu/g, which subsequently increased during fermentation up to 1.04×10^5 cfu/g at the 96th hours of fermentation and thereafter decreased gradually. No fungi growth was detected after boiling but gradual and slow growth which peaked at 4.8×10^3 at the 120 hours was observed. However, soaking in 5% sodium metabisulphite(SM) for 25-30 minutes drastically reduced the bioload of samples fermented for 48 hours (preferred sample) from 1.03×10^3 cfu/g to 0.9×10^1 cfu/g. Extended storage at ambient laboratory temperature (30±2^{o}C) witnessed steady increase up till the 21st day (1.11×10^5) and thereafter decreased. However, the microbial count was fairly stable in samples stored in the refrigerator at 10^{o}C. Nine (9) bacteria genera (*Bacillus, Staphylococcus, Streptococcus, Leuconostoc, Lactobacillus, Corynebacterium, E. Coli, klebsiella* and *Salmonella*) and four fungi genera (*Condida, Geotrichum, Aspergillus* and *Penicillium*) were detected and isolated during processing and fermentation. However, *Salmonella, Klebsiella* and *E. Coli* were eliminated following boiling. Only t hera (3) bacteria genera (*Bacillus, Streptococcus* and *Lactobacillus* and (2) Fungi genera (*Geotrichum* and *Aspergillus*) were detected and isolated in the post fermentation and extended storage phase (Table 1). The P^H decreased from 5.08±0.05 to 4.58±0.01 after boiling and thereafter decreased gradually till the end of the fermentation period (3.75±0.02). Conversely, the titrable acidity (TA) increased all though the period of fermentation. However, slight increase from 4.01±0.05 to 4.31±0.2 was recorded in the P^H after treatment with SM. Nevertheless, gradual decrease was recorded in samples stored at ambient temperature till the end of the storage period (28 days), whereas the P^H was fairly stable in the refrigerated samples. Although, slight increase was detected in the titrable acidity in ambient stored samples, no noticeable changes were observed in the refrigerated samples.

The moisture content increased through out the fermentation period from 36.24±0.5 to 46.61±0.2 (Table 1). Similarly, slight increase was noticed during extended storage period from 40.30±0.5 to 42.80±0.2 in samples stored at 30±2^{o}C while refrigerated samples were fairly stable. The hydrocyanic acid (HCN) decreased all through the fermentation and post fermentation Ex tended storage period. The various sensory attributes evaluated at the end of the fermentation were significant at different level ($p <= 0.001, 0.01, 0.05$). However preference was in the order 48hrs> 72hrs>24hrs>96hrs> 120hrs samples. Extended storage of 48hrs fermented samples showed that refrigerated samples was highly acceptable even though freshly prepared samples were preferred. Comparative preference evaluation indicates acceptability to be in the order fresh samples> refrigerated sample> ambient stored samples.

Discussion

The initial sharp decrease recorded in the bioload may be related to injuries, sublethal, lethal, distorted homeostasis and other negative effects potentiated by the boiling process on the associated microorganisms. However, the subsequent steady increase till the 96th hour of fermentation suggest favourable microenviromental condition, recovery of injured cells and return of balanced homeostasis. Whereas the final decrease observed thereafter could be traced to negative effects of accumulated by products of metabolism leading to unfavorable environmental conditions and possible nutrient depletion. Furthermore the drastic reduction recorded in day (0) in the post fermentation extended storage phase may be attributed to antimicrobial effect of (SM) during soaking and the decongestion effect of the sanitization process and hygienic handling. However, the gradual but slow increase up to the 21st day of storage in samples held at 30±2^{o}C (ambient temperature) may be associated with favourable environmental conditions. Nevertheless, the low and stable count recorded in the refrigerated samples demonstrates the benefits of low temperature storage for food quality and the ability to retard microbial growth. These findings corroborate previous reports for other food items (Leistner, 1994; Gould, 1988; Ogiehor *et al*., 1988; Ogiehor *et al.*, 1999; Ogiehor *et al.,* 2003). The increase recorded in the moisture content all through the fermentation period may be traced partly to the absorption properties of the substrates (bobozi) itself and partly to the activities of the associated microorganisms which tends to soften and weakened the tissues, thereby permitting water entrance. Furthermore the slight increase observed in the post fermented sample stored at 30±2^{o}C may be related to the low microbial activities and permeability properties of the packaging material used. While the fairly stable

Table 1: Microbiology and Physico-chemical quality changes in Bobozi during fermentation at ambient temperature (30±2°C)

	Fermentation period						
Parameters	Row sample	0	24	48	72	96	120
TVC (bacteria)cfu/g	$3.7x10^1$	$0.3x10^1$	$4.6x10^1$	$1.02x10^3$	$1.12x10^4$	$1.04x10^5$	$9.6x10^4$
TVC (fungi)cfu/g		NG	$0.2x10^1$	$0.9x10^1$	$2.5x10^2$	$3.41x10^3$	$4.8x10^3$
MC (%)	31.20±0.5	36.24±0.2	38.40±0.1	40.30±0.2	43.41±0.3	45.10±0.5	46.61±0.2
P^H	5.08±0.05	4.58±0.02	4.21±0.02	4.01±0.05	3.98±0.01	3.86±0.03	3.75±0.02
TA (%)	0.01±0.001	0.01±0.01	0.01±0.01	0.02±0.01	0.02±0.01	0.03±0.01	0.03±0.001
HCN (mg/kg)	31.60±0.5	16.45±0.05	12.10±0.6	8.40± 0.04	6.25±0.6	5.10±0.3	4.56±0.5

TVC = Total Viable Count; MC = Moisture content; TA = Titratable acidity, hence hydrocyanic acid.

Table 2: Post fermentation quality changes in Bobozi during storage at ambient laboratory temperature (30±2°C)

	Period of storage (days)							
Parameters	0	2	4	7	10	14	21	28
TVC (cfu/g)	$0.9x10^1$	$3.6x10^1$	$5.4x10^2$	$8.3x10^2$	$9.6x10^3$	$1.10x10^3$	$1.11x10^4$	$9.8x10^4$
MC	4.30±0.5	40.35±0.3	40.50±0.2	40.75±0.1	40.95±0.4	41.20±0.2	42.10±0.3	42.80±0.2
pH	4.31±0.02	4.18±0.02	4.06±0.01	3.92±0.02	3.81±0.01	3.76±0.02	3.70±0.005	3.61±0.004
TA	0.02±0.001	0.002±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.03±0.01	0.03±0.001	0.3±0001
HCN	8.40±0.5	8.15±0.3	7.65±0.5	7.02± 0.4	6.20±0.6	5.91±0.5	5.46±0.3	4.01±0.6

TVC = Total Viable Count; MC = Moisture content; TA = Titratable acidity; hydrocyanic acid

Table 3: Post fermentation quality changes in Bobozi during storage at refrigeration temperature (10°C)

	Period of storage (days)							
Parameters	0	2	4	7	10	14	21	28
TVC (cfu/g)	$0.9x10^1$	$1.2x10^1$	$1.5x10^1$	$2.1x10^1$	$2.5x10^1$	$3.6x10^1$	$4.21x10^1$	$6.6x10^1$
MC (%)	4.30±0.5	4.32±0.2	4.32±0.2	40.36±0.5	40.41±0.6	40.44±0.6	40.49±0.3	40.50±0.5
P^H	4.31±0.02	4.20±0.02	4.10±0.02	4.05±0.02	4.00±0.02	3.98±0.03	3.95±0.02	3.92±0.021
TA (%)	0.02±0.001	0.02±0.01	0.02±0.001	0.02±0.001	0.02±0.001	0.02±0.01	0.02±0.001	0.2±0.02
HCN (mg/kg)	8.40±0.5±0.5	8.25±0.5	8.20±0.2	8.10± 0.3	8.02±0.4±0.2	7.96±0.3	7.92±0.4	7.84±0.5

TVC = Total Viable Count; MC = Moisture content; TA = Titratable acidity; hydrocyanic acid

Table 4: Sensory quality of Bobozi after fermentation

Period of fermentation	Attributes					
(hrs)	Taste	Aroma	Texture	Mouthfeel	Appearance	Overall Score
24	4.36±0.3	5.04±0.5	4.90±0.2	4.06±0.4	7.41±0.2	5.15±0.3 +
48	6.47±0.3	6.65±0.5	5.64±0.6	5.34±0.4	6.24±0.2	6.07±0.4 +++
72	6.25±0.5	6.24±0.6	5.03±0.2	5.65±0.5	6.05±0.5	5.84±0.5 ++
96	4.24±0.2	5.61±0.3	4.40±0.6	4.66±0.4	5.41±0.3	4.86±0.4 F
120	3.96±0.7	4.38±0.2	4.20±0.4	4.40±0.2	5.05±0.5	4.40±0.4 NA

+++ = Preferred sample, ++ = Highly acceptable, + = Acceptable, F = Fairly acceptable, NA = Not acceptable

Table 5: Overall Acceptability Score

	Attributes					
Type of product	Taste	Aroma	Texture	Mouthfeel	Appearance	Overall score
Freshly prepared Bobozi (48hrs Fermented)	$7.63±0.3^a$	$6.76±0.4^a$	$7.04±0.2^a$	$6.15±0.5^a$	$7.12±0.3^a$	6.94±0.34 ***
Post fermented Extended storage at Ambient temperature(30±2°C)	$5.01±0.5^b$	$5.10±0.2^b$	$5.16±0.4^b$	$4.52±0.3^c$	$5.20±0.3^b$	5.00±0.34 *
Post fermented Extended storage at 10°c (28days)	$6.15±0.5^a$	$5.71±0.3^b$	$5.34±0.4^b$	$5.02±0.2^b$	$5.75±0.5^b$	5.60±0.38 **

a = Significant (p<0.01), b = Significant (p<0.05), c = Not Significant, *** = Preferred sample , ** = Highly acceptable, * = Acceptable

and insignificant increase recorded in the refrigerated samples may be due to the seemingly lack of microbial activities recorded. The decrease recorded in the P^H throughout the fermentation period may be associated with the production of some organic acids by the associated microorganism during fermentation.

However, the slight increase recorded in the early storage phase of the post fermented sample could be related to the presence of SM used in soaking and sanitization. The subsequent decrease in the sample held at 30±2°C may be due to the microbial activities, whereas, the fairly stable values observed in the refrigerated same could be traced to the lack of microbial proliferation potentiated by the low temperature. Conversely, the high titrable acidity recorded during fermentation and stability observed in the post fermented samples may be similarly explained. These reports support previous findings during processing of related indigenous foods (Ogiehor *et al.,* 1999; Ogiehor *et al.,* 2003). The continuous decrease observed and recorded in the hydrocyanic acid content may be linked with the combined effects of boiling, fermentation, microbial activities and post process storage conditions. Similar reports have been documented in the processing of other cassava based food items (Okafor and Ejiotar, 1990; Tyllerskar *et al.*, 1992). The various quality attributes of colour, aroma, texture, taste and mouthfeel evaluated were significant at various level ($p<0.001$, 0.01, 0.05). Amongst the various samples at the end of the fermentation period. The order of preference was 48hrs >72 hrs > 24 hrs > 96 hrs>120hrs. Extend storage of 48hrs fermented samples after soaking and sanitization with SM at ambient and refrigeration temperature similarly indicates that the various attributes were significant at different levels ($p<0.01$, 0.05) amongst the samples. Overall acceptability scores shows that samples stored at 10°C were highly acceptable even though freshly prepared sample were preferred. The order of preference was fresh samples > refrigerated sample > ambient (30±2°C) temperature stored samples.

This investigation has shown that noticeable microbiological, physico-chemical and organoleptic quality changes occur in bobozi during processing, fermentation and storage. Furthermore combinahoy of soaking in SM and refrigeration enhanced microbial safety and shelf stability for 28 days compared to the usual shelf life of 24-72 hours. Data obtained can be harnessed and utilized for safe processing, handling and storage of bobozi.

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