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PRODUCTION OF UGBA CUBES FROM FERMENTED Pentaclethramacrophylla SEEDS

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ABSTRACT

This research was designed as an innovation in the use of ugba as a food condiment. Production of "ugba" cubes from fermented seeds of Pentaclethramacrophylla ("ugba") was successfully carried out. Ugba seeds were dehulled after soaking overnight in warm water and shredded. Fermentation of the shredded "ugba" seeds was done using Bacillus subtilis pure culturedisolated from already fermented "ugba". Initial microbial study of the unfermented "ugba" showed the presence of Bacillus, Enterobacter, Penicillium, Aspergillus and yeast species. The initial bacterial count of the unfermented seed was 1.4 x 10^5cfu/g. Microbial counts of the "ugba" seeds after three days fermentation showed a disappearance of other microbial species except Bacillus species which was up to 9.60 x 10^4cfu/g. The shredded and fermented ugbaseeds were later milled and formed into cubes in a mould and oven dried.Sodium benzoate was used as a preservative. There was a drastic reduction of microbial count from 9.60 x 10^4cfu/g to 4.80 x 10^4cfu/g in "ugba" cubes preserved with sodium benzoate. Proximate composition of the "ugba" cubes showed increased levels of carbohydrates, ash and fibre content and a reduction in moisture protein and fats. Toxicological analysis of Bacillus fermented ugba presented low levels of hydrocyanic acid and soluble oxalates when compared with the unfermented sample. An overall acceptability of the ugba cubes was rated very highly when sensory evaluation was tested. This study recommends the use of Bacillus subtilis fermented nutritionally rich ugba cubes as a new innovation that will promote its acceptability worldwide.

KEYWORDS: Ugba, fermentation, Bacillus subtilis, sodium benzoate

INTRODUCTION

African oil bean, botanically known as Pentaclethra macrophylla Bentham is a tropical tree crop found mostly in the southern and middle belt regions of Nigeria and in other coastal parts of West and Central Africa (Key, 1989). It belongs to the family Leguminosae and its tree was recognized by Okafor (1982) as a food tree. The seeds comprise a grey coloured cotyledon, embedded in a glossy brownish, seed coat. Seeds are contained in a brownish, flattened pod which explodes at maturity and disperses the seeds (Enujiugha, 2003). A pod contains an average of 6-10 seeds; depending on the length of the pod. The fermented seeds of Pentaclethramarcrophylla are called "ugba" or "ukpaka" in Igbo language of Eastern Nigeria. It is widely consumed in this region as reported by Odunfa andOyeyiola (1985). It is known as "ukana" by the Efik and Ibibio people of Southern Nigeria and "ukpaka" in Benue state of North Central Nigeria. According to Tico (2005), ugba is a low acid food which could be prepared into flour and cubes and explored in food fortification and confectionaries.

The unfermented seeds are known to harbor a variety of microbial species such as Aspergillus, Staphylococcus, Micrococcus, PenicilliumandBacillus. However, it is belived that only bacteria species are involved in the fermentation of the seeds (Obeta, 1983). Other species disappear in the course of fermentation. This was confirmed byOlasupoet al., (2016), who noted that no fungi or yeast has been implicated in the fermentation of ugba. Oil bean seeds require careful processing and fermentation before they can be eaten. Odunfa and Oyeyiola (1985) and Enujiugha (2000) showed that seeds must be boiled in water before removal of the seedcoat. Sokari and Wachukwu (1997) removed seed coats byroasting seeds on hot sand at 100degrees centigrade for 30 minutes. Ugba is known to be rich in proteins, carbohydrates and lipids. Minerals and vitamins are reported to be in small quantity in unfermented ugba. Kabuo et al., (2007) reported that ugba contains some flavourand aroma components like ethyl oleate and ethylphenol. This makes ugba a good flavouring and seasoning agent in soups and local dishes. The high moisture content of ugba as reported by Odunfa and Oyeyiola (1985) predisposes it to rapid spoilage. This is a great concern to many researchers although not much information is available on how to effectively preserve the delicacy. Therefore, this research is an innovation aimed at devising means of producing nutritionally and microbiologically safeugba cubes with a longer shelflife. The success of this

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research will bring about increased availability of the product – ugba cubes,with its rich nutritional and organoleptic properties.

MATERIALS AND METHODS

Materials: - The materials used in this research include among others: oven, anaerobic jar, pressure pot, electric blender, kitchen knife, culture media, reagents and apparatus for biochemical and toxicological analysis of samples.

Sample Collection: - Dry seeds of Pentaclethramarcrophylla (ugba) were purchased from Nkwolagwa market in Umunukwu, Orwuato village of Mbaise Local Government Area of Imo State, Nigeria.

Sample Preparation: - The seeds were soaked in warm water overnight and dehulled.Cotyledons were sliced and boiled for one hour and washed in water. Portions were later homogenized in an electric blender and subjected to microbiological, toxicological and proximate analysis. Sliced cotyledonwere further subjected to fermentation.

Microbiological analysis of unfermented ugba.

A one-gram sample of ground ugba seed was analyzed for bacterial and fungi colonies using appropriate growth media (Nutrient agar-and Saboroud Dextrose Agar). This was done by spread plating the fourth step of a 10-fold serially diluted sample. Plates were incubated at 37degrees centigrade for Nutrient Agar plates and 72hours for SaboroudDextrose Agar plates on the shelf at room temperature.

Identification and Characterization of Microbial Isolates

Fungal isolates were subjected to fungal staining. All bacterial isolates were subjected to gram staining, spore staining, indole test, oxidation-fermentation test, growth in 6.5% sodium chloride, motility test, catalase test, oxidase test and other useful identification and biochemical characterization tests were carried out.

Fermentation of “ugba” and production of “ugba” cubes.

The slicedugbaseeds were properly mixed with salt (NaCl), inoculated with pure cultures ofBacillus subtilisand wrapped in sterile foil to ferment. Fermentation was for 3 days maintaining a microaerophilic environment.The fermented, slicedugba cotyledon were milled and mixed with a gelling agent (maize flour) and divided into two portions (samples 1 and 2). Sample 1 was further treated with 0.5% sodium benzoate but sample 2 had no preservative. Both samples were poured into moulds to form cubes. The ugba cubes were wrapped in foils and stored on the shelf for 4 weeks before further microbiological examination.

Microbial, Proximate and Toxicological composition of ugba cubes

After four (4) weeks of shelf storage, the ugba cubes (samples 1 and 2) were analyzed for microbial (bacterial and fungal) growth and proximate composition. For the proximate composition, parameters like moisture, crude fibre, ash, crude protein, fat and carbohydrate contents were analyzed using gravimetric method (AOAC, 1990),Weadne method (James, 1995), Kjeldal method. (Chang, 2003) and gravimetric method (Kiric and Sawyer, 1998) respectively. Methods described by Wood (2007) were used for analyzing toxicants in both fermented and unfermented ugba.

Sensory Evaluation

The method of Carmen (2013) was used to evaluate the acceptability and sensory quality of fermented ugba cubes and fermented dried ugba slices. A 6-membertrained panelist was used in evaluating the sensory attributes of the product. The panelists are people who taste the food and judge it and could be made up of one person or several hundreds depending on the type of sensory method as reported by Balàzs (2012). The panelists chosen for this evaluation were a group of six adults who have been eating shredded ugba for a period of 15 years. They are familiar with the taste, aroma, colour, and texture of ugba. The evaluation was carried out independently and without bias. The organoleptic qualities of colour, taste, texture, aroma and overall acceptability was carried out using a 9-point hedonic scale. One (1) for extremely disliked and nine (9) for liked extremely. Samples were used in the preparations of a local salad called “abacha” and coded for the panelists to rate. Table 4 reflects the sensory evaluation of ugba samples.

Statistical Analysis

Mean scores of results from the hedonic rating were subjected to analysis of variance (ANOVA). The significance of the mean differences of the samples was determined by Fischer’s Least Significant Difference (LSD) as outlined by Ihekenonye and Ngoddy (1985). The LSD was defined at $P<0.05$

RESULTS AND DISCUSSIONS

Microbiological Analysis.

Table 1 shows the microbial counts of unfermented ugba, fermented ugba cubes with 0.5% sodium benzoate and fermentedugba without preservatives.

Table 1 Total Microbial Counts in Ugba samples.

Samples	Total microbial counts (cfu/g)	Bacterial counts (cfu/g)	Fungal counts (cfu/g)
Unfermented ugba	$4.2x10^5$	1.45×10^5	1.02×10^5
Fermented ugba cubes (with preservative)	4.80×10^4	4.80×10^4	Nil
Fermented ugba cubes (without preservative)	9.60×10^4	9.60×10^4	Nil

Table 2 Microbial Counts after 4 weeks storage

Samples	Shelf storage (27-30degrees centigrade)			Storage at 4degrees centigrade		
	TMC(cfu/g)	BC(sfu/g)	FC(cfu/g)	TMC (cfu/g)	BC(cfu/g	FC(cfu/g)
Fermented ugba cubes (with preservatives)	2.4×10^4	1.7×10^4	1.2×10^4	2.3×10^4	2.1×10^4	Nil
Fermented ugba cubes (without preservatives	6.3×10^4	5×10^4	8.2×10^4	4.4×10^4	3.8×10^4	1.4×10^4

Key:
TMC = Total Microbial counts,BC = Bacterial Counts, FC = Fungal Counts

Table 2 shows the outcome of the microbiological analysis of ugba cubes stored on the shelf and in the refrigerator. The sample which had no chemical preservative had a total microbial count of 6.3×10^4cfu/g on the shelf. The sample which contained 0.5% sodium benzoate had a total microbial count of 2.4×10^4cfu/g.

Table 3 Proximate composition of fermented and unfermented ugba

Parameters	Fermented ugba(percent weight)	Unfermented ugba (percent weight)
Crude protein	10.4	21.24
Carbohydrate	24.51	18.10
Crude Fat	26.06	52.67
Crude Fibre	18.67	3.12
Ash Content	16.00	2.51
Moisture Content	4.37	2.36

Table 4: Sensory Evaluation of ugba samples

Ugba Samples	Aroma	Taste	Colour	Texture	Overall Acceptability
Unfermented Slices	4	4	5	4	4
Fermented Slices	5	6	6	4	7
Fermented Cubes	7	8	7	6	8

Key: 1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither like nor dislike, 6 –like slightly, 7 – like moderately, 8 – liked extremely.

Table 5: Toxicants in unfermented and fermented ugba (Mg/100g dry matter)

Sample	Hydrocyanic acid	Soluble oxalates	Total oxalates	Tannins	Phytates
Control	1.03±0.01	11.77±0.01	20.13±0.01	0.04±0.01	0.04±0.01
Fermented in leaf	1.23±0.01	6.92±0.01	16.18±0.02	4.44±0.01	0.35±0.01
Fermented in B. subtilis broth culture	1.31±0.01	8.91±0.01	20.24±0.01	0.91±0.01	0.51±0.01

The toxicological analysis shown on Table 5 reveals that there was a slight increase in total oxalate from 20.13±0.01 to 20.24±0.01. The phylate content also increased from 0.49±0.01 to 0.51±0.01. However, fermentation of ugba with Bacillus subtilis broth culture reduced the tannin content from 0.04±0.01 to 0.19±0.01. soluble oxalate in the same sample was reduced from 11.77±0.01 to 8.91±0.01. All results were measured as milligrams per 100g dry matter (Mg/100g dry matter)

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The production of “ugba” cubes is an innovation aimed at adding value to the existing conventional method of slice, ferment and consume. This old method of eating the ugba immediately after fermentation, makes the product unacceptable to people of other cultures. When ugba is preserved in cubes as bouillion, people from other cultures will be able to assess it and derive its high nutritional potentials. This way the product will be available at off-seasons.

In this study, fermentation of the cotyledon led to the softening of the cotyledon and a drastic disappearance of other microbial species except Bacillus species which was up to 9.60 x 10^4cfu/g. Addition of preservatives brought about drastic reduction of microbes during storage at different storage conditions. According to Fawole and Osho (2002), the use of preservatives on food may be microbiocidal and kill the target organism or they may be microbiostatic and simply prevent them from growing, thus improving the shelf life of the product. This could be the reason behind the drastic reduction in the total bacterial count of ugba cubes treated with 0.5% sodium benzoate.

The results of the proximate analysis showed an increase for carbohydrates,ash and fibre, but a decrease in protein and fat content. This decrease could be due to heat treatmentwhich led to protein denaturation and melting of fats. The proximate composition of the unfermented ugba is similar to the results of Enujiugha and Akanbi (2002) where the results for crude protein, carbohydrate, lipid, crude fibre and ash contents were 22.32% 19.16%, 53.98%, 2.13% and 2.40% respectively. There was also a decrease in moisture content from 4.37% after fermentation to 2.36% moisture after oven during. At this temperature microbes rarely survive. Toxicological composition of fermented and unfermented ugba shows a decrease in soluble oxalades. The tannins and phytate contents of fermented ugba samples, increases significantly. This could be due to browning reaction caused by some enzymatic activities in the product.

In conclusion, the results and discussion of this research suggests that seeds of the African Oil bean (Pentaclethra macrophylla) tree could be fermented, milledand formed into seasoning cubes. This will increase the availability and acceptability of ugba for use in confectionaries and fortification of foods. It could also be suggested that chemical preservatives together with drying could be used to improve the keeping quality of ugba cubes. However, drying at a lower temperature (about 50^0C) may yield a more nutritious product, with less damage on the protein content.As reported by Ohiri and Bassey (2017), fermentation of ugba improved its flavor and reduced the lipid content to a healthy level. The aroma of fermented ugba, as deduced by Nwokolo, and Ugwuanyi (2015) is due to the presence of methyl esters of various long chain fatty acids. This claim was upheld with sensory evaluation. It is therefore recommended that fermentation of ugba using Bacillus subtillisbroth culture should be adopted as it helps in the improvement of the nutritional microbiological and toxicological quality of the product.

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