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Selection of starter cultures for the production of ugba, a fermented soup condiment

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Abstract Ugba is a traditional fermented African oil bean condiment that serves as flavouring in soups or as low-cost protein snack in Nigeria. A total of 56 strains of spore-forming bacteria were isolated from 21 ugba samples purchased from retail markets in southwestern Nigeria. *Bacillus subtilis* (26 strains) was identified as the dominant microorganism of the fermented samples. The total viable count for the ugba samples obtained from the retail outlets was in the range of 1.4×10^7–2.8×10^9 cfu/g, while the spore-forming bacteria had a count of 1.8×10^{10} cfu/g. Strains of *B. subtilis* were selected as potential starter cultures based on their enzymatic activities and ability to produce stickiness. The nine selected strains had a range of scores of proteolytic activity (5.2–7.4 mm), amylolytic activity (4.4–5.6 mm) and stickiness (15.1–17.3 cm). Relative viscosity was in the range of 2.3–5.3 U/ml, while protease activity was from 30.1–51.4 U/ml. However, *B. subtilis* MM-4:B12 that had the highest scores was chosen as starter culture for the laboratory preparation of ugba, and a viable count of 1.4×10^{10}, pH of 8.1 and 46.5% moisture content were obtained at the end of 72 h fermentation period. The fermented product had the peculiar characteristics of the traditionally prepared sample which is usually fermented for about 5 days. There were no significant differences in the protein (17.8–18.1%), fat (40.9–41.2) and titratable acidity (0.11–0.13%) of both starter culture fermented ugba and those obtained from the retail markets. However, organoleptic evaluation scores showed the starter-inoculated samples being rated higher by the consumers with regard to consistency, aroma and taste.

Keywords Condiments · Fermentation · *Bacillus* species · African oil bean · Starter culture sensory evaluation

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Introduction

Ugba is a fermented product of African oil bean seeds (*Pentaclethra macrophyla)* and is produced and eaten mostly in the south-eastern parts of Nigeria. The condiment is taken as delicacy or added to soups and sauces as flavouring agents. The production of ugba like other fermented foods in sub-Saharan Africa is a traditional family art done in homes with rudimentary utensils [1]. Since the fermentation process is uncontrolled, the associated microorganisms are frequently unpredictable thereby leading to products of varied quality attributes. Occasionally, enabling factors in the microenvironment of the fermenting matrix may cause delay in fermentation and in some cases, fermentation may not be initiated [2].

Microbiological studies of Nigerian fermented vegetable proteins established *Bacillus* species as the main fermenting organisms, and ugba also belong to this closely related *Bacillus* food fermentations. Other microorganisms implicated in the various fermentations have been reported to play no significant role[3, 4, 5, 6] can thus be regarded as opportunists. Sarkar and Tamang [7] also confirmed the predominance of *Bacillus* in their studies on kinema, a fermented soybean of the people of Himalayan regions.

Despite the availability of reports on the microbiological and biochemical characteristics of most of the fermented vegetable proteins of sub-Saharan Africa, there is still a dearth of information on the optimal conditions for their scientific production. Odunfa and Adewuyi [8] reported the effect of temperature, time and humidity on the production of iru, while Sanni et al [2] provided information on the optimisation of process conditions for owoh, a fermented cotton seed condiment. However, several authors on fermented vegetable condiments in the sub-region have reported different strains of *Bacillus* species both in terms of isolation and performance capability. To this end, selection and adaptation of specific starters for peculiar characteristics of each condiment are desirable.

Therefore, the main objective of this study was to screen for strains of *Bacillus* species that can be employed as starter organisms for ugba production.

Materials and methods

Traditional preparation of ugba

Africa oil bean seeds are boiled in water for 4–5 h to soften the hard brown shell. The shells are broken to remove the kernels, which are soaked in water overnight over a low flame and smouldering wood. The seeds are allowed to cool, washed several times with water and soaked in water again for a period of 6 h. They are then cut into long narrow slices about 5 cm long. The sliced beans are mixed with salt, wrapped in small packets (about 10 cm diameter) using banana leaves, transferred into an earthenware pot and covered with a jute bag. Fermentation is allowed to progress for 5 days [1], and the product if adequately fermented, is sticky with pungent aroma.

Collection of ugba samples

A total number of 21 fresh ugba samples were randomly and aseptically collected from retail markets in south-western Nigeria over a period of 4 weeks, and were kept at 5 °C pending microbiological analysis.

Isolation procedures

Homogenised samples (1 g) were diluted ten-fold serially in 0.85% sterile physiological saline and heated for 5 min in a boiling water bath. Appropriate diluted suspension (0.1 ml) was mixed with molten (45 °C) Trypticase soy agar (sporeformers) and Plate Count Agar (total viable counts) [9], before pouring into duplicate petri dishes. Incubation was at 30 °C for 48 h. Representative colonies of the sporeforming bacteria were picked, purified by repeated streaking and subjected to various tests described by Smith *et al* [10], Gordon *et al* [11] and Claus and Berkeley [12]. The tests which included Gram's staining, catalase, Voges-Proskauer, methyl-red, growth at different pH levels, temperatures and salt concentrations, starch, gelatin and casein hydrolysis, sugar utilisation and nitrate reduction, were to phenotypically characterise the sporeforming *Bacillus* into species and strains.

Selection of candidate strains

Proteolytic and amylolytic activities of the cultures monitored on surface–dried milk and starch agar plates incubated at 30 °C for 3 days [9, 11] were used as preliminary screening parameters. At the end of incubation period, the plates were flooded with iodine solution and the diameter of the cleared zones were used as an assessment of the tests [2]. Stickiness, an important parameter for assessing adequately fermented vegetable condiments in sub-Saharan Africa and Asia was determined by growing the cultures on phytone agar at 30 °C for 24 h. A sterile inoculating needle was used to touch the culture growing on the plates, pulled and the stickiness measured by the length of the thread formed. The selected strains were then subjected to protease and viscometric analysis.

The modified method as adapted by Tamang and Nikkuni [9] was employed for determination of protease activity. Isolates were cultivated in phytone broth at 30 °C for 48 h in a shaker incubator at 180 rev/min, followed by centrifugation at 13 000×g for 10 min. The assay was carried out by the azocasein digestion method. The enzyme solution diluted appropriately and 1% azocasein prepared in 0.1 M phosphate buffer (pH 6.8) were pre-incubated separately at 37 °C for 20 min, and the reaction was terminated by adding 2.5 ml of 10% ($^{w}/_{v}$) trichloroacetic acid. This was followed by centrifugation at 13 000×g for 10 min. The supernatant (2 ml) was neutralized with 2 ml of 1 N NaOH and the absorbance measured at 450 nm with a spectrophotometer. Protease activity was expressed as azocasein unit (U/ml). One unit was defined as the quantity of enzyme required to increase the absorbance by 0.1 under the stated conditions.

For viscometric measurement, the strains were grown in nutrient broth at 30 °C for 48 h in a shaker incubator at 180 rev/min followed by centrifugation at 13 000×g for 10 min. The supernatant was decanted and viscosity measured directly (Brookfield Model DV–1+Viscometer).

Production of ugba using the starter organism

Inoculum

The selected *Bacillus* strain was grown on nutrient agar slants for 24 h, after which 5 ml of sterile distilled water was added and shaken to make a suspension which was used as inoculum. The number of organisms per ml of suspension used was estimated using the Petroff-Hauser bacterial counting chamber and phase contrast microscope [2]. The suspension was prepared to a concentration of approximately 10^4 cfu/ml.

Fermentation

About 100 g of African oil bean seeds were cleaned and pressure-cooked at 121 °C for 2 h. The cotyledons were removed, sliced into narrow strips, washed and cooked again for 30 min. Approximately 50 g of the cooked cotyledons were placed in thinly perforated polythene sheet and inoculated to about 2×10^4 cfu/g. The inoculum was thoroughly mixed with the substrate using a sterile spatula. The tightly wrapped packages were incubated at 30 °C for 48 h. The uninoculated samples served as control. Fermentation trials were carried out 6 times at two-week intervals.

Analyses

Chemical composition

Changes in the pH, moisture, titratable acidity and viable count were monitored during the laboratory preparation of ugba. Total viable count was on Plate Count Agar (PCA). Appropriate weights of oven-dried, starter culture prepared and market ugba samples were analysed for protein, fat and ash [13].

Sensory evaluation

This was carried out to determine the quality attributes of the laboratory-prepared and traditionally prepared ugba samples. A 12-member panel of judges drawn from the resident community of the University, but familiar with the peculiar characteristics of highly-rated ugba was constituted. The members were asked to rate the samples for colour, consistency, texture, taste and aroma. The ratings were presented on a 9–point Hedonic scale ranging from 9=like extremely to 1=dislike extremely. The data obtained were subjected to statistical analysis.

Results and discussion

Strain isolation and characterisation of starters

A total of 56 strains of spore-forming bacteria were isolated from 23 samples of ugba obtained from retail outlets in southwestern Nigeria. They were phenotypically characterised and identified as *Bacillus subtilis* (26 strains), *B. pumilus* (11 strains), *B. licheniformis* (9 strains), *B. coagulans* (6 strains) and unidentified *Bacillus* species. The isolates were distinguished based on cultural and biochemical tests described by Smith *et al* [10], Gordon *et al* [11], Claus and Berkeley [12]. Table 1 shows the microbial load of the ugba samples. The MM samples had the highest count of spore-formers with values ranging between 1.7×10^9–12.8×10^9, while SM samples recorded the lowest count of 1.8×10^8–1.2×10^9.

Since fermented foods in sub-Saharan Africa are usually brought about by chance and spontaneous inoculation involving mixed microflora, *Bacillus* strains usually implicated in condiment fermentation may have originated from the leaves used in wrapping the fermenting vegetable seeds before the fermentation process [2, 6].

The consistent dominance of *B. subtilis* in the fermentation process of vegetable condiments in sub-Saharan Africa was confirmed in this present study. The prevalence of the species has been reported in soybean-based foods such as natto [15, 16], and kinema [17]. In Nigeria, *B. subtilis* have been reported to be predominantly associated with the fermentation of African locust bean *(Parkia biglobosa*) for iru [4], cotton seed (*Gossypium hirsutum*) for owoh [6], African oil bean (*Pentaclethra macrophylla*) for ugba [18] and *Prosopis africana* seeds for okpehe [19]. According to Pederson [20], *Bacillus* species are usually implicated in the fermentation of nitrogenous and carbohydrate products.

In Nigeria and other countries in the sub-region, production of sticky mucilage and strong proteolytic activity are the most important characteristics of fermented vegetable condiments. To this end, the dominant *B. subtilis* isolated in this study were qualitatively screened for their proteolytic activity (>5 mm), amylolytic activity (>4 mm) and ability to produce stickiness (>15 cm). Therefore, data of strains that demonstrated relatively high capability potential for the above parameters are presented in Table 2 and Table 3 as probable candidates that can be employed as starter cultures for ugba fermentation. Based on the values obtained, *B. subtilis* MM-4:B12 was chosen as the starter organism for the laboratory preparation of ugba.

Fermentation characteristics

As the fermentation progressed, there was a gradual increase in the viable count of inoculated strain culminating in the count of 10^9 at the end of 48 h when the experiment was terminated. The pH also increased from 6.8 to 8.0, while the moisture content was 45.6% (Table 4). The shorter fermentation period was due to the fact that

Table 1 Microbial load of market ugba samples

Colony forming unit (cfu/g)		
Samples	Total viable count	Spore-forming bacteria
BM	4.7×10^8–2.8×10^9	1.3×10^9–1.9×10^{10}
MM	1.2×10^8–3.1×10^9	1.7×10^9–2.8×10^{10}
SM	1.4×10^7–2.4×10^8	1.8×10^8–1.2×10^9

Data represent range of average values of duplicate determinations (n=7)
BM=Bodija market, MM=Mokola market, SM=Sango market

Table 2 Screening scores for the selected *B. subtilis* strains isolated from market ugba samples

Strains	Proteolytic activity (mm)	Amylolytic activity (mm)	Stickiness (cm)
BM–5:B7	6.4	5.1	15.8
BM–1:B24	6.8	5.2	16.3
BM–7:B6	5.3	4.4	15.2
MM–1:B47	5.7	4.4	15.6
MM–4:B12	7.4	5.6	17.3
MM–6:B31	7.0	5.2	16.4
SM–3:B21	5.3	4.4	15.1
SM–7:B23	6.7	5.1	15.5
SM–2:B11	5.2	4.5	15.3

Values represent average scores of triplicate determinations

Table 3 Protease activity and relative viscosity (U/ml) of the selected *B.subtilis* strains isolated from market ugba samples

Strains	Protease activity	Relative viscosity
BM–5:B7	40.7	3.2
BM–1:B24	42.3	4.1
BM–7:B6	32.4	2.9
MM–1:B47	32.3	3.0
MM–4:B12	51.4	5.3
MM–6:B31	47.6	4.6
SM–3:B21	30.5	2.7
SM–7:B23	38.9	3.8
SM–2:B11	30.1	2.7

Values are average scores of triplicate determinations

Table 4 Changes in the pH, moisture and viable count of fermenting ugba using *B. subtilis* MM-4:B12

Fermentation period (h)	pH	Moisture (%)	Colony forming unit (cfu/g)
0	6.8	38.4	4.1×10^4
12	7.0	40.6	2.5×10^5
24	7.4	43.3	1.9×10^7
36	7.7	44.2	6.1×10^9
48	8.1	46.5	1.4×10^{10}

Values are average scores of triplicate determinations

Table 5 Chemical composition of starter culture produced and market ugba samples

Samples	Parameters					
	Moisture (%)	Protein (%)	Fat (% DM)	Ash (% DM)	pH	Titratable acidity (%)
LA	46.81a	18.11a	41.23a	6.07a	8.01a	0.13a
BM	45.13a	17.87a	41.10a	6.18a	7.81b	0.12a
MM	44.62b	18.16a	40.94a	6.11a	7.65b	0.11a
SM	44.10b	17.93a	41.20a	6.14b	7.42b	0.11a

Values represent the mean scores (n=3). Scores followed by the same letter in a column are not significantly different (p=>0.05). LA=ugba produced using starter culture,BM=ugba from Bodija market, MM=ugba from Mokola market, SM=ugba from Sango market, DM=dry matter

Table 6 Sensory evaluation of the ugba samples

Samples	Parameters				
	Colour	Consistency	Texture	Taste	Aroma
LA	7.2a	7.3ab	7.1a	7.6ab	7.8a
BM	7.0a	6.1a	6.8a	7.1a	6.8b
MM	7.1a	6.4a	7.2a	7.4a	7.1b
SM	6.8a	6.2a	6.5a	7.0a	6.9b

Scores followed by the same letters are not significantly different by Duncan's Multiple Range test at 5% level of significance. However, higher values indicate greater preference. Interpretation of sample symbols is as stated in Table 5.

the growth level of inoculated strain (7.3×10^9), brought about the desired biochemical changes in the fermenting mash comparable to the traditional ugba that is usually fermented for about 5 days.

Table 5 shows the chemical composition of the starter culture prepared ugba and the samples obtained from the retail markets. There were no significant differences in the protein, fat and titratable acidity of the samples. However, the significant difference in the final pH of both samples could also be related to the more discernible pungent ammoniacal aroma of the starter culture fermented sample.

Sensory evaluation

For the sensory evaluation (Table 6), the starter inoculated sample was rated higher by the panelists. Of note were the significant differences observed for the consistency, taste and aroma of market ugba samples compared to the ones produced using starter organism. From reports on fermented foods in sub-Saharan Africa, there are usually unpredictability of associated microorganisms of even different batches of the same product due to uncontrolled fermentation processing carried out by natural microflora of the raw substrates. The resultant inconsistency in product quality and occasional failure of fermentation to be initiated or incomplete due to possible competition for nutrients with other opportunistic organisms [2], are some of the militating factors in the traditional processing of fermented foods in Africa.

Conclusion

Ugba similar to those available in retail outlets was produced using a selected strain of *B. subtilis*. The shorter fermentation time and relative consistency in the product's quality, are some of the positive steps in the optimal processing of fermented foods in sub-Saharan Africa that have remain largely a traditional family art.

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