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Mixed Microbial Flora as Starter Culture for the Production of Ugba from African Oil Bean Seed

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ABSTRACT
Ugba production is locally done using wild microbial flora introduced randomly through processing materials for the fermentation of African oil bean seed. Utilization of mixed microbial flora as starter culture for controlled fermentation of African oil bean seed (*Pentaclethra macrophylla*) for ugba production was evaluated. Microbial association of locally fermented ugba using wild microbial flora was isolated, purified and characterized. Isolates were used as starter cultures, singly and in combination for the laboratory production of ugba for a period of 48 h at 37°C. Organoleptic properties of laboratory produced ugba were evaluated using color, texture and taste as organoleptic indices. Use of starter cultures shortened the fermentation of ugba from 3-4 days to 2 days. While locally prepared ugba was very slimy (+++), laboratory prepared ugba using single cultures of *B. megaterium*, *A. viscolatis* and *Corynebacterium* sp., was slimy (+). Use of mixed cultures of *B. megaterium* and *Corynebacterium* sp., or *B. megaterium* and *A. viscolatis* led to the production of ugba which was light brown and soft. Use of single culture of *Bacillus megaterium* led to the production of ugba with 87% overall quality compared to locally produced ugba (100%). A combination of cultures of *B. megaterium/Corynebacterium* sp. and *B. megaterium/Alkaligenes viscolatis* led to the production of ugba with 98 and 97% overall quality, respectively while a combination of *A. viscolatis/Corynebacterium* sp. and *A. viscolatis/Micrococcus roseus* gave the overall yield of 46 and 42%, respectively. Ugba production appears to be as a result of synergistic activity of fermenting microorganisms.

Key words: Ugba, fermentation, bacillus, quality, spoilage, synergistic

INTRODUCTION
Ugba is a value added solid substrate fermentation product of African oil bean seed (*Pentaclethra macrophylla*) prepared by the Ibos in Eastern Nigeria. Fermentation detoxifies the African oil bean seed with subsequent increase in nutrient availability and digestibility. Ugba is widely consumed as a snack, side dish or food condiment (Mbata and Orji, 2008). Ugba production is locally done through a mixed wild bacteria fermentation of the sliced, boiled and soaked African oil bean seeds. Unprocessed African oil bean seeds are bitter and possess anti-nutritional factors amongst which are pancine, cyanide, oxalates, saponin, phytic acid, phytate and tannins (Achinewhu, 1983; Enujiugha and Akanbi, 2005; Onwuliri *et al.*, 2004). Microbial population of ugba is introduced through the air, water, utensil, banana leaves or the handler; no starter culture

is used for the traditional method. Microorganisms involved are predominantly *Bacillus*, *Micrococcus* and *Lactobacillus*. Other organisms isolated from ugba include *Pseudomonas*, *Staphylococcus*, *Enterobacters*, *Leuconostoc*, *Corynebacterium* and *Alkaligenes* (Enujiugha, 2009; Isu and Njoku, 1997; Isu and Ofuya, 2000; Njoku *et al*., 1990; Obeta, 1983; Sanni *et al*., 2000). Processing drastically reduces the levels of the anti-nutritional compounds in African oil bean seeds with a concomitant increase in iron, calcium, potassium, thiamine and riboflavin levels (Enujiugha and Ayodele-Oni, 2003; Achinewhu and Riley, 1986). The fermented product is rich in fats, protein and carbohydrate (Oboh and Ekperigin, 2004).

Fermentation of the seeds is normally uncontrolled and last for 3-4 days (Njoku *et al*., 1990). Uncontrolled activities of fermentative organisms' after production result to the very short shelf life of ugba (Orji *et al*., 2003). Product deterioration and spoilage is witnessed in the form of organoleptic changes in color, texture, odor and taste (Mbata and Orji, 2008).

Attempts have been made to prolong the shelf life of ugba using starter cultures, wrapping or canning of finished product and use of different preservative agents. However, much work is still necessary in this area to achieve remarkable success in product uniformity and shelf life. The current study was carried out to determine the organisms which actively participate in ugba production for use as starter cultures. Organisms isolated from ugba were used as starter cultures, singly or in different combinations to produce ugba comparable to the traditionally produced and widely accepted ugba.

MATERIALS AND METHODS

The African oil bean seed was purchased in 2009 from Ogbete market, Enugu City in Eastern Nigeria and the research lasted for a period of 4 months. Ugba fermentation studies were carried out at the Microbiology Laboratory, Caritas University, Amorji Nike, Nigeria. The traditionally fermented ugba was from a local dealer in Amechi Awkunananw town, Enugu State and was promptly assessed.

Isolation of organisms and development of starter cultures: Fermenting samples were taken aseptically (using sterile forceps) from locally purchased ugba. Exactly 10 g quantity of each sample was homogenized in a tared blender with sterilized cups using 90 mL of sterile 0.1% peptone water as diluents. Subsequently, appropriate dilutions were made. On to a Petri dish containing 0.1 mL of the inoculums, 10-15 mL of sterile medium (Nutrient Agar NA), blood agar and cystine lactose electrolyte deficient agar (CLED) was poured for the isolation of bacteria while 1 mL of the inoculum was used for the isolation of yeasts and fungi. The plates were gently rotated to distribute the inoculum evenly in the plate and left to solidify under laminar airflow. Half of the plates for each medium were incubated aerobically at 30°C for 24-48 h while the other half were incubated anaerobically at 30°C for 72 h using a Gas Pak anaerobic system. Anaerobiosis was confirmed with a BBL anaerobic indicator. Incubation of the mould and yeasts was at 25°C for 5 days. Nutrient agar (DIFCO) and plate count agar (DIFCO) were the isolation and enumeration media for the bacteria. Potato Dextrose Agar (OXOID) and Malt Extract Agar (OXOID) were used for the isolation of the fungi and yeast (Speck, 1984).

Characterization and identification of isolates: Representative colonies of isolates streaked were on nutrient agar medium. Pure cultures resulting from the isolation were subcultured and preserved on nutrient agar slants at 4°C. Cultural characteristics of the isolates on the plates were

noted. The motility of the isolates was examined by the hanging drop technique. Gram reactions and cell morphology were examined from heat-fixed smears. The microorganisms were identified by the methods described by Gordon *et al*. (1973), Cowan and Steel (1974), Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974) and Health protection Agency (2007).

Pure cultures of *Bacillus megaterium*, *Corynebacterium* sp., *Alcaligenes viscolatis* and *Micrococcus varians* isolated from traditionally produced ugba were developed by inoculating each of the organisms into 40 mL peptone water in 100 mL Erlenmeyer flask and incubated for 24 h at room temperature. The organism were transferred into 100 mL Peptone Water Ugba Extract Broth (PWUEB) in 250 mL flask and incubated for 24 h at room temperature (Mbata and Orji, 2008). Each of the cultures was finally grown in 250 mL sterile Ugba Extract Broth (UEB) in 500 mL Erlenmeyer flask for 18 h at room temperature. The 18 h old cultures were then used as the starter cultures.

Production of Ugba: Ugba was produced in the laboratory according to the method described by Mbata and Orji (2008). Exactly 1 kg of seeds of African oil bean seed tree (*Pentaclethra macrophylla*) was boiled for 5 h. The seeds were de-hulled and the cotyledons washed in sterile water and then sliced longitudinally into 4-5×0.1-0.2 cm slices using sharp table knife. The slices were washed and introduced into wide mouth bottles and aluminum cups of 40 g sizes and capped tightly with their lids. The bottles and cups containing the slices were then boiled for 2 h. On cooling down to room temperature, each bottle and cup was aseptically inoculated with 3 mL suspension each of the inocula using sterile syringes and needles. Fermentation was allowed to proceed for 48 h at room temperature. After fermentation the cups and bottles containing the ugba was boiled at 98-100°C for 30 min. The pasteurized product was allowed to cool down to room temperature before samples were taken for physicochemical, microbiological and organoleptic analysis. Un-inoculated treated and sealed ugba samples and traditionally produced samples of ugba were used as controls.

Traditional method: The traditional method of producing ugba usually involves boiling some quantities of seeds of oil bean tree (*Pentaclethra macrophylla*) for 5-8 h to ease the removal of the hard shell. When the shells are removed, the cotyledons are washed with water and sliced into sizes of 4-5×0.1-0.5 cm or more. Ugba slices are washed, boiled for about 1-3 h and then soaked in water for about 10-12 h. Slices are washed again and allowed to drain for ½ - 1 h in a basket lined with banana leaves (*Musa sapietum* Linn). This is followed by wrapping with ororompo leaves (*Mallotus oppositifolius* Mull) in 40-50 g wraps. The wraps are allowed to ferment for 3-4 days at room temperature. Product is known as ugba.

Determination of physicochemical and organoleptic properties: The pH was determined using a WPAC10 pH meter. The product texture (softness) was determined instrumentally using a portable penetrometer and subjectively by pressing the slices between the fingers. The organoleptic tests for taste, aroma, color, softness and sliminess were carried out using a six man taste panelist who is very familiar with ugba. The rating test method was used and scoring was done using a 5-point Hedonic scale in a well lit room at room temperature. Each panelist was provided with 2 g of the test sample and asked to freely evaluate, comment and score the samples taste, color, aroma, softness and sliminess. Scale used was as follows, 5- very good, 4-good, 3-fair, 1-very poor/unacceptable. To eliminate bias, un-labeled samples were presented to panelist

individually with sufficient privacy to guarantee independent judgment. The acceptability of the samples was based on the scores and remarks made by the panelists. The result of the test was assessed using the Hedonic preference test. The scores for the samples were analyzed statistically using the method of analysis-ANOVA (Snedecor and Cochran, 1976).

RESULTS AND DISCUSSION

Bacterial content of locally produced ugba were isolated, characterized and identified as *Alkaligenes viscolatis*, *Micrococcus varians*, *Bacillus cereus*, *Bacillus megaterium*, *Staphylococcus aureus* and *Corynebacterium* sp. Traditional production of ugba is as a result of mixed fermentation activities of different organisms randomly introduced into food through water, materials and equipments used, leaves for wrapping, air and human handler. Given the traditional method of production and wide sources of inoculum, some of the microbial populations of ugba are contaminants introduced into the food during production which probably play no role in ugba fermentation. *Staphylococcus aureus* is a major human pathogen capable of producing diseases by their ability to multiply or through the production of extracellular substances such as enterotoxins in contaminated foods (Jawetz *et al.*, 1984). *Bacillus cereus* is a common contaminating bacterium isolated readily from a wide variety of foods, however the organism has been implicated in food poisoning when extended multiplication has occurred (Mbajunwa *et al.*, 1998). Mbajunwa *et al.* (1998) reported that *S. saprophyticus* and *B. cereus* were not adequate fermenters of ugba and did not soften ugba nor produce desirable qualities; moreover presence of *S. aureus* and *B. cereus* in food constitutes health risk to consumers. Furthermore, from the extensive work carried out in our laboratory, we deduced that *S. aureus* are microbial contaminants whose growth decreases with increase in keeping time, showing that they do not possess the necessary requirements for continued proliferation and spoilage of fermented ugba. The reduction in their numbers is thought to be as a result of toxic by-products of active fermenters present in food or inability to compete effectively for nutrients with the fermentative organisms. For these reasons, *B. cereus* and *S. aureus* were not applied as starter cultures in the proceeding part of our work.

Color, texture and aroma are organoleptic indices of a well fermented ugba and were used for product qualification in our study. The use of single cultures for ugba fermentation led to products with varying physical properties depending on the fermenting organism. Table 1 shows that while locally prepared ugba was very slimy (+++) after 3-4 days fermentation, laboratory prepared ugba using *B. megaterium*, *Alcaligenes viscolatis*, *Corynebacterium* sp., each was slimy (+), however ugba produced by *Micrococcus varians* was not slimy at all. Vegetative cells were used as starter cultures in order to reduce the lag period prior to microbial growth and product fermentation; using starter culture fermentation of ugba was for 2 days. We set out to achieve a product with similar physical and qualitative properties as the locally produced ugba under a shorter period of time to

Table 1: Physical properties of ugba produced using a single organism as starter culture

Starter culture	Color	Texture	Sliminess
Bacillus megaterium	Light brown	Soft	+
Micrococcus varians	Dark	Hard	-
Alkaligenes viscolatis	Dark brown	Hard	+
Corynebacterium sp.	Dark brown	Hard	+
Locally prepared	Light brown	Soft	+++
Boiled-uninoculated (control)	Dark	Hard	-

+: Slimy, +++: Very slimy, -: Not slimy

reduce chances of product contamination. The prolonged incubation for 3-4 days during traditional fermentation of ugba is probably responsible for the very slimy nature. Locally produced ugba was light brown and soft which is in agreement with the report by Mbata and Orji (2008). It is worthy to note that among microbial isolates used for the laboratory production, only *Bacillus megaterium* was capable of softening the ugba slices. This study is in agreement with that of Obeta (1983), *Bacillus* sp. were reportedly the principal organisms responsible for softening of ugba during production. Mbajunwa *et al.* (1998) reported that ugba fermentation using *Pseudomonas chlororaphis* as starter culture led to the softening of ugba, however the product was green and unacceptable as a result of the production of a green phenazine pigment known as chlororaphin. *Pseudomonas* sp., was not isolated in this work and following previous reports, it is likely that presence of *Pseudomonas* sp., is not responsible for the production of ugba with the proper physical and organoleptic qualities. Also according to the report by Enujiugha *et al.* (2008) the effect of *Pseudomonas fluorescens* when used as starter culture for the production of ugba was insignificant. Micrococcus species have been reported severally as part of the microbial population of ugba (Obeta, 1983; Njoku *et al.*, 1990), however their role in ugba production remains unclear. Using *Micrococcus varians* as starter culture, product was dark, hard and not slimy. This is in agreement with the report by Mbajunwa *et al.* (1998) that ugba produced using *Micrococcus* sp., as starter culture was poor with respect to aroma, texture and overall acceptability. Table 2 showed that production of ugba in the absence of Bacillus was not successful, ugba produced with *A. viscolatis/Corynebacterium* sp. and *A. viscolatis/M. varians* was dark brown and hard. Though a combination of *B. megaterium/Corynebacterium* sp. and *B. megaterium/A. viscolatis* gave similar qualities in terms of colour (light brown) and texture (soft), the product from the combination of *B. megaterium* and Corynebacterium was most similar to the locally produced ugba. This was based on the sliminess which was (+++) for the ugba with *B. megaterium/Corynebacterium* sp., similar to the locally fermented ugba. To the best of our knowledge, this is the first work implicating *Corynebacterium* sp. and *A. viscolatis* in the production of ugba. This does not correlate with the report by Njoku *et al.* (1990) that *Bacillus* sp. were the organisms capable of fermenting African oil bean seeds either separately or in combination. Moreover, *B. megaterium* was used in our study while *B. licheniformis* and *B. subtilis* were used as starter cultures by Enujiugha (2009). This shows the diversity of wild type bacteria found in ugba due to the practice of random inoculation through air, water, leaves or the handler. This is in agreement with the report by Sanni *et al.* (2000) who isolated *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. pumulus*, *B. brevis* and *B. polymyxa* from 45 samples of three Nigeria fermented soup condiments ugba, iru and ogiri. *Bacillus megaterium* as a single culture had the highest overall scores (87%) compared to locally prepared ugba (100%), however product was lacking in terms of taste (25/30), aroma (23/29) and softness (24/28) as shown in Table 3. This is a clear indication that other organisms present during fermentation play some roles in the adequate fermentation of ugba. This corresponds with the study of Enujiugha *et al.* (2008) that the fermentative activity of a bacterium is not responsible for proper ugba quality, rather production of ugba is as a result of synergistic action of microbial isolates. We further produced ugba using a combination of microorganisms as starter culture. Ugba produced by a combination of *B. megaterium* and *Corynebacterium* sp. or *B. megaterium* and *A. viscolatis* had the highest overall acceptability of 98 and 97%, respectively according to marks awarded by the panelists (Table 4). Based on organoleptic indices, results of laboratory produced ugba using *B. megaterium* and *Corynebacterium* sp. were taste 30/30, colour 28/28, aroma 27/29 and softness, 28/28 with an overall yield of 98%. Similar results were obtained when a combination

Table 2: Physical properties of ugba produced using mixed microbial population as starter culture

Starter cultures	Color	Texture	Sliminess
B. megaterium and *Corynebacterium* sp.	Light brown	Soft	+++
M. varians and *A. viscolatis*	Dark brown	Hard	++
A. visocolatis and *Corynebacterium* sp.	Dark brown	Hard	+++
B. megaterium and *A. viscolatis*	Light brown	Soft	++
Locally prepared	Light brown	Soft	+++
Boiled-uninoculated (control)	Dark	Hard	-

+": Slimy, +++: Very slimy, -: Not slimy

Table 3: Organoleptic properties of ugba produced using a single organism as starter culture

Starter culture	Taste	Color	Aroma	Softness	Overall score (%)
B. megaterium	25	28	23	24	87
M. varians	14	10	16	6	40
Alkaligenes viscolatis	18	11	17	19	57
Corynebacterium sp.	19	14	18	19	61
Locally prepared ugba	30	28	29	28	100

30: Maximum additive score of the panelist for each parameter, 6: The least additive score of the panelist for each parameter

Table 4: Organoleptic properties of ugba produced using a single organism as starter culture

Starter culture	Taste	Color	Aroma	Softness	Overall score (%)
B. megaterium and *Corynebacterium* sp.	30	28	27	28	98
M. varians and *A. viscolatis*	8	9	9	22	42
A. viscolatis and *Corynebacterium* sp.	11	10	8	24	46
B. megaterium and *Alkaligenes viscolatis*	27	28	28	28	97
Locally prepared ugba	30	28	29	28	100

30: Maximum additive score of the panelist for each parameter, 6: The least additive score of the panelist for each parameter

of *B. megaterium* and *A. alkaligenes* were used, taste was 27/30, colour 28/28, aroma 28/29 and softness, 28/28 with an overall yield of 97% as compared to the locally fermented ugba. A combination of *A. viscolatis* and *M. varians* gave the overall yield of 42% while that of *A. viscolatis* and *Corynebacterium* sp. gave the overall acceptability of 46% according to the report of the panelists. The current work corresponds with previous reports that *Bacillus* plays a major role in ugba production (Obeta, 1983) and with that of Njoku *et al.* (1990) that *Bacillus* sp. are primarily responsible for African oil seed fermentation and ugba production. From our analysis, 87% overall product quality obtained using *B. megaterium* as starter culture is indicative that organisms work synergistically towards the production of ugba with the proper qualities. Indeed, this was confirmed by results obtained from combining *B. megaterium* and Corynebacterium or *B. megaterium* and *A. viscolatis* as starter cultures in ugba production. This is in agreement with the report by Enujiugha (2009) and Mbata and Orji (2008) who used the combined bacterial culture of *B. megaterium* and *B. subtilis* for ugba production. Use of starter cultures for ugba production will limit the microbial population of ugba to those strictly necessary for proper ugba quality while reducing drastically the chances of food contamination by pathogenic microorganisms. Prepackaging and heating prior to fermentation further ensures that extraneous organisms do not have access into the food (Mbata and Orji, 2008). Additionally, use of proper starter cultures will help towards achieving uniformity of product. One of the major problems in traditional production of ugba is the lack of uniformity in starter cultures which results to products of varying qualities with short shelf-life.

CONCLUSION

Present findings indicate that a number of organisms play different roles in ugba fermentation, primarily Bacillus species in this case *B. megaterium* and to a lesser extent *Corynebacterium* sp. and *Alkaligenes viscolatis*. Use of starter culture will enable uniformity in product quality and could set the foundation for mass production of ugba. Work is currently been carried out on product pasteurization and other methods to inactivate the uncontrolled activities of this organisms after production in order to prolong shelf-life. Mass production using starter cultures will reduce chances of microbial contamination, product variation, food borne diseases and intoxication. Increased production will also necessitate increased availability of ugba with better quality at cheaper rate. This will go a long way towards addressing the 'Protein energy malnutrition' issues in developing countries such as Nigeria.

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