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Bioinhibition of Phenotypes of Gram-negative Food Indicator Bacteria from Nigerian Fermented Food Condiments

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ABSTRACT

Identification of a total of 440 Gram-negative food-borne bacterial strains from four Nigerian fermented condiments revealed that: 77 (17.5%) were *Escherichia coli*, 96 (21.8%) *Klebsiella pneumoniae*, 89 (20.2%) *Enterobacter aerogenes*, 10 (2.3%) *Enterobacter cloacae*, 34 (7.73%) *Proteus mirabilis*, 48 (10.9%) *Salmonella* spp., 38 (8.64%) *Citrobacter* sp. and 48 (10.9%) *Pseudomonas aeruginosa*. The bacterial strains were mostly resistant to cotrimoxazole (50.0–65.0%) and tetracycline (40.0–70.0%), while moderate to high multiple antibiotic resistance (MAR) of 37.5–75.0% was also exhibited by the food-borne bacteria. The inhibitory activities of crude extracts of selected plant spices, *Allium cepa*, *Allium sativum*, *Aframomum melegueta*, *Eugenia aromatica*, *Xylopia aethiopica*, *Monodora myristica*, nutmeg, *Zingiber officinale* were very minimal *in vitro* (7.1–29.0%), except for *Piper guineense*, which exhibited moderate inhibitory activities (20.0–54.0%). No *in vitro* inhibitory activity was recorded against the food indicator bacteria by *Lactobacillus acidophilus*, *L. brevis*, *L. plantarum*, *L. fermentum* and *Leuconostoc mesenteroides* isolated from the condiments. However, three *Lactobacillus* strains from Nigerian fermented beverages, *L. plantarum* LbOG1, *L. delbrueckii* LbOG2 from *ogi* and *L. brevis* LbKN1 from *kunun zarki* were able to inhibit about 65.0% of the selected Gram-negative food-borne bacterial pathogens. Sensory analyses indicated that there was no significant difference between the level of acceptance of the biopreserved condiment and the control by the respondents. Antimicrobial-producing *Lactobacillus* strains would therefore, be of considerable effect in enhancing the bioinhbition and preservative quality of Nigerian indigenous fermented food condiments both at the cottage and industrial levels of production, for local consumption and for exportation.

Keywords: biopreservation, food-borne bacteria, food protection, food safety, lactic acid bacteria

INTRODUCTION

Fermented food condiments are popular strong-smelling, fermented agri-food culinary products that give a pleasant aroma to soups, sauces and other prepared dishes. Many of them are known to contribute to the calorie and protein intake and are generously added to soups as a low-cost meat substitute by low-income families in most African countries. They are good sources of fatty acid and gross energy (Mbajunwa 1995), and have also constituted a significant proportion of diet to many people. Many proteinaceous oily seeds that are fermented to produce food condiments include African locust beans, cotton seeds, melon seeds, castor oil seeds, mesquite beans and soybeans (Obeta 1985; Sanni and Ogbonna 1991; Barber and Achinewhu 1992; Omafuvbe *et al*. 2000; Ogunshe *et al*. 2006, 2007, 2008).

Fermentation of these food condiments is widespread; however, the production of such foods is still largely a traditional family art done in homes in indigenous manners, as a cottage industry, under highly variable conditions. The methods employed in the manufacture of fermented condiments differ from one region to the other because these processes are based on traditional systems, according to local custom, climate conditions, type of substrates used and the specific process variations. In general, their fermentation takes place under conditions which the producers have found to be favourable for the appropriate growth and activity of the fermenting microorganisms (Achi 2005). All of the indigenous fermented condiments were originally fermented by natural microorganisms which have been transferred from generation to generation; however, in recent times, food-borne bacteria have been implicated in most Nigerian indigenous fermented condiments (Campbell-Platt 1980; Nwafor 1985; Barber *et al*. 1988; Ogbadu and Okagbue 1988; Odibo *et al*. 1992; Ogunshe *et al*. 2006, 2007).

The food-borne bacteria have also shown resistance to antibiotics (Ogunshe *et al*. 2008). Antibiotic resistance in food-borne pathogens is a reality and the recovery of strains of resistant food-borne pathogens to a variety of antimicrobials has become a major health concern (Kiessling *et al*. 2002), and the rise in carriage of antibiotic genes in virtually every species of bacterium has also been documented. As mentioned earlier by Adams and Mitchell (2002), identifying which pathogenic agents may be transmitted by fermented foods is clearly an important step in the overall risk assessment process, which generally requires expert knowledge and appraisal of data from a variety of sources, so that a significant pathogen is not disregarded. This study therefore, tries to determine the possibility of some food-borne pathogens surviving and growing in some of the Nigerian fermented food condiments, as well as the likely bioinhibition of the associated food-borne Gram-negative bacterial flora by lactic acid bacterial species of fermented food origin.

MATERIALS AND METHODS

Collection of samples and isolation of food-borne bacterial isolates

One hundred and eighty nine samples of the four most popular Nigerian fermented food condiments [*iru* (67) from *Parkia biglobosa*; *ogiri* (58) from *Citrullus vulgaris*; *ugba* (22) from *Pentaclethra macrophylla* and *okpehe* (42) from *Prosopis africana* were

Received: 11 June, 2008. Accepted: 12 July, 2008.

Original Research Paper

obtained from two cities of the middle belt and six major cities of South-Western Nigeria over a period of 36 months.

Microorganisms

The Gram-negative bacterial isolates from the fermented condiments were isolated and characterised using standard phenotypic taxonomic tools, based on their colonial morphology, microscopic, biochemical and physiological characteristics.

Antibiotic susceptibility determination of the Gram-negative bacteria

The antibiotic susceptibility determination of the Gram-negative, food-borne indicator bacterial strains, using some already prepared but commonly used imported antibiotics (discs) in Nigeria; AMX (amoxicillin; 25 µg), COT (cotrimoxazole; 25 µg), NIT (nitrofurantoin; 250 µg), GEN (gentamicin; 10 µg), NAL (nalidixic acid; 30 µg), OFL (ofloxacin; 30 µg), AUG (augmentin; 30 µg) and TET (tetracycline; 30 µg), obtained from ABTEK Biologicals Ltd. (Liverpool, UK), was carried out on the Gram-negative bacterial isolates using a modified agar disc-diffusion method.

The Gram-negative indicator bacterial isolates were separately inoculated into sterile petone water and incubated at 35°C for 24 h, after which the entire surface of each sterile Mueller-Hinton agar plate was streaked with 250 µl of each Gram-negative bacterial culture in brain heart infusion (BHI) broth, using sterile swab sticks. The plates were left for about 15 min before aseptically placing the antibiotic discs on the agar surfaces. The plates were then incubated at 35°C for 24-36 h. Zones of inhibition were measured and the diameter of each zone of inhibition was recorded in mm (Bauer *et al*. 1966; NCCLS 2003). Plates having no inhibition zones or zones less than 10 mm were recorded as negative (resistant).

Antimicrobial assays of spices and lactic acid bacteria

The modified agar well-diffusion method of Tagg (1976) was used. A lawn of each indicator bacterial isolate was prepared by spreading 500 µl of an active culture in BHI broth culture on the surface of Mueller-Hinton agar (LAB M, UK) in 12 cm diameter Petri dishes. Wells (6 mm in diameter) were punched into each agar plate and 500 µl of each crude extract of *Allium cepa*, *Allium sativum*, *Aframomum melegueta*, *Eugenia aromatica*, *Xylopia aethiopica*, *Monodora myristica*, *Zingiber officinale* and *Piper guineense*, was introduced aseptically into the agar well. 500 µl of each the active broth cultures of the putative lactic acid bacterial strains was introduced aseptically into the agar wells of sterile BHI agar plates. Seeded plates were left at room temperature for about 15 min before incubating at 35°C for 24–48 h. Zones of inhibition were measured after the period of incubation. Plates having no inhibition zones or zones less than 10 mm were recorded as negative (resistant).

Evaluation of acceptance of treated condiments

The fermented condiments were treated singly with the different selected *Lactobacillus* species and later evaluated for overall acceptability with regard to odour (smell), texture, flavour (taste) and appearance (colour) by two sets of panels of 20 judges who were regular consumers of the condiments. The judges who participated in this study were healthy volunteers 31–53 years of age and had no previous experience with sensory evaluations.

The attributes used in the sensory descriptive analyses of the biocontrol and the market (control) *iru* samples include appearance (colour, i.e., the actual dark brownish colour of *iru*); smell/aroma (the characteristic pungent, strong ammoniacal aroma); flavour (the characteristic fresh and pasty taste); texture (soft/tender fermented cotyledons, reminiscent, chewiness of fermented cotyledons); pasty mouth-feel of softness, gritty mouth-feel of presence of small particles. A structured Hedonic scale (Anzaldua-Morales 1994) with numerical scores 9 (like very much) to 1 (dislike very much) was used. A score of 5 was considered as the borderline of acceptability. The consumers were served the samples as 3-digit-coded on white plates, while the purchase of intent of the consumers toward the products was rated using the 5-point scale with 1 = definitely would not buy and 5 = definitely would buy (Meilgaard *et al*. 1999).

The Hedonic scale used in the consumer tests for sensory descriptive analyses using a control group include, the overall acceptability with the values rated as 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither dislike nor like; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely, while the scale for purchase intent was rated as 1 = definitely would not buy; 2 = probably would not buy; 3 = may or may not buy; 4 = probably would buy; 5 = definitely would buy.

RESULTS

The pH values of the indigenous fermented food condiments were in the neutral and slightly alkaline range, *iru* (7.3–8.9), *ogiri* (7.5–9.1), *okpehe* (7.1–7.9) and *ugba* (7.1–7.8), while a total of 440 Gram-negative food-borne bacterial strains identified as *Escherichia coli* 77 (17.5%), *Klebsiella pneumoniae* 96 (21.8%), *Enterobacter* species 99 (22.5%), *Proteus mirabilis* 34 (7.73%), *Salmonella* spp. 48 (10.9%), *Citrobacter* sp. 38 (8.63%) and *Pseudomonas aeruginosa* 48 (10.9%) were randomly recovered from the four most-consumed Nigerian indigenous fermented food condiments – *iru*, *ugba*, *ogiri* and *okpehe*.

The 138 Gram-negative bacterial isolates from *iru* samples were identified as *E. coli* 12 (8.70%), *Klebsiella pneumonia* 33 (23.9%), *Enterobacter* sp. 28 (20.3%), *Proteus mirabilis* 11 (7.97%), *Salmonella* spp. 23 (16.7%), *Citrobacter* sp. 16 (11.6%) and *Pseudomonas aeruginosa* 15 (10.9%). The 119 isolates from *ogiri* samples were identified as *E. coli* 21 (17.6%), *Klebsiella pneumonia* 31 (26.1%), *Enterobacter* sp. 25 (21.0%), *Proteus mirabilis* 9 (7.56%), *Salmonella* spp. 11 (9.24%), *Citrobacter* sp. 9 (7.56%) and *Pseudomonas aeruginosa* 13 (10.9%). The 73 isolates from *okpehe* samples were identified as *E. coli* 28 (38.3%), *Klebsiella pneumonia* 12 (16.4%), *Enterobacter* sp. 5 (6.8%), *Proteus mirabilis* 5 (6.8%), *Salmonella* spp. 8 (11.0%), *Citrobacter* sp. 7 (9.59%) and *Pseudomonas aeruginosa* 8 (11.0%), while the 110 isolates from *ugba* samples were identified as *E. coli* 16 (14.5%), *Klebsiella pneumonia* 20 (18.1%), *Enterobacter* sp. 41 (37.3%), *Proteus mirabilis* 9 (8.1%), *Salmonella* spp. 6 (5.5%), *Citrobacter* sp. 6 (5.5%) and *Pseudomonas aeruginosa* 12 (10.9%).

The Gram-negative bacteria isolates from the fermented condiments were generally highly resistant to COT and TET (**Figs. 1A–E**). In this study, low antibiotic resistance values were taken as ≤25%, moderate resistance as 25-35% and high as ≥35%, the food-borne bacteria species from the *iru* recorded overall moderate to high antibiotic resistance against TET (37.5%) and COT (45.8%) respectively; the bacterial flora from *okpehe* samples exhibited high resistance against COT (54.5%) but moderate resistance against TET (27.3%). The bacterial species from *ogiri* exhibited high resistance against COT (40.0%) but moderate resistance against NAL (30.0%) and tetracycline (30.0%), while the food-borne bacteria from *ugba* similarly exhibited high resistance against COT (41.1%) but moderate resistance against TET (23.5%) and AMX (29.4%) as shown in **Fig. 1F**. However, no resistance against ofloxacillin was recorded in all the *iru*, *ugba* and *okpehe* samples. A relatively high multiple antibiotic resistance (MAR) was also exhibited by the food-borne bacteria from the fermented food condiments. *Klebsiella* and *Pseudomonas* strains exhibited a MAR of 25.0-50.0%, *Enterobacter*, 25.0-75.0% while *Citrobacter* and *Salmonella* strains also exhibited a 25.0-37.5% MAR (**Table 1**).

The inhibitory activities of crude extracts of selected plant spices, *A. cepa*, *A. sativum*, *A. melegueta*, *E. aromatica*, *X. aethiopica*, *M. myristica*, nutmeg, *Z. officinale* were very minimal *in vitro* (7.1–29.0%), except *P. guineense*, which exhibited moderate inhibitory activities (20.0–54.0%).

The lactic acid bacteria (LAB) isolated from the

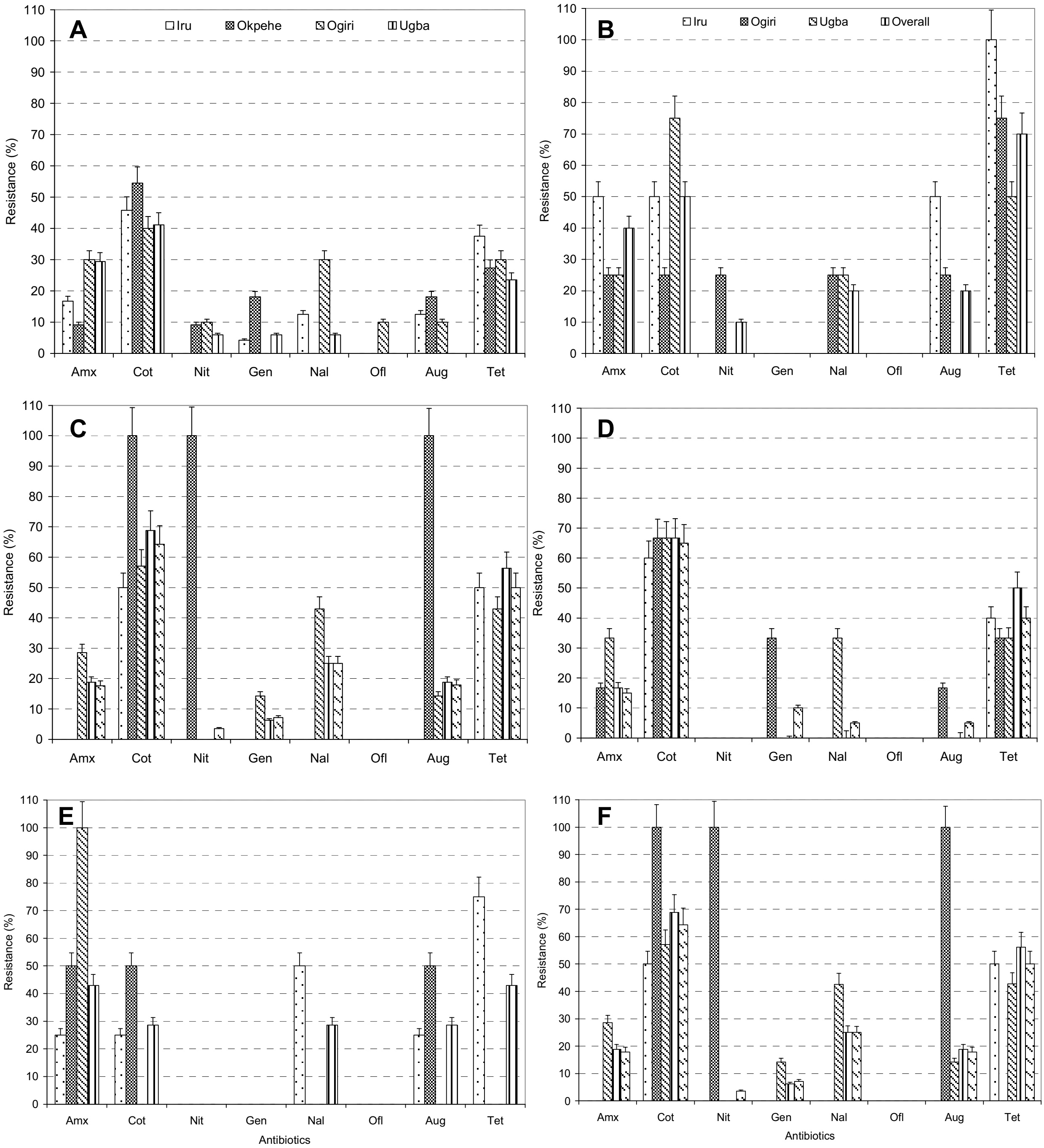

Fig. 1 *In vitro* overall (**A**) antibiotic resistance profiles of food-borne strains from condiments. (**B**) *Klebsiella* strains. (**C**) *Enterobacter* strains. (**D**) *Salmonella* strains. (**E**) *Citrobacter* strains. (**F**) *Pseudomonas* strains.

indigenous fermented food condiments in this study were *Leuconostoc* sp., *Lactobacillus* sp., *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Leuconostoc mesenteroides* from *iru*; *Leuconostoc* sp. and *Lactobacillus plantarum* from *okpehe*, *L. brevis* from *ugba* and *L. fermentum* from *ogiri*. The *in vitro* inhibitory activities of 12 LAB isolated from the fermented food condiments towards the Gram-negative food-borne bacteria were assayed for but there were no *in vitro* inhibitory activities against the food-borne Gram-negative bacteria from same or similar food condiments (**Table 2**). However, three *Lactobacillus* species from two Nigerian fermented beverages, *L. plantarum* LbOG1 and *L. delbrueckii* LbOG2 from *ogi*, a Nigerian fermented food from maize and *L. brevis* LbKN1from *kunun zarki*, a Nige-rian fermented alcoholic beverage from sorghum, millet and maize were able to inhibit 51.6–55.9% of the food-borne Gram-negative bacterial strains with inhibition zones ran-ging between 10.0–22.0 mm in diameter.

Sensory analyses results indicated that there was no significant difference between the level of acceptance of the biopreserved condiment and the market (control) *iru* samples by the respondents since $p > 0.05$ for all the organoleptic parameters (**Table 3**).

DISCUSSION

Among the requirements for foods to be of good sanitary quality, they must be shown to be free of hazardous micro-organisms, or those present should be at a safe level. Numerous instances of food-borne diseases have been recorded, and in spite of our knowledge of microbiology, as well as the implementation of safety procedures such as HACCP, the worldwide incidence of food poisoning has increasing (Baird-Parker 1994). Food microbiologists are usually interested in the studies of microbial flora of industrial importance, especially in selection of starter cultures for fermented foods; including fermented condiments. This study therefore, tries to determine the effect of microbial presservation of the four most-consumed Nigerian indigenous fermented food condiments – *iru*, *ugba*, *ogiri* and *okpehe*

Table 1 Antimicrobial profiles of crude plant extracts and salt-based spices against food indicator bacterial isolates using the agar well-diffusion method.

Spices	**% susceptibility with zones of inhibition (mm diameter)**			
	Iru	***Ogiri***	***Okpehe***	***Ugba***
Allium cepa	0.0 [a]	0.0 [a]	0.0 [a]	0.0 [a]
	(-)	(-)	(-)	(-)
Allium sativum	25.8 [b]	27.0 [b]	14.2 [b]	20.0 [b]
	(10.0-30.0) [c]	(12.0-30.0) [c]	(12.0-20.0) [c]	(20.0-30.0) [c]
Aframomum melegueta	3.2 [b]	16.2 [b]	7.1 [b]	0.0 [a]
	(10.0-15.0) [c]	(10.0-18.0) [c]	(12.0-20.0) [c]	(-)
Eugenia aromatica	29.0 [b]	18.9 [b]	7.1 [b]	60.0 [b]
	(10.0-15.0) [c]	(10.0-22.0) [c]	(12.0-16.0) [c]	(12.0-20.0) [c]
Xylopia aethiopica	0.0	2.7 [b]	0.0	0.0
	(-)	(12.0) [c]	(-)	(-)
Monodora myristica	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)
Piper guineense	25.8 [b]	54.0 [b]	28.6 [b]	20.0 [b]
	(10.0-17.0) [c]	(10.0-30.0) [c]	(10.0-12.0) [c]	(10.0-12.0) [c]
Nutme	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)
Zingiber officinale	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)
Maggi	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)
Royco	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)
Ajinomoto	0.0	0.0	0.0	0.0
	(-)	(-)	(-)	(-)

[a] = no zones of inhibition; [b] = values (total number and % inhibition) indicating the diameter of inhibition zones; [c] = zones of inhibition in mm using 500 µl of the lactobacilli broth culture on Mueller Hinton agar

Table 2 *In vitro* inhibition of the foodborne Gram-negative bacterial species by the antimicrobial-producing *Lactobacillus* strains using the agar well–diffusion method.

Gram-negative bacteria	**% susceptibility with zones of inhibition (mm diameter)**		
	***L. plantarum* LbOG1**	***L. delbrueckii* LbOG2**	***L. brevis* LbKN1**
Klebsiella spp. [10]	4 (40.0) [b]	3 (30.0) [b]	5 (50.0) [b]
	(12.0-18.0) [c]	(12.0-16.0) [c]	(10.0-20.0) [c]
Enterobacter spp. p [28]	15 (53.6) [b]	14 (50.0) [b]	12 (42.9) [b]
	(10.0-18.0) [c]	(10.0-18.0) [c]	(10.0-18.0) [c]
Citrobacter spp. [7]	4 (57.1) [b]	5 (71.4) [b]	4 (57.1) [b]
	(10.0-16.0) [c]	(10.0-16.0) [c]	(12.0-22.0) [c]
Salmonella spp. [20]	11 (55.0) [b]	14 (70.0) [b]	11 (55.0) [b]
	(10.0-18.0) [c]	(10.0-22.0) [c]	(12.0-16.0) [c]
Pseudomonas spp. [28]	14 (50.0) [b]	16 (57.1) [b]	18 (64.3) [b]
	(10.0-18.0) [c]	(12.0-16.0) [c]	(12.0-18.0) [c]
Total [93]	48 (51.6)	52 (55.9)	50 (53.8)

[b] = values (total number and % inhibition) indicating the diameter of inhibition zones; [c] = zones of inhibition in mm using 500 µl of the lactobacilli broth culture on Mueller Hinton agar.

using food-compatible *Lactobacillus* strains.

It has however, been noted earlier that fermented condiments may pose a health problem to consumers, especially from the concept of food hygiene, due to the recovery of high prevalence of food-borne pathogens from some African fermented foods and beverages (Campbell-Platt 1980; Shehu and Adesiyun 1990; Odibo *et al*. 1992; Gadaga *et al*. 2004; Ogunshe *et al*. 2006). The Gram-negative food-borne bacteria recovered from the fermented condiments in this study were *E. coli*, *Enterobacter*, *Klebsiella*, *Citrobacter*, *Proteus*, *Pseudomonas* and *Salmonella* species, which are similar to those earlier obtained from the same or similar food products at different stages of fermentation (Kolawole and Okonkwo 1985; Nwafor 1985; Barber *et al*. 1988; Ogbadu and Okagbue 1988; Ikenebomeh 1989; Odibo *et al*. 1992; Ogunshe *et al*. 2006, 2007). In addition, the food-borne bacteria have been implicated as aetiologic agents of gastrointestinal infections and food poisonings (Mead *et al*. 1999; Okeke and Nataro 2001; Lucas and Gills 2003).

The bacterial species also displayed varied phenotypic antibiotic resistance profiles but highest resistance was exhibited against cotrimoxazole and tetracycline. Similar trend of high antibiotic resistance was observed in a previous study of Ogunshe *et al*. (2008) at various periods of surveillance between 2006 and 2007. There is an increase in carriage of antibiotic genes in virtually every bacterial species and while antibiotic resistance in food-borne bacterial pathogens is a reality, the recovery of resistant food-borne strains to a variety of antimicrobials has become a major health concern (Schwarz and Charius-Dancla 2001; Kiessling *et al*. 2002).

Under favourable conditions, microorganisms can alter the flavour, texture and aroma of foods, therefore, in product development, and it is important to apply the knowledge of the type of microorganisms which contaminate foods to ensure that such food products are safe to eat and of required quality (Barnett 1996), as well as increasing their shelf life (Gould 1996). A number of traditional preservative techniques are currently being used in Nigeria to control food spoilage and pathogenic microorganisms associated with the traditional fermented food condiments. A common post-fermentation treatment is drying. The extent of drying however varies with locality, with moisture content found to vary between 9 and 19% (Campbell-Platt 1980). Smoking is another traditional method applied as means of preserving indigenous fermented condiments and though it appears to be a more effective approach (Wachukwu *et al*. 2003), the associated Gram-negative food-borne bacterial flora were still isolated from smoked condiments, especially *okpehe*, which is the condiment that is mostly

Table 3 Results of parameters in the tested sensory analysis of the bio-preserved condiments in the consumer tests and scale for purchase of intent using a control group.

Parameters	Biocontrol	Control
Appearance	8.45 ± 0.15	8.50 ± 0.15
Smell	8.25 ± 0.099	8.35 ± 0.11
Flavour	8.30 ± 0.16	8.35 ± 0.17
Texture	8.60 ± 0.13	8.55 ± 0.19
Intent of purchase	4.95 ± 0.022	4.90 ± 0.031

preserved by smoking. A study carried out by Wachukwu *et al.* (2003) reported that microorganisms associated with *iru* (*daddawa*) fermentation could not survive beyond the first day of smoking but for sun drying, a reduction in contaminant level was a gradual process. Sun drying and smoking however, yields a dark brown to black fermented condiments which is usually not as desirable as the fresh moist brownish samples.

The addition of salt to the condiments is also believed to serve as a temporary preservative step and this is because salt is known to exert some osmotic force on spoilage microorganisms. Salting was also reported to safeguard against food pathogens such as *Clostridium* and *Salmonella* as well as halting the development of undesirable volatile amines, which gives a putrefactive odour to fermented foods (Mossel 1982). It is believed that the use of salt as a preservative could be more effective if the right concentration is used but there is the possibility of over-salting which will affect the sauce, soup or other delicacies prepared with the condiments. Meanwhile, in this study, table salt and industrial salt-based spices, Maggi, (Nestlé, Nigeria Plc.), Royco (Unilever Nigeria Plc) and Aji-no-moto (Ajinomoto Co., Inc., Tokyo, Japan) were not inhibitory against any of the bacterial isolates *in vitro*. The three salt-based industrial products are taste-enhancing super seasoning, therefore, their inability to inhibit the food-borne bacterial flora further confirm that salt and salt-based food products have no inhibitory potentials on the food-borne indicator Gram-negative bacterial flora. Despite the application of the traditional preservative techniques, quite a number of food spoilage and pathogenic microorganisms were still found to be associated with the Nigerian indigenous fermented food condiments.

The introduction of spices with antimicrobial properties has notably obtained emphasis for possible application in food production in order to prevent bacterial and fungal growth (Adegoke *et al.* 1988; Sagdic *et al.* 2003). Moreover, plants' volatiles have been generally regarded as safe (GRAS) (Newberne *et al.* 2002). Aqueous and crude extracts of the spices used in this study were non-inhibitory against the food-borne bacterial pathogens *in vitro*, except for the crude extracts of *Allium sativum* and *Eugenia aromatica*, which were minimally inhibitory *in vitro* (7.1–29.0%), while *Piper guineense* exhibited moderate inhibitory activities (20.0–54.0%).

Consumers in developed countries have a more critical attitude about what they eat and drink as a consequence of modern life, while food microbiologists are currently facing a huge challenge regarding food 'freshness' implicit in the consumer's demand for more natural products. Food with less severe processing, that is additive-free, safer, with satisfactory shelf life and easy to prepare is sought because of higher consciousness about nutrition and health (Loureiro and Querol 2000). A number of studies have shown that using starter cultures increases the safety of many fermented foods. The food-borne bacterial flora obtained from the fermented food condiments in this study were therefore, subjected to inhibition assays, using LAB isolated from same and similar condiments but it was observed that though, LAB have found an ecological niche in foods, especially fermented foods (Santos *et al.* 1996; Konings *et al.* 2000) and have an essential preservation role in most food and beverage fermentation processes (Savadogo 2004), which is usually achieved by inhibition of contaminating spoilage bacteria (El-Gazzar and Marth 1992; Ray and Daeschel 1992; Vignolo *et al.* 1993; Chiang *et al.* 2000), none of the associated Gram-negative food-borne flora was inhibited *in vitro* by the *Lactobacillus* strains.

Bacterial pathogens are generally studied individually, although in their natural environment they often co-exist or compete with multiple other microbial species (Pericone *et al.* 2000). The frequency of occurrence of the isolated LAB from the fermented condiments in this study was found to be relatively less than other indigenous fermented foods As previously documented (Campbell-Platt 1984; Achinewhu 1987; Okagbue and Ogbadu 1998; Ogunshe *et al.* 2006), the pH of various indigenous fermented food condiments, depending on the vegetable seeds, mode of fermentation and the fermenting microbial flora was in the neutral (7.1) and slightly alkaline (9.1) range, which is quite common with the fermentation of vegetable proteins (Whitaker 1978). The liberation of ammonia and increased pH condition had been earlier noted to encourage the growth of food-borne bacteria in the condiments, as similarly observed in this study. It is strongly believed that the alkaline pH range of the condiments is responsible for the low recovery rates of the LAB.

Reported mechanisms for microorganisms that control food-borne pathogens have been found to include the reduction of pH (Brashears and Durre 1999), as in the application of LAB. Lactic acid, as produced by lactic acid starter culture bacteria or as an additive to foods, functions as a natural antimicrobial, having a generally recognised as safe status. Members of the genera *Lactobacillus* were isolated from the fermented condiments in this study but contrary to the earlier reports of Doores (1993) that lactic acid produced by LAB are able to inhibit the growth of many types of food spoilage bacteria, including Gram-negative species of the families *Enterobacteriaceae* and *Pseudomonadaceae*. Odusote (1980) and Obeta (1983) also confirmed the presence of *Lactobacillus* in the fermentation process of *ogiri*; however, the LAB isolated from fermented condiments in this study were all found to be non-inhibitory towards the food-borne bacteria isolated from same or similar condiments.

The lack of inhibitory activities of the LAB obtained from the fermented condiments as reported in this study indicates that the Gram-negative food-borne bacterial species were resistant to the inhibitory metabolites produced by the LAB or that the inhibitory activities of the LAB were not effective in the alkaline fermenting environment of the condiments, as opposed to the acidic environment in which the LAB thrive and produce organic acids and other inhibitory metabolites. This *in vitro* resistance of the Gram-negative bacterial isolates against the metabolites produced by the LAB explains the high recovery rate of these food-borne bacteria from the fermented food condiments.

Microbial strains traditionally used to ferment food have a long history of safe use and are, therefore, recognised as safe (Aureliand and Franciosa 2002), while antimicrobial activities of LAB isolated from fermented foods have been the subject of intensive research due to the potential application of these bacteria as protective cultures in biological preservation (Holzapfel *et al.* 1995; Drosinos *et al.* 2007). In a review by Holzapfel *et al.* (2000) on the status of biological preservation of foods, recent developments on underlying mechanisms of inhibition by 'protective' cultures, with special reference made to LAB and their 'food-grade' safety were discussed. The addition of antimicrobial-producing bacterial species can create a built-in food preservation system (Zhou *et al.* 2006) and they may therefore, act as biocontrol agents (Ray and Sandine 1992; Reuter 2001). Scientific advances, such as the addition of antimicrobial-producing microorganisms will help food processors ensure that the production lots being released to distribution for consumption are the safest foods possible. In this study, only three strains, *Lactobacillus plantarum* (LbOG1), *Lac-*

tobacillus delbrueckii (LbOG2) and *Lactobacillus brevis* (LbKN1) out of over 50 isolates from fermented condiments, foods and beverages were able to inhibit about 65% of the Gram-negative food indicator bacteria. In a similar study by Drosinos *et al*. (2007), out of 300 LAB isolated from spontaneously fermented sausages made by two medium-sized enterprises (MSE) located in southern Greece, only 3 strains were found to be able to produce antimicrobial compounds of proteinaceous nature against *Listeria monocytogenes*.

Prospects for applying advanced technologies to indigenous fermentation had been reviewed earlier by workers like Owusu-Ansah *et al*. (1980), therefore, indigenous fermented food condiments if properly developed, have a strong potential of increasing food production, improving the nutritional status of the rural population and decreasing food imports. Adams (1990) emphasised the variety of fermented foods and their quantitative importance in the human diet, while Achi (2005) also reported that fermented food condiments are good sources of nutrients and could be used to produce complementary food supplements. The popularity of the Nigerian indigenous fermented condiments is based specifically on their organoleptic properties, i.e., their aroma, flavour, taste and colour (Ogunshe and Olasugba 2008). This work brings to light the possibility of including *Lactobacillus* cultures in the processing of indigenous fermented food condiments to effectively reduce their contamination and increase their shelf-life, thereby controlling infections from food-borne pathogens. There was no noticeable effect of the inhibitory *Lactobacillus* flora on the sensory properties of the fermented condiments, which was generally acceptable to the panelists, therefore, the safety of the Nigerian indigenous fermented food condiments may be improved by biopreservation without affecting their organoleptic properties. The selected antibacterial-producing *Lactobacillus* strains would therefore, be of considerable effect in enhancing the preservative quality of the Nigerian indigenous fermented food condiments both at the cottage and industrial levels of production, for local consumption and export.

ACKNOWLEDGEMENTS

Mr. Donatus Esimekhuai of the herbarium, Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria for taxonomic assistance on the spices.

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