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Evaluation of the effects of in vitro antibiotic resistance patterns of Gram-negative, food borne indicator bacterial species

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Abstract

Varied but multiple antibiotic resistance rates were exhibited by all the foodborne indicator bacterial species, *Citrobacter, Escherichia coli, Enterobacter, Klebsiella, Morganella morganii, Salmonella, Shigella, Pseudomonas* isolated from four most-popular Nigerian indigenous fermented plant food condiments *ogiri, iru, okpehe* and *ugba*. The generally most-resisted antibiotics (discs) were tetracycline (44.0 - 63.0%), cotrimoxazole (56.0 - 70.3%), nalidixic acid (28.0 - 74.1%), amoxicillin (28.0 - 88.9%) and augmentin (16.0 - 94.4%), while the least resisted antibiotics were ofloxacillin (0.0% - 1.85%) and gentamicin (4.0 - 16.7%). Antibacterial activities of crude extracts of local spices and essential oils of plant origin, as well as carvone, lactic and acetic acids on the multiple antibiotic resistant Gramnegative foodborne bacterial species were determined using modified agar well-diffusion assay. Crude extracts of *Eugenia aromatica* (92.0%) and *Allium sativum* (72.0%) were maximally inhibitory; lactic (44.4%) and acetic (46.3%) acids were moderately inhibitory but essential oils of *Eugenia uniflora* (24.0%), *Ageratum conyzoides* (16.7%) and *Chrsophylum albidium* juice (13.0%) were minimally inhibitory *in vitro*, indicating that crude extracts of *E. aromatica* and *A. sativum* can serve as easily prepared, non-chemical, plant-based, adjunct preservatives of fermented condiments both domestically and industrially, even by the traditional producers of the indigenous and similar condiments.

Keywords: Cottage food production, essential oils, food safety, food processing, multiple antibiotic resistance, Nigerian indigenous fermented foods, plant foods.

INTRODUCTION

New strategic research areas have emerged and been developed, with profound effects on the capacity to produce food and manage natural resources and the environment (Taeymans, 2011), while the world Health Organisation food safety unit has also given high priority to the research area of fermentation as a technique for preparation and storage of food. In Africa and Asia, the art of fermentation is widespread, including the processing of plant vegetable seeds into food condiments, which in Nigeria and many other countries of west and central Africa are popular strong-smelling fermented food culinary products that give pleasant aroma to soups, sauces and other prepared dishes.

It is evident that fermented food condiments are good sources of nutrients and could be used to produce complementary food supplements (Achi, 2005); however, it is quite obvious that fermented food condiments may harbour some bacterial pathogens that can be hazardous to human health. Nevertheless, the demand for locally processed Nigerian fermented food condiments (NIFFC) such as iru, ogiri, ugba, okpehe, etc. (from seeds of biglobosa, Citrullus vulgaris, Parkia Pentaclethra macrophylla and Prosopis africana respectively) is currently on the increase; even among the elites, probably because none of the industrial seasonings or spices has been able to meet the indigenous organoleptic qualities of these fermented condiments of plant origin. Microorganisms have successfully adapted to changes in food production, processing and preservation techniques, thereby resulting in a number of new and emerging foodborne pathogens, as well as re-emergence of the ones that have been problematic in the past. To protect the public health, science must therefore, meet the challenges that result from the remarkable adaptability of foodborne pathogens to foods (Woteki and Kineman, 2003). In addition to recovery of foodborne bacterial species from these condiments, there is also the emergence of multiple antibiotic resistant bacteria, which pose a challenge in food systems (White et al., 2002, 2004). Food safety therefore, remains a major challenge to producers and consumers of these fermented food condiments. Consumers are also currently skeptical of chemical preservatives in their foods; thus, the need to discover other more acceptable means of protecting and preserving these highly popular indigenous foods becomes pertinent, more so since plant extracts are more natural.

Certain plant-based spices have been found to impart flavour and prolong the storage life of foods by bacteriostatic or bactericidal activities (López et al., 2007; Shan et al., 2007). Being natural foodstuffs, they also appeal to consumers who tend to question the safety of synthetic / chemical food additives (Vanit et al., 2010). The purpose of this study therefore, was to determine the in vitro antibiotic resistance patterns of Gram-negative, foodborne indicator bacterial species isolated from four most popular Nigerian fermented food condiments, iru, ogiri, ugba, okpehe. Using an easily reproducible method, the inhibitory and preservative activities of some local spices, organic acids and essential oils against the foodborne bacterial pathogens isolated from the fermented food condiments shall be evaluated.

MATERIALS AND METHODS

Collection of samples

The Nigerian fermented food condiments used in this study were market *iru* produced (from locust bean, *Parkia biglobosa*), ogiri (from melon seeds, *Citrullus vulgaris*), okpehe (from *Prosopis africana*) and *ugba* (from oil-bean seeds, *Pentaclethra macrophylla*), while the fresh food spices (*Allium sativum, Eugenia aromatica, Monodora frangrans, Mytristica frangrans, Piper guineensis, Xylopia aethiopica* and *Zingiber officinalis*) were all purchased from various markets in South-West Nigeria. The spices, two organic acids (lactic and acetic acids) and essential oils of

Ageratum, Eugenia, Carvone, Anacardium plants and Chysophyllum albidium juice were assayed for their inhibitory effects on the foodborne pathogens from the four fermented condiments in this study. The condiments were obtained at 12-month interval between the first samples' collection (A) and the second samples' collection (B).

Isolation, characterisation and preservation of Gram-negative bacteria from fermented condiments

The Gram-negative bacteria isolates were selectively recovered from the various fermented food condiment samples, using sterile MacConkey broth and agar (MCC, Lab M, England), *Salmonella-Shigella* agar (SS, Lab M, England), *Pseudomonas* selective agar (BHI, Oxoid, England), thiosulphate citrate bile sucrose agar (TCBS, Lab M, England), while differential identification was determined on eosin merhylene blue (EMB, Lab M, England) and triple sugar iron agar (TSI, Lab M, England). All the pure bacterial isolates were kept in triplicates on Brain Heart Infusion (BHI, Oxoid, England) agar slants, as working and stock cultures. Taxonomic studies were carried out on the purified bacterial isolates on the basis of their cultural, morphological, biochemical and physiological characteristics. General keys with reference were used for the identification (Buchanan and Gibbons, 1974; Crichton, 1996).

Antibiotic susceptibility determination of the Gram-negative bacteria

Antibiotic susceptibility / resistance of the Gram-negative foodborne bacterial species to various antibiotics AMX (Amoxicillin; 25 μ g), COT (Cotrimoxazole; 25 μ g), NIT (Nitrofurantoin; 250 μ g), GEN (Gentamicin; 10 μ g), NAL (Nalidixic acid; 30 μ g), OFL (Ofloxacillin; 30 μ g), AUG (Augmentin; 30 μ g) and TET (Tetracycline; 30 μ g) was determined according to the Bauer et al. (1996) and NCCLS (2003) methods. The entire surface of each sterile Mueller-Hinton agar plate was seeded with each Gram-negative bacteria isolate using sterile swab sticks, after which the plates were left for about 15 min before aseptically placing the antibiotic discs on the agar surfaces with sterile forceps, followed by incubation at 35°C for 18 to 24 h. Zones of inhibition around the agar wells after incubation were measured and recorded in millimeter diameter, while zones of inhibition less than 10.0 mm in diameter or absence of zones of inhibition were recorded as resistant or negative.

In vitro antimicrobial inhibition bioassays of the Gramnegative bacteria isolates by local spices

Fresh local food spices (Z. officinalis, A. sativum, X. aethiopia, P. guineensis, M. frangrans, M. frangrans, E. aromatica, Aframomum melegueta) were surface-sterilised with ethanol and later cleaned with wet, sterile cotton wool. In order to obtain the spice's extracts, about 150 g of each cleaned spice were dry-milled with blender, which was hot-washed (boiled water at 100°C) five times after each use, based on the microbial loads of the rinses. A set of the milled extracts were soaked in warm sterile distilled water for 12 h to obtain the aqueous extracts of the spices and the other set were slightly wet with sterile distilled water to obtain the crude extracts (pastes) of the spices. About 10 ml of C. albidium juice was obtained by squeezing out the juice into 15 ml of sterile distilled water, while the essential oils of Ageratum convzoides, Eugenia uniflora, Anacardium occidentals, carvone used in this study were prepared in the organic unit of the Department of Chemistry, University of Ibadan. The organic acids - lactic and acetic acids

Antibiotic disc (μg ⁻¹)									
S/N	Bacterial isolate	AMX	СОТ	NIT	GEN	NAL	OFL	AUG	TET
1	Enterobacter spp. [8]	12.5	50	0	12.5	25	0	12.5	37.5
2	<i>Klebsiella</i> spp. [6]	50	66.7	33.3	0	33.3	0	16.7	66.7
3	Citrobacter spp. [3]	33.3	33.3	0	0	66.7	0	33.3	100
4	Salmonella sp. [5]	0	80	0	0	0	0	0	20
5	Ps. aeruginosa [3]	66.7	33.3	33.3	0	33.3	0	33.3	0
	No. / % Total resistance	7 (28.0)	14 (56.0)	3 (12.0)	1 (4.0)	7 (28.0)	0 (0.0)	4 (16.0)	11 (44.0)

Table 1. Percentage *in vitro* antibiotic resistance patterns of Gram-negative foodborne bacteria species isolated from fermented food condiments (A).

Amx = amoxicillin; Cot = cotrimoxazole; Nit = nitrofurantoin; Gen = gentamicin; Nal = nalidixic acid; Ofl = ofloxacillin; Aug = augmentin; Tet = tetracycline.

were BDH products.

In vitro antimicrobial inhibition bioassay on the Gram-negative bacteria species by the local spices, essential oils and organic acids was determined by the modification of the agar well-diffusion method of Tagg and McGiven (1971), in which 0.5% agar powder dissolved in appropriate quantity of water was sterilised and added (40°C) to the aqueous extracts of the spices, essential oils and organic acids to avoid spreading of the extracts and acids on the surface of the seeded agar plates. Using sterile cork borers, 6.0 mm wells in diameter were bored into sterile Mueller-Hinton agar plates followed by surface flaming of the agar surfaces. The entire surface of each sterile plate was seeded by streaking with each selected Gram-negative bacterial strains (previously inoculated into sterile tryptone soy broth and incubated at 37°C for 18 to 24 h). 500 µl of the different aqueous extracts and 0.5 gm of the crude extracts of the local spices, essential oils and organic acids were dispensed into each set of agar wells and then incubated un-inverted at 35°C for 24 to 48 h. Zones of inhibition surrounding the wells after incubation were measured and recorded in millimeters (mm) diameter, while wells with no inhibition zones or less than 10.0 mm in diameter were recorded as resistant.

RESULTS

Varied antibiotic resistance patterns recorded among the individual bacterial species and the overall phenotypic antibiotic resistance patterns exhibited by the foodborne indicator bacteria from both sets A and B were as shown in Tables 1 and 2. The foodborne bacteria from the first set (A) of sampled fermented food condiments exhibited lower overall antibiotic resistance towards the test antibiotics- nitrofurantoin (12.0%), augmentin (16.0%), amoxicillin and nalidixic acid (28.0%), with moderate antibiotic resistance exhibited towards tetracvcline (44.0%) and cotrimoxazole (56.0%). All the bacterial isolates were however, susceptible to ofloxacillin. The most resisted antibiotics by the bacterial flora from set B of the sampled fermented food condiments were tetracycline (63.0%), cotrimoxazole (70.3%), nalidixic acid (74.1%), amoxicillin (88.9%) and augmentin (94.4%) but lowest resistance was recorded towards ofloxacillin (1.85%).

In set (A), relatively lower antibiotic resistance patterns were recorded- Enterobacter strains (12.5-50.0%) except in nitrofurantoin and ofloxacillin (0.0%); Klebsiella and Pseudomonas strains (16.7 - 66.7% and 33.3 to 66.7%) respectively except in gentamicin and ofloxacillin, as well as tetracycline in *Pseudomonas* strains (0.0%); Citrobacter strains (33.0 to 100.0%) except towards nitrofurantoin, gentamicin and ofloxacillin (0.0%). Most of the Salmonella strains were susceptible to the test antibiotics but resistance rates of between 20.0 and 80.0% were still recorded among them towards tetracycline and cotrimoxazole respectively (Table 1). Multiple antibiotic resistance (MAR) was however, exhibited by all the bacterial species. Among the bacteria isolated in set B, low to very high phenotypic antibiotic resistance patterns were recorded in Escherichia coli strains (27.3 to 90.9%) except against ofloxacillin (0.0%); Salmonella strains (71.4 to 100.0%) except against gentamicin and ofloxacillin (0.0%); Shigella strains (16.7 except against nitrofurantoin (0.0%); to100.0%) Citrobacter strains (22.2 to 100.0%) and Klebsiella strains (23.0 to 100.0%) except against ofloxacillin (0.0%); Enterobacter aerogenes strains (28.6 to 85.7%) and Morganella morganii except against gentamicin and ofloxacillin (0.0%) (Table 2).

In this study, the foodborne bacteria from the four types of fermented food condiments of plant origin were assayed *in vitro* against some local Nigerian spices to determine the possibility of the spices being used as domestic and industrial, non-chemical preservatives but only the crude extracts of the spices were inhibitory *in vitro* against the foodborne Gram-negative bacteria, while the aqueous extracts of the spices were non-inhibitory or exhibited very minimal inhibitory activities *in vitro*. Meanwhile, the overall *in vitro* susceptibility patterns of crude extracts of the spices indicated that the foodborne bacteria were mostly susceptible to *E. aromatic* and *A. sativum* but mostly resistant to *M. frangrans*, *M. frangrans*, *P. guineensis*, *X. aethiopia*, *Z. officinalis* and *A. melegueta* (Table 3).

		Antibiotic disc (μg ⁻¹)								
S/N	Bacterial isolate	AMX	СОТ	NIT	GEN	NAL	OFL	AUG	TET	
1	<i>E. coli</i> [11]	81.8	72.7	36.4	27.3	63.6	0.0	90.9	45.5	
2	Salmonella spp. [7]	100.0	85.7	85.7	0.0	100.0	0.0	100.0	71.4	
3	Shigella spp. [6]	83.3	16.7	0.0	16.7	50.0	16.7	100.0	50.0	
4	Citrobacter [9]	100.0	77.8	44.4	22.2	88.9	0.0	100.0	77.8	
5	<i>Klebsiella</i> spp. [13]	92.3	84.6	61.5	23.0	76.9	0.0	100.0	84.6	
6	Ent. aerogenes [7	85.7	71.4	28.6	0.0	71.4	0.0	85.7	42.9	
7	Morg. morganii [1]	100	100	100	0.0	100	0.0	100	100	
	No. / % Total resistance	48 (88.9)	38 (70.3)	24 (44.4)	9 (16.7)	40 (74.1)	1 (1.85)	51 (94.4)	34 (63.0)	

Table 2. Percentage *in vitro* antibiotic resistance patterns of Gram-negative food borne bacterial species isolated from fermented food condiments (B).

AMX = Amoxicillin; COT = Cotrimoxazole; NIT = Nitrofurantoin; GEN = gentamicin; NAL = Nalidixic acid; OFL = Ofloxacillin; AUG = Augmentin; TET = Tetracycline. Values in parenthesis are percentage resistance.

Table 3. Percentage *in vitro* inhibition rates of crude extracts of spices against Gram-negative food borne bacterial species isolated from fermented food condiments

		Crude extract of spices (g ml ⁻¹)								
S/N	Lab codes of isolate	ZGO	ALS	XYA	PPG	MYF	MOF	EUA	AMG	
1	Enterobacter [8]	0.0	87.5	0.0	25.0	0.0	0.0	87.5	12.5	
2	Klebsiella [6]	16.7	66.7	16.7	0.0	0.0	0.0	100.0	33.3	
3	Citrobacter [3]	0.0	100.0	0.0	33.3	0.0	0.0	66.7	0.0	
4	Salmonella spp. [5]	0.0	40.0	20.0	0.0	0.0	0.0	100.0	0.0	
5	Pseudomonas [3]	0.0	66.7	0.0	33.3	0.0	0.0	100.0	0.0	
	No. / % Total susceptibility	1 (4.0)	18 (72.0)	5 (20.0)	4 (16.0)	0 (0.0)	0 (0.0)	23 (92.0)	3 (12.0)	

ZGO = Zingiber officinalis; ALS = Allium sativum; XYA = Xylopia aethiopia; PPG = Piper guineensis; MYF = Myristica frangrans, MOF = Monodora frangrans; EUA = Eugenia aromatica; AMG = Aframomum melegueta.

An overall total of 44.4 and 46.3% of the bacterial strains from the fermented food condiments were inhibited by lactic acid and acetic acid respectively (Table 4), while 24.0, 16.7 and 13.0% of the bacteria were minimally inhibited by the essential oils of *E. uniflora*, *A. conyzoides* and *C. albidium* juice respectively (Table 4).

DISCUSSION

Foodborne pathogens have been found to be widely distributed in nature and causing considerable mortality and morbidity in the population (Indu et al., 2006); so, authors have started to focus on the study of survival and growth of foodborne pathogens including some of the indigenous / ethnic fermented foods and beverages of plant origin (Odibo et al., 1992; Ogunshe et al., 2006, 2007, Ogunshe and Olasugba, 2008). As earlier reported, the most commonly encountered bacterial pathogens in the Nigerian fermented food condiments include *Bacillus cereus, E. coli, Salmonella* sp., *Staphylococcus aureus, Vibrio cholerae, Aeromonas, Klebsiella, Campylobacter*

and Shigella spp., Alkaligenes viscolactis, Corynebacterium spp., Enterobacter cloacae, Citrobacter cloacae E. aerogenes, Pseudomonas aeruginosa, Proteus mirabilis and Micrococcus sp. (Kolawole and Okonkwo, 1985; Nwafor, 1985; Barber et al., 1988). However, these Gram-negative foodborne bacteria have been implicated in diseases of public health importance, such as gastroenteritis (Ogunshe and Olasugba, 2008; Jiva et al., 1988; Salyers and Whitt, 1994; Tauxe, 2002), and have become increasingly important as causes of diarrhoeal diseases.

In addition to the health implications of the survival of these food indicator bacteria in the Nigerian food condiments, the issue of their antibiotic multi-resistance is also of great concern. According to Kiessling et al. (2002), antibiotic resistance in foodborne pathogens is a reality and recovery of strains of resistant foodborne pathogens to a variety of antimicrobials has become a major health concern, and similarly, the rise in carriage of antibiotic genes in virtually every species of bacterium has also been consistently documented. In this study, the Gram-negative bacteria species from the fermented food

		О	ganic acids	5	Essential oils			
S/N	Bacterial isolate	LA	AA	AGR	EUG	ANC	CAR	CHR
1	E. coli [11]	54.5	63.6	9.09	9.09	0.0	0.0	0.0
2	Shigella dysentariae [6]	33.3	16.7	16.7	0.0	0.0	0.0	33.3
3	Citrobacter sp. [9]	66.7	66.7	22.2	44.4	11.1	11.1	11.1
4	Salmonella sp. [7]	28.6	28.6	14.3	0.0	14.3	14.3	14.3
5	Klebsiella aerogenes [13]	38.5	46.2	15.4	30.8	15.4	7.7	7.7
6	Ent. aerogenes [7]	28.6	42.9	28.6	57.1	0.0	0.0	28.6
7	Morg. morganii [1]	1	0	0	0	0	0	0
	No. / % Total susceptibility	24 (44.4)	25 (46.3)	9 (16.7)	13 (24.0)	4 (7.4)	3 (5.56)	7 (13.0)

Table 4. Percentage *in vitro* inhibition rates of organic acids and essential oils on Gram-negative bacterial species from fermented condiments.

LA = lactic acid; AA =acetic acid; CHR = Chrsophylum albidium; AGR = Ageratum conyzoides; EUG = Eugenia uniflora; ANC = Anacardium occidentals CAR = carvone.

condiments were also found to exhibit antibiotic resistance, and in addition, almost all the bacterial strains were found to be resistant to multiple antibiotics. This is of considerable medical significance because they may also serve as hosts of antibiotic resistance genes that can be transferred to other bacteria and ultimately humans who consume such foods that harbour them. Search for new antimicrobials is therefore, very important in recent times, considering the escalating levels of antibiotic resistance among the foodborne bacteria.

Several food additives, such as herbs and spices, and which are generally regarded as safe (GRAS), are used delay or inhibit the growth of pathogenic to microorganisms and also to extend the shelf life of food (Zaika, 1988; Arora and Kaur, 1999; Newberne et al., 2000). In this study, crude extracts of garlic (A. sativum) and clove (E. aromatica) exhibited 72.0 and 92.0% in vitro inhibitory activities respectively against the Gramnegative foodborne bacterial species. These results agree with observations of previous researchers in which garlic was reported of having inhibitory activities against E. coli O157:H7, Salmonella typhymurium, E. coli, S. aureus, B. cereus, Bacillus subtilis, mycotoxigenic Aspergillus and Candida albicans. Moderate activities of ginger extract on S. paratyphi and S. enteritidis; onion extract against E. coli, S. paratyphi and S. typhimurium and antibacterial activities of extracts of garlic (A. sativum), nutmeg (M. fragrans), ginger (Z. officinale), onion (Allium cepa) and pepper (Piper nigrum) against different strains of E. coli and Salmonella [Dankert et al., 1979; Sasaki et al., 1979; Arora and Kaur, 1999; Indu et al., 2006).

Contrary to the studies of Indu et al. (2006), extracts of ginger (4.0%) and pepper (16.0%) did not show significant antibacterial activity against the test bacterial strains in the present study; although results obtained indicated that the *in vitro* inhibitory activities of the spices were not bacterial species dependent. Also as observed

in the current study, inhibitory activities were prominent in the crude extracts of the spices, which support the earlier assertions of Shelef (1983) that in general, higher spice levels are required to effect inhibition in foods than in culture media, and although the inhibitory activities of some spices and herbs are documented, the normal amounts added to foods for flavour is not sufficient to completely inhibit microbial growth because the antimicrobial activities vary widely, depending on the type of spice or herb, test medium and microorganisms.

In this study as well, the foodborne bacterial species from the fermented food condiments were found to be partially inhibited in vitro by lactic and acetic acids. Other studies which reported antibacterial potentials / bactericidal effects of organic acids include those of Cherrington et al. (1991), Hirshfield et al. (2003). Plants essential oils and phenolic secondary metabolites possess antimicrobial activities (Prabuseenivasan et al., 2006), and some of these plant extracts are used in the aroma and flavor industry, and are also generally regarded as safe (Shelef, 1983). Based on the result findings of the present study, it was observed that the addition of some commonly obtainable spices such as A. sativum and E. aromatica to fermented condiments can serve as biopreservatives against foodborne bacterial pathogens to a larger extent but the organoleptic results (results not shown) indicated that addition of E. aromatica to the fermented condiments was preferred to A. sativum in supporting dishes such as ewedu, a Nigerian soup prepared from Corchorus olitorius L. and ila, a Nigerian soup prepared from okra (Abelmoschus esculentus (L.) Moench), while the addition of A. sativum in minimal quantities was preferred in main and supporting stews like various vegetable soups such as apon / ogbonno, another Nigerian soup prepared from Irvingia gabonensis, as well as ikokore (a Nigerian indigenous delicacy prepared from bitter yam (Dioscorea dumetorum (Kunth) Pax) and popular among the liebus).

This indicates that *A. sativum* was acceptable in stews, soups and dishes that can mask its smell.

There are several industrial food spices in Nigeria, but they are mostly salt and aroma based; therefore, the popularities of the indigenous condiments such as ogiri, iru / dawadawa, ugba, okpehe etc. cannot be compared with the less desired industrial food spices (Ogunshe et al., 2008a, b). Consumers are so traditionally inclined to their ethnic fermented foods and beverages, such that it is very difficult to accept industrial alternatives to these indigenous foods. About two decades ago, an industrial condiment named dadawa was launched into the Nigerian markets with very captivating advertisements but less than a year afterwards, it was out of market because of its non-acceptance by the general populace, and till date, no industrially-prepared fermented condiment has been prepared for the Nigerian market. Consumers demand convenient, innovative, fresh foods, including new "minimally processed" products, while the food industry will utilise novel technologies to provide the new quality attributes demanded by consumers and ensure the all-important and expected assurance of food safety (Taeymans, 2011). The popularity of the Nigerian plant-based indigenous fermented condiments is specifically due to their organoleptic properties, that is, their aroma, flavour, taste and colour; though, few of the elites do not like these nutrient rich food products because of their pungent smell prior to cooking.

A sensory evaluation panel preferred the uncooked fermented condiments that were preserved with the essential oils in their evaluation for smells (results not shown). Therefore, the addition of such well-accepted local spices as, A. sativum and E. aromatica, essential oils of some spices and some natural organic acids, which have inhibitory characteristics on foodborne indicator bacteria incorporated as non-chemical, adjunct preservatives to domestically or industrially prepared condiments can alleviate the fear of non-safety of such enriched, indigenous fermented food products of plant origin. Addition of salt is the preservative currently employed by the traditional producers but it has not been effective in inhibiting the foodborne pathogens; thus, suggestion of adding crude plant extracts having inhibitory properties to the prepared condiments is imperative.

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