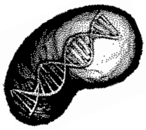

Journal of Microbiology, Biotechnology and Food Sciences

Falegan 2011 1 (2) 187-203

REGULAR ARTICLE

MICROBIOLOGY PROFILE AND BIOCHEMICAL CHARACTERISTICS OF COMMERCIAL 'OGIRI' SAMPLES FROM SOUTH-WESTERN, NIGERIA

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ABSTRACT

The microbiological profile and biochemical properties of commercial samples of `ogiri'(a fermented soup condiment) from five states in Southwest, Nigeria were analysed. The total microbial load in each of the twenty-three (23) samples ranged between 9 x 10^{6} CFUg^{-1} and 1.2 x 10^{9} CFUg^{-1}. The organisms associated with the fermented product were identified as *Bacillus megatarium, Bacillus cereus, Bacillus lichenformis. Bacillus subtilis. Staphylococcus aureus*., *Escherichia coli, Proteus* spp., *Pseudomonas* spp., *Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus casei.* On the basis of starter culture experiments best 'ogiri' product was obtained using strains of *Bacillus subtilis*. In most of the 'ogiri' samples the population of *Bacillus* spp. ranged between 63% and 90% of total microflora; while other organisms constituted the remaining 10 to 37%. The highest percentage 37% and least percentage (9.6%) of contaminants were recorded in samples from Ogun and Oyo State respectively. The pH of samples ranged between 6.0 and 6.6.

The soluble protein content varied from 4.19% - 8.1%. The samples from Ekiti State had the highest percentage of soluble protein.

Keywords: 'Ogiri', *Bacillus subtilis*, condiment, southwest, *Lactobacillus*

INTRODUCTION

Fermented foods are essential parts of diets in all parts of the world, particularly in Africa **(Odunfa, 1985)**. Fruits, vegetable, cereals, root crops, legumes and oil seeds are used in the production of fermented foods. Fermentation is one of the oldest and most economical methods of producing and preserving foods in developing countries **(David and Aderibigbe, 2010)**. In Africa, many proteinaceous oily seeds such as cotton seed (*Gossypium hirsutum)*. African locust bean (*Parkia biglobosa*) and melon seed (*Citrullus vulgaris*) are fermented to produce soup condiments **(Odunfa, 1981a&b)**, which give pleasant aroma to soups and sauces. In many countries especially Nigeria and India where protein/calories malnutrition is a major problem, these condiments serve as good sources of energy, low-cost protein and fatty acids in diets **(Odumodu, 2007)**. Thereby supplement the nutritive quality of the respective diets where they are consumed.

'Ogiri' is one of the condiments consumed in the Eastern and Western parts of Nigeria, especially the `Ijebu' ethnic groups. `Ogiri' is an oily paste produced by fermenting melon seeds (*Citrullus vulgaris*) in the western part of Nigeria. **Oyenuga (1986)** have the composition of melon seed to be: dry weight (88.9%); crude protein (32.6%); ether extract (50.2%); Crude fibre (3.7%); Silica-free ash (3.45%). Minerals (mg/100g) content of shelled melon seed were; calcium (112); phosphorus (1,777); magnesium (578); potassium (538); sodium (5); chlorine (32); vitamins (μ/g): A (30.65); D (11.20) and E (0.25). Melon seed has high protein and low carbohydrate content. *Citrullus vulgaris* is a member of the family

cucurbitaceae **(Alfred, 1986)**. 'Ogiri' is characterized with very strong pungent odour. Among the consumer, there are preferences for 'ogiri' produced from specific locality. The production process being a local art makes the quality of product varies. The fermented products are also stored at ambient temperature (28±2) ^{o}C for varied length of time (days or weeks). The population and types of microorganisms involved during fermentation and storage could have affected the quality of the product.

This research is necessary to improve on the available information on relationship of different microbes involved in fermentation of *Citrullus vulgaris* for the production of 'ogiri' in southwestern part of Nigeria. This also elucidate reasons data for preferences in 'ogiri' from different part of Southwestern Nigeria.

MATERIAL AND METHODS

Source of Samples

'Ogiri' samples were bought from local markets in different towns located in five States namely: Ekiti, Oyo, Ogun, Osun and Ondo (Table 1). All these States are in the Southwest part of Nigeria. The samples were bought and kept in cellophane bags, and stored in a refrigerator at 4°C.

Tab 1 Sources and state of 'ogiri' samples

S/N	Sample code	State	Town	Market	State of samples
1	OIOF	Ondo	Ita-Ogbolu	Oja-Oba	Fresh
2	OIOS	Ondo	Ita-Ogbolu	Oja-Oba	Stale
3	OANF	Ondo	Akure	NEPA-Market	Fresh
4	OANS	Ondo	Akure	NEPA-Market	Stale
5	OOIF	Osun	Osogbo	Igbona	Fresh
6	OOIS	Osun	Osogbo	Igbona	Stale
7	OWOF	Osun	Iwo	Ojori	Fresh
8	OWOS	Osun	Iwo	Ojori	Stale
9	EAMF	Ekiti	Ado-Ekiti	Main Market	Fresh
10	EAMS	Ekiti	Ado-Ekiti	Main Market	Stale
11	OOMF	Oyo	Oyo	Main Market	Fresh
12	OOMS	Oyo	Oyo	Gege	Stale
13	OIGF	Oyo	Ibadan	Oke-Ado	Fresh
14	OBOF	Oyo	Ibadan	Oje	Stale
15	OBJS	Oyo	Ibadan	Itoku	Fresh
16	OATS	Ogun	Abeokuta	Itoku	Stale
17	OATF	Ogun	Abeokuta	Kuto	Fresh
18	OAKF	Ogun	Abeokuta	Kuto	Fresh
19	OAKS	Ogun	Abeokuta	Oke-Aje	Fresh
20	OJOF	Ogun	Ijebu-Ode	Oke-Aje	Fresh
21	OJOS	Ogun	Ijebu-Ode	Idobi	Stale
22	OJDS	Ogun	Ijebu-Ode	Ita-Osun	Stale
23	OEIS	Ogun	Ijebu-Ode		Stale

Legend: Fresh: 3-4 days old product sample; Stale: More than 1 week after production of sample

Microbiological analysis

One gram of ogiri was diluted serially in ten fold dilution blanks and properly mixed with sterile glass rod **(Meynell and Meynell, 1970)**. The 0.1 ml of diluted sample was pipetted into sterile plate and molten sterile agar medium (45°C) was poured **(Harrigan and McCance, 1966)**. The media used were plate count agar (PCA, Biotech), nutrient agar (NA Biotech) and DeMan Rogosa Sharpe agar (MRS, Biotech). The plates were rotated gently to disperse inoculum in medium and allowed to solidify. This was done in triplicates and plates were incubated at 37°C. The MRS plates were also incubated anaerobically at 37°C for isolation of Lactic acid bacteria (LAB).

Characterization of Isolates

Colonies that developed on the plates were grouped on the bases of their cultural characteristics. Pure cultures of all bacterial isolates were obtained by repeated streaking on NA, PCA and MRS plates. Morphological characteristics of each isolate were examined after Gram-staining, spore stain, and motility under the light microscope (X1000) using oil immersion objectives. For the purpose of identification the following biochemical tests were performed on the isolates: gelatin hydrolysis, catalase, indole, nitrate reduction, Voges Proskauer, methyl red and sugar utilization (glucose, lactose, galactose, maltose, mannitol, sorbose,cellobiose, arabinose, raffinose sorbitol, fructose, xylose and sucrose).

Determination of pH

Five grams (5g) of each sample was weighed into a sterile mortar and mashed with pestle. The paste was transferred into a clean beaker and 50ml of distilled water was added. It was mixed thoroughly to form slurry. A standard buffer solution (pH 5.0) was prepared by dissolving a pellet of buffer in 100 ml of distilled water in a volumetric flask and this was

used to standardize the pH meter (Pye Unicam, Model PW 9409). The electrode of the digital pH meter was dipped in the slurry. The pH readings were recorded.

Determination of moisture content

Preweighted clean, dry Petri dishes (W_1); and reweighed (W_2). The samples were dried at 70°C for 72h in type of oven used. After 3 days the dried samples were allowed to cool down in a dessicator and reweighed (W_3). Drying was continued until a constant weight was obtained. The percentage moisture content was calculated thus:

$$\text{Moisture content (\%)} = \frac{\text{loss in weight due to drying x 100}}{\text{Weight of wet samples x 1}}$$

$$\frac{W_2 - W_3 \times 100}{W_2 - W_1 \times 1}$$

Determination of soluble protein

Lowery *et al.*, (1951) method was used. Reagent A: was 2% $NaCO_3$ in 0.1 N NaOH; Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% Na or K tartarate; Reagent C: 100ml of Reagent A + 2 ml of reagent B; Reagent E: 1:2 dilution of John's reagent water. Graded concentrations of BSA in tubes were prepared. Then 0.3 ml of each concentration was to test tubes. 3 ml of reagent C was added, mixed and left for 10 min. Then 0.3ml of reagent E was added, mixed and then left for 30 mins. The optical density at 600nm was read. The graph of OD versus concentration of BSA was obtained and standard curve of BSA. The same was done for unknown substance and the protein concentrations from the standard curve are read off and obtain concentration by multiplying with dilution factor. All readings were obtained in triplicates.

Starter culture fermentation

The bacterial isolates from the 'ogiri' samples were; *Bacillus megatarium, Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Staphylococcus aureus, Eschereichia coli, Proteus* spp., *Pseudomonas* spp., *Bacillus subtilis, Lactobacillus brevis, Lactobacillus plantarum* and *Lactobacillus casei*. They were all used for starter culture fermentation.

The method used by **Odunfa and Adewuyi (1985)** and **Sarkar and Tamang (1994)** was adopted to prepare the inocula. Twenty four hour old cultures of isolates on PCA slant was added to 5ml of sterile distilled water and shaken. The suspension was used as inoculum for 200g of sterile melon seeds in crystallizing dish. The number of organisms per ml of suspension used was estimated to be 3 x 10^6 cell per ml using the Petroff-Hauser counting chamber.

Determination of Organoleptic properties of 'Ogiri' Samples

The sensory attributes of the 'ogiri' produced by starter culture fermentation were evaluated. Ten panelists assessed 5g of each sample and scored parameters including texture, odor and color on a 100 point score sheet adapted from **Sarkar and Tamang (1994)**.

RESULTS AND DISCUSSION

On the basis of cultural, morphological and biochemical characteristics, eight isolates were recovered during aerobic incubation from the twenty-three (23) samples of `ogiri'. The isolate were tentatively identified a strains of *Bacillus megaterium*. *B. cereus, B. licheniformis, B. subtilis, Staphylococcus aureus, Escherichia coli, Proteus* spp. and *Pseudomonas* spp. (Table 2). **Odunfa (1981a)** had reported that members of the *Bacillus subtilis* groups are involved in the fermentation.

Tab 2 Morphological and biochemical characteristics of aerobic bacterial isolates

											Sugar Fermentation						
Isolates	Shapes & arrangement of cells	Grams reaction	Spore staining & location	Motility test	Gelatin hydrolysis	Catalase test	Indole test	Nitrate reduction test	V.P Test	Methyl red	Glucose	Lactose	Maltose	Manitol	Arabinose	Nylose	Probable Identity
OB 1	Rod in chains	+	+ central	+	+	+	-	+	+	-	+	+	-	+	+	-	*B.megaterium*
OB 2	Rods in chains	+	+ subterminals	+	+	+	-	+	+	-	+	-	+	-	-	-	*Bacillus cereus*
OB 3	Rod in chains	+	+ central	+	+	+	-	+	+	-	+	-	-	+	+	+	*B. subtilis*
OB 4	Rod in chains	+	+ central	+	+	+	-	+	+	+	+	+	+	+	+	+	*B. lichenformis*
OB 5	Cocci in	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	*S. aureus*
OB 6	cluster	-	-	+	+	+	+	-	-	+	+	-	-	-	-	+	*E.coli*
OB 7	Rod in chains	-	-	+	+	+	+	-	-	-	+	-	-	-	-	-	*Proteus* spp.
OB 8	Swaming Rod in cluster	-	-	+	+	+	+	-	+	-	+	+	+	+	+	-	*Pseudomonas* spp.

Legend: + - positive, - - negative

The total aerobic microbial load in the ogiri (Tables 3) samples ranged between 9.0 x 10^6 and 1.2 x 10^9 $CFUg^{-1}$. The highest total microbial load was found in sample 3, while the least was observed in sample 19 which had few non-*Bacillus* spp. Lowest level of non-*Bacillus* spp. 9.6% was observed in sample 13, which could be rated as the most hygienic of all the twenty three `ogiri' samples. **Campbell-Platt (1980)** reported 83%-93% of the total isolates in iru to be *Bacillus* spp.*,* while other organisms constituted 7% to 17% of the isolates. When diluted 'ogiri' samples were inoculated on MRS agar and incubated anaerobically three species of *Lactobacillus* were obtained viz: - *Lactobacillus plantarum, L. brevis* and *L. casei* (Table 4). The percentage of the *Bacillus* spp. was highest in sample 13 (19.4%) but least in sample 23 (52.6%). The highest percentage of contaminants was recorded in sample 23, followed by sample 8 (Table 3).

All the samples had pH values ranging from 6.0 to 6.6. In most cases, the stale samples had pH values lower than the fresh samples. This phenomenon might be due to activities of *Lactobacillus* spp. which produce Lactic acid. The reduced pH thereby helped to keep down the population of competing organisms **(Aderiye and Laleye, 2003)**. The moisture content ranged from 30.0% to 41.9%, the moisture content of fresh samples was generally higher than the stale samples (Table 5). This might partly be due to the mode of storage which is either by smoking or drying. These two methods reduce the moisture content of the samples. The soluble protein content varied from 4.9 to 8.1%.

The samples from Ado-Ekiti, Ekiti State had the highest percentage of soluble protein followed by the samples from Ogun State. This variation might due to differences in strains of organisms involved, quality of substrate used and in methods of preparation.

Tab 3 Percentage of aerobic microbial population of isolates in the `ogiri' samples

Ogiri	*Baillus stutilis*	*B. licheniformis*	*B. megaterium*	*B. cereus*	*Staphylococcus aureus*	*Escherichia coil*	*Proteus* spp.	*Pseudomonas* spp.	*Total population x* 10^6
1	10.1	50.6	-	1.3	7.6	10.1	-	-	7.9
2	14.3	73.8	-	1.2	3.6	2.4	4.8	-	8.4
3	-	48.3	-	44.2	6.7	-	-	0.8	12.0
4	-	85	-	-	15.0	-	-	-	4.0
5	58.5	22	-	-	19.5	-	-	-	4.1
6	66.7	-	-	22.2	-	11.1	-	-	3.6
7	-	81.5	-	-	-	-	18.5	-	2.7
8	-	-	60.0	-	10.	-	30.0	-	2.0
9	-	2.17	54.3	32.6	10.9	4.4	-	-	4.6
10	61.5	-	-	-	7.7	-	30.8	-	1.3
11	-	91.7	-	-	-	-	30.8	-	1.2
12	-	88.0	-	-	12	-	-	-	5.0
13	28.8	64.0	-	-	9.6	-	-	-	5.2
14	16.0	-	-	74.0	4.0	6.0	-	-	5.0
15	-	71.4	-	-	-	-	28.6	-	1.4
16	-	90.6	-	-	5.7	3.8	-	-	5.3
17	-	-	-	88.9	11.1	-	-	-	0.9
18	-	81.4	-	-	13.6	-	5.1	-	5.9
19	-	75.0	-	-	25.0	-	-	-	1.6

Tab 4 Biochemical characteristics of anaerobic isolates from `ogiri' samples

Isolates	Ogiri samples	Gram's R xn	Catalase	Oxidase	Motility	Indole production	Gelatin hydrolysis	Glucose	Lactose	Galatose	Maltose	Mannitol	Sorbose	Cellobiose	Arabinose	Raffinose	Sorbitol	Fructose	Xylose	Sucrose	Probable identity	Total load
CR 50	3	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	*Lactobacillus plantarum*	3×10^4
CR 51	11	+	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-	-	*L. brevis*	6×10^4
CR 52	17	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	-	+	-	N0 Rxn	*L. casei*	5×10^5
CR 53	20	+	-	-	-	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	*L. plantarum*	2×10^5

Tab 5 pH, water content and soluble protein of `ogiri' samples

Samples	pH	Moisture content (%)	Soluble protein (%)
1	6.40	41.81	6.3
2	6.00	30.02	6.2
3	6.45	41.26	6.0
4	6.30	32.05	5.7
5	6.43	32.45	4.9
6	6.20	31.06	6.0
7	6.50	41.19	6.1
8	6.09	31.26	6.5
9	6.58	41.20	7.1
10	6.19	32.06	8.2
11	6.60	41.66	4.9
12	6.17	30.25	5.8
13	6.57	41.87	5.5
14	6.58	41.58	6.3
15	6.08	30.35	6.0
16	6.55	41.16	7.4
17	6.57	41.76	8.1
18	6.60	41.01	7.5
19	6.11	33.01	6.3
20	6.59	41.66	6.0
21	6.12	32.05	6.1
22	6.00	33.17	6.3
23	6.20	32.18	6.5

The result of starter culture experiments showed that products of *Bacillus cereus* and *B. licheniformis* fermentation were fairly acceptable (Table 6). The 'ogiri' produced by the *B. subtilis* stains were rated as the best in quality. Samples inoculated with other organisms resulted in production of objectionable odour and products with coarse textures (i.e. non-

fermentation). This result confirms the earlier report by **Odunfa (1985)** that strains of *Bacillus subtilis* are primarily responsible for fermentation to produce `ogiri'. Although other *Bacillus* spp. fermented the substrate, the products were not of good quality. *Bacillus* species might have been introduced by chance inoculation from the air, the calabash tray, the water used for processing the inner lining of wrapping leaves could also be source of the *Bacillus* species. The presence and isolation of pathogenic organisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas* species and *B. cereus* indicated poor hygienic practices during production. These organisms could have been introduced by the personnels, utensils and also from the banana leaves (*Musa sapientum*) used in wrapping the products. However, no fungi were isolated from the commercial samples. The low oxygen tension which was prevalent in the fermenting mash could have been partly responsible for non-isolation of mold. The pH of the fermentation of vegetable protein is not always conducive for fungal growth **(Arora, 2004)**. Thus 'ogiri' can be presumed to be free of mycotoxins, `Ogiri' has not been implicated in any form of mycotoxicity unlike fermented foods of South East Asia that were fermented mainly by moulds **(Ogbadu and Okagbue, 1982)**. *Bacillus species* have been reported to be involved in fermentation of vegetable protein such as African locust bean **(Odunfa, 1981)** and African oil bean **(Obeta, 1983)**. *Bacillus* species are known specificially for their ability to initiate fermentation of both nitrogenous and carbohydrate products **(Aderibigbe, 2010b)**. The presence of these microbes in such fermentation supports their ubiquitous nature. **Campbell-platt (1980)** reported that dominance of *B. subtilis* in a vegetable protein could be attributed to its ability to produce antimicrobial compounds which are active against other microorganisms such as *Lactococcus lactis, Saccharomyces uvarum* and others.

Tab 6 Sensory scores of starter-culture fermented ogiri samples

Attributes	**Organisms used in starter culture experiment**										
	BM	BC	BL	BS	SA	EC	PR	PS	LP	LB	LC
Flavour (50)	20.0	20.0	39.0	45.0	20.4	25.0	18.1	15.7	16.2	15.6	20.0
Body and texture (45)	19.0	24.1	35.0	40.2	10.3	18.0	15.0	10.0	12.4	11.6	13.8
Colour (5)	1.0	2.0	3.0	4.5	3.0	2.6	3.0	2.0	2.5	3.0	3.2
Total Score (100)	40.0	46.1	77.5	89.7	33.7	45.6	36.3	18.7	31.1	30.2	29.0

Legend

BM *Bacillus megaterium*
BC *Bacillus cereus*
BL *Bacillus licheniformis*
BS *Bacillus subtilis*
EC *Escherichia coli*
SA *Staphylococus aureus*
PR *Proteus* spp.
PS *Pseudomonas* spp.
LP *Lactobacillus planetarium*
LB *Lactobacillus brevus*
LC *Lactobacillus casei*

CONCLUSION

The research work revealed *Bacillus* species as the major microbes responsible for the fermentation of *Citrullus vulgaris* for the production of 'ogiri' as soup condiment. Other organisms which were associated with the fermentation were contaminants, which resulted to the traditional methods of producing 'ogiri'. Samples from Ogun State were the poorest in term of hygene while the samples from Ekiti State had the highest percentage of soluble protein. 'Ogiri' from Ekiti State is best recommended for consumption. The possible solution to this varied standard in the quality of 'ogiri' samples from the market of Southwestern Nigeria is to develop a starter culture of *Bacillus subtilis* strains which has been proved to have the best fermenting quality according to the result of sensory evaluation which was 89.7%. The processing conditions for the fermentation of *Citrullus vulgaris* must be standardized i.e. duration, temperature and methods of aeration during fermentation of the substrate must be standardized. Further research on better understanding of extracellular secretion of enzymes and condition for optimum production of the enzymes by the fermenting microbes will be thoroughly investigated.

Acknowledgments: I sincerely appreciate the assistance of Prof (Mrs.) E.Y. Aderibigbe and Mr. A.T. Odeyemi of the Department of Microbiology, Ekiti State University, Ado-Ekiti, for their technical contributions during the course of this research.

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