PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 11 (3): 231-236, 2012
ISSN 1680-5194

Microbial, Nutritional and Sensory Evaluation of Traditional Sundried Okra (Orunla) in Selected Markets in South-Western Nigeria

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Abstract: The aim of this study was to investigate the microbial load and evaluate the nutritional content and sensory property of traditional sun dried okra ("orunla" - *Yoruba*, Nigeria). Samples were purchased from four different markets at different time in South-western Nigeria. Microorganisms present in the samples were analyzed on nutrient agar, potato dextrose agar and malt extract. Proximate evaluation of the sun dried okra was also carried out to determine the protein, ascorbic acid, ash and crude fibre contents. Nutritional evaluation revealed considerable amount of measured nutrient without significant difference ($p>0.05$) inspite of the time and place of purchase. Sensory evaluation of the samples showed that they were generally acceptable to a taste panel. Microbial study identified *Staphylococcus aureus*, *Aspergillus niger*, *Aspergills tamari*, *Fusarium compactum*, *Rhizopus nigricans* and *Bacillus lichiniforms* as some of the microorganisms present in the sun-dried vegetable. This study thus, suggested that sun-dried okra could be consumed but when processed under control microbial condition.

Key words: Okra, nutrients, microbial load, taste panel

INTRODUCTION

One of the most important vegetable crops in Nigeria contributing substantially to the Nigerian diet as a main constituent of soup and stew is okra (Bobbi, 1998). Okra, *Abelmoschus esculentus* belongs to mallow family (Bobbi, 1998) and is known by many names like lady fingers, gambo, okra, ila etc. Okra is a fruit vegetable rich in energy and also contain protein, carbohydrate, calcium, thiamine, iron, beta-carotene, riboflavin, niacin and ascorbic acid (Adetuyi *et al*., 2011). Despite all the important nutritional characteristics, okra is a perishable commodity that suffers heavy losses in terms of quality and quantity during post-harvesting handling processes. Because it is normally available in large quantities during rainy season and unavailable during dry season, there is therefore the need to preserve them during the season of plenty. The method commonly adopted in okra preservation includes canning, freezing and dehydration. In Nigeria, dehydration has been found suitable, as this method reduces weight and volume by significant amount. Dehydration involves sun-drying. This method of preservation has been adopted in many part of Nigeria, especially the south-western part. The okra is diced and dried. The dried-diced okra is then ground into powder and use for soup making. However, despite its wide acceptability, little is known regarding the nutrient content of the powdered, sun-dried okra. The objective of this work is thus to evaluate the nutritional and sensory characteristics as well as the microbial load of the powdered, sun-dried okra obtained in south western Nigeria.

MATERIALS AND METHODS

Sun-dried Okra: The sun-dried okra used were purchased from Oja-Oba market, Akure, Oje market, Ibadan, Bodija market, Ibadan and Tejuosho market, Lagos, all in South-western Nigeria, in the months of December, early and late April and middle of May. The sun-dried okra samples were grouped after purchase based on the time and place of purchase.

Physiochemical analysis: The protein, crude fibre, ash, moisture and ascorbic contents were determined according to the method of the Association of Official Analytical Chemist (1980). The crude protein content was calculated by multiplying the total nitrogen content by factor 6.25. The reduced ascorbic acids were assayed using the method of the Association of Official Analytical Chemist (1994). In this procedure, ascorbic acid was oxidized with 2,6-dichlorophenol (DPIP) dye at pH 6.8 and the absorbance reading was taken using UV spectrophotometer Cecil series 2 at 620 nm wavelength.

Sensory evaluation: Sensory tests were carried out on colour, flavour, taste and aroma by a panel of 9 assessors using a multiple comparison difference

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analysis (Larmond, 1977) on a hedonic scale. The sensory evaluation results were subjected to statistical analysis using Duncan's multiple procedure to determine which sample differed significantly to another.

Microbial evaluation

Media preparation: The media prepared for the analysis include:

Nutrient agar: 28 g of nutrient agar powder was weighed into a conical flask and 1 litre of distilled water was added and covered with cotton wool and aluminum foil. It was dissolved by heating and autoclaved at 121°C for 15 min (151 bs/sq inch). The media was allowed to cool to about 45°C and 15-20 ml of it was dispensed into petridishes (Akujobi and Njoku, 2010).

Potato Dextrose Agar (PDA): 39 g of PDA powder was weighed into a conical flask. The same procedure used for nutrient Agar was employed and the media was dispensed into petridishes in aliquot and allowed to solidify (Ayolabi and Fagade, 2010).

Malt extract agar: 8 g of malt extract was weighed into a conical flask containing 5 g of mycological peptone, and 15 g of Agar. 1 litre of distilled water was added and covered with cotton wool. The pH was adjusted to 5.4 and the flask autoclaved at 1.05 kg/cm^3 for 1 min (Solomon *et al*., 1999).

Total viable count: 1 g of pulverized sundried okra was weighed aseptically into 10 ml of sterile distilled water in a test-tube and a serial dilution method was used by pipetting 1 ml of the dilution into 9 ml of sterile distilled water; dilution was prepared up to 10-5 dilutions. The 10-3 and 10-5 dilutions were plated by transferring 1 ml of the dilution into separate petridish and 20 ml of sterile cool molten nutrient agar was added and mixed by swirling the plate (Bridson, 2006). The plates were incubated at 37°C for 24 hrs and were examined for growth and colonies, counted and recorded as total bacterial count. The morphology of the different colonies was recorded and a pure culture was obtained using the streak method.

Yeast and mould count: 1 g of each pulverized sundried okra samples was weighed and 10 ml of sterile distilled water was added into each test-tube. A serial dilution was carried out and 20 ml of sterile cool molten malt extract agar was added. This was mixed by swirling the plate to obtain homogeneity. This was then incubated at 25-30°C for 24-48 hrs. The fungal growth and colonies was obtained and recorded. The pure culture was obtained by streak method (Himelbloom and Crapo, 1998).

Coliform count: Coliform load of each of the sundried okra was estimated using the plate count method (APHA, 1992). The same procedure of serial dilution above was employed and eosin methylene blue agar (Bridson, 2006) was added and were incubated at 37°C for 48 hrs and the plates were examined for total coliform.

Identification of bacterial and fungal isolates: The biochemical test for identification of the bacterial isolates include:

Gram staining: A smear of bacterial isolates was made with a drop of distilled water and sterile incubating loop on a clean grease free slide. The smear was air-dried and heat fixed. It was then flooded with crystal violet solution and allowed to stand for seconds, and was later washed off and iodine was flooded on the slide which was later drained and washed off with water. 95% alcohol was used to flood the slide to avoid decolorization. It was counter stained with safaranine and left for another 60 seconds before it was washed off with water. The slide was then air-dried and examined under the oil immersion objective lens of light microscope. The gram positive bacteria stained purple while the gram negative bacteria stained red (Fawole and Oso, 1988).

Slide coagulase test: A smooth emulsion of the organism was made with normal saline. The emulsion was mixed with reactive human plasma. The appearance of agglutination and clumping indicates a positive test while Streptococcus was used for the negative control (HPA, 2007).

Catalase test: A portion of the isolated culture was emulsified on a clean slide and 3% hydrogen peroxide was added. Effervescence indicated the presence of the enzyme catalase. *Staphylococus aureus* served as the positive control while *Klebsiella pneumoniae* served as the negative control (Cheesbrough, 1989).

Indole production test: The medium used contained 0.5% NaCl and tryptone. It was sterilized at 121°C for 15 min and incubated with each of the isolated species. A stripe of filter paper impregnated with oxalic acid was kept above the medium by the use of cotton plug. The test-tube was incubated again at 37°C for 24 hrs. *Escherichia coli* served as the positive indole control test which gave a pink colouration of the paper strip and *Staphylococus aureus* was the negative control which gave no colour change (Cheesbrough, 1989).

Motility test: The hanging drop method was used. A drop of the emulsified incubated bacteria culture was placed on a cover slip. A thin film of vaseline was applied around the edges of the cover slip. The slide was then inverted on the cover slip. The culture was in

the form of hanging drop and it was observed under x40 objective lens microscope. Motility was observed by directional movement of the motile cells for *Samlonella* while *Shigella* was non-motile (HPA, 2007).

Voges proskaeur test: A test-tube containing 2 ml of glucose phosphate peptone water was incubated with the bacterial isolate for 48 hrs at 37°C. 0.6 ml creatine and 3 ml of NaOH was added and mixed. It was allowed to stand for 1 hr and a slow colour change from pink to red was observed (Ljutov, 1963). *Klebsiella pneumoniae* was for the positive test and *Escherichia coli* for the negative control.

Oxidase test: Several spots were stained on a filter paper with drops of oxidase reagent and each isolate streaked on it. A positive result gave a sharp purple colour while a negative result remained brown after a few seconds.

Urease test: Christensen's urea agar medium was employed for this test.

Citrate utilization test: A plate of Simmonss citrate culture agar was inoculated by streaking pure culture on bacteria isolate on a medium for 48 hrs at 37°C and a change in colour of the agar from green to blue indicated citrate utilization (Cheesbrough, 1989).

Identification of fungal isolates: Pure culture of fungal isolates was examined under the microscope. The microscope study of each colony was carried out by wet-mount method. A flamed incubating needle was used to pierce a piece of the colony and was transferred in a drop of distilled water to a clean glass slide. It was covered with cover slip and examined under the microscope using x40 objective lens. Another mount was also made which was stained with cotton plug, in lactophenol on a clean slide and KOH was used as a clearing agent as described by Cheesbrough (1989). The somatic and reproductive structures were observed. The relationship of the hyphe, size and shape of the reproductive structures were also noted. It was identified according to Davis's Medically Important Fungi: A Guide to Identification (1976).

RESULTS AND DISCUSSION

The proximate composition of the various samples of sun dried okra purchased from the four different markets and at different times is shown in Table 1. The protein content of the samples range from 18.38-24.07 with samples 133 and 143 from Bodija market in Ibadan having the least protein content of 19.70 and 18.38 respectively while sample 131 bought from Akure market in early April have the highest protein content of 24.07. In general, there was no significant difference ($p>0.05$) in the overall protein content though quite low. The low protein content of the okra samples is in agreement with the report that the protein content of okra is generally low (Bryant *et al*., 1988), however, sun-dried okra still contains trace amount of protein because of reduced water content. This low protein content (sun-dried Okra) is attributed to the difference in geographical, edaphic and climatic factors (Ologhobo and Fatuga, 1993) in the different markets. The method of preservation and structure of the markets could also be a significant factor. The moisture content ranged from 9.49-15.20. Apart from samples 121, 122, 123 and 124 from each markets bought in the month of December that have the lowest moisture content (4.49, 9.75, 10.05 and 11.92 respectively), it was observed that the samples bought in the months of April and May were significantly higher in moisture content ($p<0.05$) when compared to samples 121-124. The difference in the moisture content of sundried okra of April and May could have

Table 1: Proximate composition of traditional sun-dried Okra

Sample	Month of purchase	Market of purchase	Protein content	Moisture content	Ash content	Vit. C content (mg)	Crude fibre (%)
121	December	Akure	20.14	9.49	0.91	20.00	8.18
122	E.April	Oje	21.76	9.75	0.83	17.60	9.70
123	L.April	Bodija	22.72	10.05	0.94	14.70	13.10
124	May	Tejuosho	21.58	11.92	0.96	17.64	11.75
131	December	Akure	24.07	13.99	0.93	20.58	8.35
132	E.April	Oje	20.52	14.41	1.08	17.64	8.35
133	L.April	Bodija	19.70	12.40	0.99	20.58	13.70
134	May	Tejuosho	20.13	12.23	0.90	17.64	8.35
141	December	Akure	20.13	14.78	0.95	14.70	13.23
142	E.April	Oje	20.57	14.83	0.93	17.64	8.10
143	L.April	Bodija	18.38	14.57	1.02	20.58	13.28
144	May	Tejuosho	20.13	12.23	0.92	17.64	11.80
151	December	Akure	21.44	15.00	0.93	14.70	8.10
152	E.April	Oje	20.26	15.20	0.95	17.64	8.10
153	L.April	Bodija	20.26	15.20	0.95	14.70	11.35
154	May	Tejuosho	20.61	13.99	0.93	17.64	13.70

E.April = Early April, L.April= Late April

been influenced by the season of purchase, since December period is usually characterized with absence of rainfall (dry season) thus, the moisture content of the okra is reduced considerably unlike the month of April and May where rainfall is experienced, enhancing increase in moisture content of the sun-dried okra.

The ash content was relatively low when compared to fresh okra and other legumes (Ene-obong and Carnovale, 1992). The lowest ash value was obtained for sample 122 (0.83) and the highest for sample 132 (1.08) both purchased from Oje market Ibadan and yet there was no significant difference ($p>0.05$) in the ash content. An appreciable amount of minerals can be obtained from the sun-dried okra which can play major role in many biochemical reactions where they function as co-enzyme and aids physiological functioning of major metabolic processes in the body. Also there was no significant difference ($p>0.05$) in the overall ascorbic acid concentration. It is generally known that vitamin C is a water-soluble vitamin, however, its instability may not allow for long storage by drying especially in air. The high concentration of ascorbic acid in Akure sample is as result of reduction and total elimination of moisture by open drying. The high crude fibre content from Tejuosho market, Lagos in early April could also be attributed to the environmental, climatic and edaphic factors while the low fibre content for samples from Akure could be due to the use of snapping and matured fresh okra for processing of sun-dried okra.

Table 2 showed the mean values for multiple comparison analysis for selected quality attributes of sun-dried okra samples from the four respective markets. The panel preferred the sun-dried okra of Akure and Oje markets bought in the month of December as they were the same in colour than other ones of early and late April and middle May. This preference is attributed to the time of purchase and market structure which impacted on the quality of the sun-dried okra. There was also no significant difference in the aroma ($p>0.05$) with respect to time for samples bought in December, early and late April from Akure, Oje and Bodija markets.

There was no variation in the score of acceptability by the panelist across the markets. This observed trend in the score of acceptability by the panelist conform with the report of Olorunda and Tung (1997) who observed only minor differences in the textural acceptability or behaviour of fresh and frozen okra dispersions and concluded that soup made from frozen okra would not be inferior in viscometric properties to that made from fresh okra. The taste attribute gave no significant difference in flavor ($p>0.05$) between the sun-dried okra samples when compared with each other. In terms of overall acceptability, none of the sun-dried okra samples showed any level of significance ($p>0.05$). The taste attributes by the panelist may be due to susceptibility to chilling injury (Bramlage and Meir, 1990) by frozen okra. Thus it is suspected that okra would be better preserved by sun drying.

The results of the microbial load, coliform counts, yeast and mould counts obtained are presented in Table 3. It was observed that the total bacterial count ranged from 1.0×10^5 to 22×10^5 cfu/g in which sample 122 bought from Oje market in late April have the highest mean count of 22×10^5 cfu/g while sample bought from Akure in early April have the least mean bacterial count of 1.0×10^5 cfu/g. Some of the microorganisms isolated include *Stasphylococus aureus*, *Aspergillus niger*, *Aspergillus tamari, Fusarium compactum*, *Rhizopus nigricans*, *Bacillus lichenifomms*.

Table 2: Sensory attributes of traditional sun-dried Okra from selected markets in South-west Nigeria

Characteristics	Time of purchase	Akure	Oje market	Bodija market	Tejuosho market
Colour	December	7.33^{a}	7.00^{a}	2.89^{c}	4.67^{a}
	E.April	6.33^{ab}	5.89^{ab}	4.11^{ab}	4.56^{a}
	L.April	5.67^{b}	5.00^{a}	4.56^{a}	5.56^{a}
	May	6.78^{a}	6.00^{ab}	3.44^{bc}	4.56^{a}
Aroma	December	6.78^{a}	6.89^{a}	4.44^{a}	5.11^{a}
	E.April	6.67^{a}	5.78^{b}	5.11^{a}	5.00^{a}
	L.April	6.11^{a}	5.56^{b}	5.33^{a}	4.56^{a}
	May	6.78^{a}	6.44^{ab}	4.22^{a}	4.89^{a}
Taste	December	6.78^{a}	6.78^{a}	3.44^{a}	4.44^{a}
	E.April	7.22^{a}	6.22^{a}	3.56^{a}	4.22^{a}
	L.April	7.00^{a}	6.67^{a}	3.78^{a}	4.33^{a}
	May	7.22^{a}	6.56^{a}	3.56^{a}	3.88^{a}
Textural acceptability	December	7.78^{a}	7.11^{a}	3.44^{a}	4.44^{a}
	E.April	7.67^{a}	7.00^{ab}	3.56^{a}	4.22^{a}
	L.April	7.89^{a}	7.33^{a}	3.78^{a}	4.33^{a}
	May	7.44^{a}	6.67^{a}	3.56^{a}	3.88^{a}
Overall acceptability	December	7.56^{a}	7.00^{a}	4.11^{a}	4.22^{a}
	E.April	7.56^{a}	6.78^{a}	3.78^{a}	4.22^{a}
	L.April	7.22^{a}	6.78^{a}	3.67^{a}	3.78^{a}
	May	7.56^{a}	6.89^{a}	3.89^{a}	4.11^{a}

Means within a column and row for each attributes followed by the same letter are not significantly different at $\alpha = 0.05$. E.April = Early April, L.April = Late April

Table 3: Microbial evaluation of sun dried Okra from South west, Nigeria

Sample	MOP	MAOP	TVC (cfgu^{-1})	CC (cfgu^{-1})	YAMC (cfgu^{-1})	Micro-organisms isiolated
121	December	Akure	$1.8x10^5$	$18.0x10^5$	$0.5x10^5$	*Staphylococcus aureus, Aspergillus niger, Aspergillus tamari, Fusarium compactium*
122	E.April	Oje	$22.0x10^5$	$30.0x10^5$	$0.6x10^5$	*Rhizopus nigricans, Fusarium oxysporum, Fusarium compactum, Cladosphorium sphaerosphorum*
123	L.April	Bodija	$12.0x10^5$	$28.0x10^5$	$0.8x10^5$	*Cladosphorum sphaerosphorum, Penicillium oxalicum*
124	May	Tejuosho	$12.0x10^5$	$25.0x10^5$	$0.8x10^5$	*Klebsiella aerogens, Fusarium compactum*
131	December	Akure	$1.0x10^5$	$12.0x10^5$	$0.9x10^5$	*Staphylococcus aureus, Proteus morganii, Streptococcus faecalis, Klebsiella areogenes*
132	E.April	Oje	$1.8x10^5$	$27.5x10^5$	$0.4x10^5$	*Micrococcus sp Streptococcus faecium, Streptococcus lactis*
133	L.April	Bodija	$11.0x10^5$	$24.0x10^5$	$0.6x10^5$	*Micrococcus sp Pseudomonas fluorescen, Serralta*
134	May	Tejuosho	$18.0x10^5$	$30.0x10^5$	$1.0x10^5$	*Micrococcus sp Bacillus cereus*
141	December	Akure	$1.8x10^5$	$18.0x10^5$	$0.6x10^5$	*Aerobacter aerogens, Bacillus cereus*
142	E.April	Oje	$22.0x10^5$	$30.0x10^5$	$0.6x10^5$	*Bacillus farmus, Proteus vulgarius, Pseudomonas aureginsia, Escherichia coli, Klebsiella aerogenes*
143	L.April	Bodija	$12.0x10^5$	$28.0x10^5$	$0.8x10^5$	*Proteus vulgarius, Pseudomonas fluorescens*
144	May	Tejuosho	$12.0x10^5$	$25.0x10^5$	$0.8x10^5$	*Staphylococcus aureus, Bacillus*
151	December	Akure	$1.0x10^5$	$12.0x10^5$	$0.9x10^5$	*Staphylococcus aureus, Proteus morganii, Streptoloccus faecalis, Klebsiella aerogenes*
152	E.April	Oje	$1.8x10^5$	$27.5x10^5$	$0.4x10^5$	*Micrococcus sp Sterptococcus faecium, Steptolococcus lactis*
153	L.April	Bodija	$11.0x10^5$	$24.0x10^5$	$0.6x10^5$	*Micrococcus sp, Pseudomonas fluorescen, serratia marcescens*
154	May	Tejuosho	$18.0x10^5$	$30.0x10^5$	$1.0x10^5$	*Micrococcus sp Acillus cereus*

E.April = Early April, L.April= Late April; MOP = Month of Purchase; MAOP = Market of Purchase; TVC = Total Viable Count (cfgu^{-1}); CC = Coliform Count (cfgu^{-1}); YAMC = Yeast and Mould Count (cfgu^{-1})

The trend of the total bacterial count obtained was significantly high thus indicating a large extent of contamination of the sun-dried okra which is liable to predispose consumers to some physiological effects. *Staphylococcus aureus* enters food during processing from the handler, grows and produces enterotoxins which are relatively stable to heat (Prescott *et al*., 1999). According to Eklund *et al*. (2004), *Staphylococcus aureus* usually exceeds 10^2 cfu/g as a result of gross mishandling of food substance. It can be observed from the result that the contamination of the sun-dried okra by the microbes vary with location as reflected in the level of contamination of the sun-dried okra bought from Akure, which is lower when compared to others. Thus the methods of preservation and handling have a great influence on the level of contamination. Other bacterial isolates and fungal isolates such as *Proteus* sp., *Aspergillus* sp., *Penicillium* sp., etc obtained are derived from external sources like soil, roof and air. The fungal species of *Aspergillus* is responsible for aspergillosis, and also the production of afflation by *A. flavis*, which is carcinogenic and is responsible for respiratory infections in immuno-compromised patients, hypersensitivity or allergic reactions (Prescott *et al*., 1996).

The results obtained from this study showed that the sun-dried okra samples from Tejuosho market in Lagos bought at different period of time and samples from Oje market bought in December have the highest microbial counts while samples from Akure market bought in December and early April have the lowest microbial count, this is attributed to the processing technique adopted and the types of market (whether 'open or close') and the potentiality of harboring micro-organisms in the okra samples as a result of the amount of moisture that is present. Both the coliform count and the yeast and mould count were found to also be relatively high among the sun-dried okra samples. The results of the bacterial isolates obtained was found to be significantly higher (105) when compared to the international microbiological standards/recommended limits of bacterial contaminates for food and food sources (10^1 to 10^2 cfu/g) (Gunasekaran, 1995) thus signifying a high level of contamination of the sun-dried okra samples which could be attributed to the method of preservation, method of handling, type of market and level of exposure.

Conclusion: The time and place of production of sun-dried okra do not significantly affect its nutrient composition; therefore, it can be purchased from anywhere, at anytime for use in soup making. However, the level of contamination by microorganism of the sun-dried okra is high. Thus, caution and protective measures must be taking before consumption.

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