Microbiological And Proximate Analysis of Dried Okra Sold Within Owerri Metropolis IMO State, Nigeria

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Abstract - 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. This study investigated the microbiological and proximate qualities of dried okra sold within Owerri Metropolis, Imo State, Nigeria. Three market samples and one laboratory-prepared control were analyzed. Proximate analysis revealed moisture content ranging from 12.94–13.24%, crude protein 24.26–24.60%, crude fat 2.29–2.42%, crude fibre 18.50–18.66%, crude ash 5.18–5.30%, and carbohydrate 35.98–36.24%. Microbiological assessment showed total viable bacterial counts of 8.0 × 10³ to 5.8 × 10⁴ cfu/g, with no coliforms detected. Fungal counts ranged from 4.0 × 10³ to 1.2 × 10⁴ cfu/g. The predominant bacterial isolates were* Pseudomonas, Staphylococcus, Micrococcus, Corynebacterium, *and* Bacillus *species, while fungal isolates comprised of* Aspergillus, Mucor, Fusarium, Penicillium, *and* Rhizopus *species. The laboratory dried control sample had significantly lower microbial loads than the market samples. The findings reveal that despite its nutritional value, dried okra sold in markets may be contaminated by pathogenic microorganisms due to poor handling and drying methods. Enhanced hygienic practices, improved drying techniques, and proper packaging are recommended to ensure safety and quality.

Keywords: Dried Okra, Proximate Analysis, Microbial Quality, Food Safety.

I. INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) also known as lady's finger, is an important vegetable crop under tropical and subtropical conditions and is among the most important vegetable crops in Nigeria contributing substantially to the Nigerian diet as a main constituent of soup and stew is okra. It is a fruit vegetable that is rich in energy and also contains protein, carbohydrate, calcium, thiamine, iron, beta-carotene, riboflavin, niacin and ascorbic acid (Adetuyi *et al.,* 2011).

Abelmoschus esculentus (Okra) belongs to the family Malvaceae. It is highly grown as a vegetable crop in the tropics and sub-tropics and also in the warmer temperate areas. In Nigeria, it is known as "ila" in "Yoruba", "kubewa" in "Hausa" and "okúrú" in "Igbo" land (Draper, 2007). Okra is a prominent fruit and leafy vegetable grown for domestic consumption of the highly nutritious immature leaves and fruits in Nigeria (Olabode *et al.,* 2010). Most of the cultivation is done during the dry season either as a follow up crop to early maize or in fadama cultivation, especially in the South West Nigeria (Mays *et al.,* 2007).

The nutritional composition of ripe seed of okra has been reported to contain 20% edible oil, 2.0% protein, vitamin A (0.2mg/100g), vitamin C (25mg/100g), high calcium content (92mg/100mg) (Mays *et al.,* 2007). The edible portion of the fruit on average contains approximately 86.1% moisture, 2.2% protein, 9.7% carbohydrate, 1.0% fibre, 0.2% fat and 0.9% ash. Okra is a good source of Vitamin A and B and also contains Vitamin C and Minerals especially iodine. Vitamin A is usually retained during drying; however, because vitamin A is light sensitive, food containing it should be stored in dark places. Vitamin C is destroyed by exposure to heat, although pretreatment of foods before drying increases the Vitamin C content (Kolawole *et al.,* 2011).

Nutritionally, dried okra contains; moisture (13.33%), crude protein (24.16%), crude fat (2.29%), crude fiber (18.96%), crude ash (5.30%), carbohydrate (36.16%), vitamin A (0.2mg/100g), vitamin C (25mg/100g), calcium (92mg/100mg), iron (18.50mg/100g), potassium (122.59mg/100g), zinc (3.83mg/100g), phosphorus (25.62mg/100g), sodium (5.4mg/100g) (Adernipekun and Oyetunji, 2010). Fresh okra contains; moisture (39.10%), crude protein (24.16%), crude fat (2.29%), crude fiber (13.69%), crude ash (4.44%), carbohydrate (16.27%), vitamin A (0.6mg/100g), vitamin C (25mg/100g), calcium (111.11mg/100mg), iron (13.50mg/100g), potassium (104.59mg/100g), zinc (5.83mg/100g), phosphorus (22.62mg/100g), sodium (4.4mg/100g) (Gemede *et al.,* 2015).

Medicinally, okra is rich in vitamins which help to support healthy immune function, decrease risk of heart problems, stroke, reduce brain inflammation, lowers blood cholesterol and prevents blot clots. Similarly, okra contains beneficial antioxidants such as polyphenols, which help protect the brain from symptoms of aging and improve cognition, learning and memory. Okra is beneficial for pregnant women because it contains folate (vitamin B9) which helps to lower the risk of a neural tube defect, which affects the brain and spine of a developing foetus (Arise *et al.,* 2012).

Vegetables are metabolically active, perishable fresh commodities that have shorter shelf-life. Vegetables such as okra contain living tissues which make them prone is decay and eventually spoilage. However, post-harvest treatments are employed to minimize the loss of fresh vegetables as well as maintain the quality, thereby increasing the shelf-life. Some of the post-harvest treatments include; chemical treatments (such as; hydrogen peroxide, chlorine-based solutions, nitric oxide etc) physical treatments (such as; heat treatments, edible coating and irradiation) and gaseous treatments (such as; ozonation, modified atmospheric packaging etc). Due to unavailability of some of these post-harvest treatment methods as well as the exposure of humans to chemical agents, the use of drying is most preferred in the preservation of some vegetables in some localities.

Drying is the simplest technology for preserving green leafy vegetable, especially when they are abundantly available. Drying of food materials has advantageous benefit such as; extending the shelf life, inhibiting microbial growth, less transport and storage cost. Some of these techniques include the use of different solar drying system. Vegetable drying is generally done either for preserving the perishable raw commodity against deterioration or to reduce the cost of packaging, handling, storing and transporting. The aim of this study was to determine the proximate and microbiological quality of dried okra sold within Owerri metropolis.

II. MATERIALS AND METHODS

Collection of Samples

Three (3) okra samples were purchased from three (3) different vendors at Nkwo-Ukwu Ihiagwa market, Owerri-West L.G.A, Imo State. One sample of dried okra was prepared which served as the control. The samples were labeled as “A”, “B” and “C” and were taken to the laboratory for proximate and nutritional quality analysis.

Microbiological Analysis

The method described by Arise *et al.* (2012) was adopted with slight modifications in the isolation of microorganisms associated with the dried okra. Ten grams (10g) each of the dried okra samples were weighed and added to 90 ml of sterile water in clean sterile 250 ml beakers. The beakers were allowed for 30 minutes with stirring at intervals. The samples were serially diluted in ten folds. The serial dilution method as described by Cheesbrough (2010) was adopted. Fifteen (4) sets of sterile test tubes each containing six (6) test tubes were arranged on test tube racks and were labelled appropriately. Nine milliliters (9ml) of sterile water was added to each of the test tubes. One milliliter (1ml) of each of the urine was transferred to the appropriately labelled first test tubes (10^{-1}) containing nine milliliters of sterile water using different sterile Pasteur pipette. The test tubes were shaken and one milliliter was transferred to the second test tubes labelled 10^{-2}. This procedure was continued to the last test tubes labelled 10^{-6} and one milliliter (1ml) was discarded from each of the last test tubes.

Inoculation of Samples

The spread plate technique as described by Cheesbrough (2010) was used in the inoculation of the plates. 0.1 milliliter aliquot of the serially diluted samples was pipette from each of the test tubes labeled 10^{-3} and was dropped onto the different media in the plates. A sterile bent glass rod was used to spread the aliquot evenly on the media (nutrient agar, eosin methylene blue agar and Sabouraud’s dextrose agar). The plates were labeled accordingly. The inoculated plates were inverted and incubated in the incubator at a temperature of 37 ^{0}C for 24 hours except Sabouraud’s dextrose agar plates that were incubated at room temperature (28^{0}C) for three days.

2.4 Microbial Plate Count

After the incubation of the different plates, the different colonies formed on the media were counted using the digital colony counter. The total population of the colonies was expressed as colony forming unit per gram (cfu/g).

2.5 Purification and Preservation of Isolates

After the various colony counts, bacterial isolates were pick with a wire loop based on their morphological appearances. The picked colonies were sub cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24h at 37 ^{0}C. After incubation pure cultures were stored in McCartney bottle in a refrigerator at 4 ^{0}C. Fungal isolates were sub cultured onto freshly prepared Sabouraud's dextrose medium.

Colonial Morphology Identification

The method described by Cheesbrough (2010) was adopted in the colonial morphology identification. Presumptive identification of the colonies was done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation. Colonies with characteristic metallic sheen on EMB agar and lactose fermenters on MacConkey agar were noted. The colonies were preserved in sterile agar slants in test tubes. Purified colonies were further characterized using Gram stain and biochemical tests.The Gram staining techniques described by Cheesbrough (2010) was adopted.

Biochemical Tests with the Bacterial Isolates

All the biochemical tests were carried out following the method described by Cheesbrough (2010). Catalase Test, Oxidase Test, Citrate Utilization Test, Indole Production Test, Motility Test, Coagulase Test, Sugar Fermentation Test were all carried out.

Lactophenol Cotton Blue Staining for Identification of the Fungal Isolates

The fungal isolates were identified by morphological characteristics on Sabouraud's Dextrose agar (SDA) and microscope examination after lacto-phenol cotton blue staining technique. Each of the fungal isolates were separately collected with a sterile inoculating needle and transferred to a glass slide, it was then emulsified with a sterile inoculating needle and then covered with a cover slip gently, to avoid air bubbles. Observation under low and high power objective lens was carried out, the observation includes, searching for different features of fungi including, the hyphae, conidia, sporangiophore (reproductive structure), and identification was carried out microscopically by examining the colonies using x10 and x40 objective lenses (Yaradua *et al.,* 2018).

Determination of Proximate Analysis of the Dried Okra

The proximate composition of the samples produced were analyzed for the following parameters: ash content, protein content, crude fibre content, crude fat content, moisture content and carbohydrate content.

Ash Content Determination

The method described by AOAC (2005) was adopted. 2.0g of the sample was weighed out using digital electronic balance in a crucible which was washed and dried in an oven, cooled and weighed. The porcelain crucible containing the sample was heated on a Bunsen flame inside a furnace cupboard until no fume was observed. The crucible with the residue was transferred to a preheated mantle furnace at 600^{o}C for 2 hours until complete ashing and constant weights were achieved with intermittent cooling and weighing. The percentage of the ash was calculated.

Nitrogen and Crude Protein Determination

The method described by AOAC (2005) was adopted. 2.0g of the sample was weighed into a Kjelhadh flask. Five gram of anhydrous sodium sulphate and one gram of Copper sulphate were added (as catalyst). Then 25 millilitre of concentrated H_2SO_4 was added to the sample and moved into a furnace cupboard and heated for 1 hour using Thermo regulated heating mantle at temperature of 25 ^{0}C with intermittent shaking till green color emerged. It was filtered to remove impurities. The filtrate was reheated gently until green color disappeared and was allowed to cool. The digest was transferred with several washings into a 250 millilitres volumetric flask and made up to the mark with distilled water. It was however distilled using distillation apparatus. 100 millilitres capacity conical flask containing 5milliliter of boric indicator was placed under the liquid. 10 millilitre of the digest was measured and transferred to the cap of the Kjeldah distiller, followed by addition of 10 millilitres of 40% 0.1 mole NaOH solutions. The solution was shaken thoroughly for 15 minutes to collect enough ammonium sulphate, evidenced by colorless solution. The receiving flask was removed and the tip of the condenser was washed down into the flask. The solution was titrated in the receiving flask using 0.01 ml HCl until blue colour appeared.

Crude Fibre Determination

The method described by AOAC (2005) was adopted. 2.0g of the sample was weighed out using digital electronic balance and boiled with 200ml 0f 1.25% H_2SO_4 for 30 minutes in a flask using water bath. It was filtered using Whatmann 54 filter paper through a funnel. The residue was washed with hot water to remove acid from it. The residue was then transferred to another beaker and boiled for 30 minutes in a water bath using 200ml of 1.25% NaOH. It was again filtered and progressively washed with boiled water. The residue was also transferred to a crucible and dried in the oven to a constant mass. It was at this stage incinerated using mantle furnace, cooled and reweighed. The percentage of the fibre was calculated.

Crude Fat Determination

The method described by AOAC (2005) was adopted. This involves the use of methanol, the wet ground mustard seed samples are dissolved in the methanol to give a single phase, miscible with water. Additionally, chloroform is added to give a separation of the phases and then centrifuged to separate the solvents. The residue left behind after the chloroform layer containing the dissolved fat is removed and reweighed. The percentage of the fat was calculated.

Determination of Moisture Content

The method described by AOAC (2005) was adopted. A crucible was properly washed and allowed to dry in an air oven at 110^0 C for 10 minutes to a constant weight. Then the crucible was allowed to cool in desiccators for 30 minutes and was labelled and weighed (W1). 2.0g of the sample was accurately weighed (W2). The crucible containing the sample was placed in an oven maintained at 103 ^{0}C for 14 hours. It was then removed and allowed in the dessicator then finally weighed (W3). The percentage moisture content was calculated.

Determination of Carbohydrate

The method described by AOAC (2005) was adopted. The total carbohydrate content was determined by difference method. The sum of the percentage moisture, ash, crude lipid, crude protein and crude fibre was subtracted from 100%. Carbohydrate = 100- (% moisture + % ash + % protein + % lipids + % fibre).

III. RESULTS/DISCUSSION

Table 1 showed the proximate composition of the dried okra samples used in this study. The proximate composition of the samples was; moisture (12.94-13.24%), crude protein (24.46-24.60%), crude fat (2.29-2.42%), crude fiber (18.50-18.66%), crude ash (5.18-5.30%) and carbohydrate (35.98-36.06%). Sample A had the highest crude fibre and crude ash; sample B had the highest moisture, crude protein and crude fat while sample C had the highest carbohydrate. Adernipekun and Oyetunji (2010) reported on nutritional values of some tropical vegetables. Their result recorded that dried okra had proximate composition of; moisture (13.33%), crude protein (24.16%), crude fat (2.29%), crude fiber (18.96%), crude ash (5.30%) and carbohydrate (36.16%). On the other hand, Kolawole *et al.* (2011) reported that the edible portion of fresh okra fruit on average contains approximately 86.1% moisture, 2.2% protein, 9.7% carbohydrate, 1.0% fibre, 0.2% fat and 0.9% ash. This shows that drying of okra reduces the moisture content which helps to prevent decay and spoilage.

Moisture content is an index of water activity of many foods. The recommended dietary allowance (RDA) for moisture in foods is ≤5% (FAO/WHO, 1998). High moisture content during storage encourages the growth of certain harmful yeast, moulds and bacteria (Dowswell *et al.,* 2019). Moisture content also affects the physical, chemical aspects of food which relates with the freshness and stability for the storage of the food for a long period of time and the moisture content determine the actual quality of the food before consumption and to the subsequent processing in the food sector by the food producers (Suzanne, 2010).

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. Determining the ash content of a food is part of proximate analysis for nutritional evaluation and it is an important quality attribute for some food ingredients (Suzanne, 2010). It is a determinant of the mineral content of a particular food. The ash content recorded in this study was 1.08%. The recommended dietary allowance (RDA) for ash in foods is ≤5.0 mg/100 g (FAO/WHO, 1998).

Proteins play a particularly important role in human nutrition. The amino acid contents, proportions, and their digestibility by humans characterize a protein's biological value (Ewa, 2011). The amino acid

composition of okra seed protein is comparable to that of soybean and the amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets. Okra seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources. Hence, it plays a vital role in the human diet (Farinde *et al.*, 2007). Fat is an important food composition. The body uses fat as a fuel source and it is the major storage form of energy in the body. Fat also has many other important functions as a moderate amount is needed in the diet for good health.

Fibre is an important dietary component in preventing overweight, constipation, cardiovascular diseases, and diabetes. High dietary fibre content has been reported to impair protein and mineral digestion and absorption in human subjects (Satter *et al.,* 2013). Okra contains high fiber, which "helps to stabilize blood sugar by regulating the rate at which sugar is absorbed from the intestinal tract". Because of fiber along with other nutrition, okra shows useful for minimizing blood sugar levels within the body, assisting along with diabetes. The fiber likewise helps support blood sugar levels level simply by slowing down sugar assimilation through the intestines (Ngoc *et al*., 2008).

Table 1: Proximate quality of dried okra samples

Proximate parameters	Samples/Compositions (%)		
	A	B	C
Moisture	12.94	13.24	13.23
Crude protein	24.46	24.60	24.26
Crude fat	2.29	2.42	2.38
Crude fibre	18.66	18.50	18.62
Crude ash	5.30	5.18	5.26
Carbohydrate	35.98	36.06	36.24

Key: A – C = Dried okra samples % = Percentage

Table 2 showed the microbial quality of the dried okra samples used in this study. Total viable bacterial counts recorded ranged from 8.0 x 10^3 cfu/g to 5.8 x 10^4 cfu/g. There were no coliform counts recorded. Total fungal counts recorded ranged from 6.0 x 10^3 cfu/g to 1.2 x 10^4 cfu/g. From the result of the microbial quality of the dried okra (Table 4.2), the control dried okra had the least microbial load (8.0 x 10^3 cfu/g) compared to the ones sold at market conditions (5.8 x 10^4 cfu/g).

Table 2: Microbial quality of the dried okra

Dried okra	TVBC (cfu/g)	TCC(cfu/g)	TFC (cfu/g)
D1	2.8 x 10^4	NG	4.0 x 10^3
D2	3.6 x 10^4	NG	6.0 x 10^3
D3	5.8 x 10^4	NG	1.2 x 10^4
Control	8.0 x 10^3	NG	NG

Keys: NG= No Growth TVBC= Total viable bacterial counts
TCC= Total coliform counts TFC= Total fungal counts
cfu/ml= Colony forming unit per milliliter D1-D3 = Dried okra

Table 3 showed the result of the cultural morphology and biochemical characteristics of the bacterial isolates from the dried okra samples. The bacterial isolates such as; *Pseudomonas, Staphylococcus, Micrococcus, Corynebacterium* and *Bacillus* species from the dried okra samples are shown in Table 3. Previous researchers have reported the isolation of bacteria from dried and fresh okra samples sold at market condition. The results of this study is in agreement with the report of Ajayi *et al.* (2013) who reported the isolation of *Pseudomonas* species, *Salmonella* species and *Staphylococcus* species from dried edible mushrooms.

Table 3: Cultural Morphology and Biochemical Characteristics of the bacterial isolates from the dried okra samples

Morphological Characteristics	Gram reaction	Oxidase test	Indole test	Spore test	Catalase test	Citrate test	Coaguase test	Motility test	S FT				Possible bacteria
									S	B	G	H_2S	
Milkish, flat, rhizoid-like dry-surface colonies	Gram positive rods in short chains	-	-	+	+	-	-	+	Y	Y	+	-	*Bacillus* species
Milkish, raised, non- mucoid colonies in clusters	Gram positive cocci	-	-	-	+	-	+	-	No Reaction		-	-	*Staphylococcus* species
Milkish, raised, non-mucoid regular shaped colonies	Gram positive rods	-	-	-	+	-	-	-	No Reaction		-	-	*Corynebacterium* species
Bluish-green, flat, non-mucoidcolonies	Gram negative rods in diploids	+	-	-	+	+	-	+	R	R	-	-	*Pseudomonas* species
Milkish, raised, non-mucoid circular colonies of about 3mm in size	Gram positive cocci in pairs	-	-	-	+	-	-	-	No Reaction		-	-	*Micrococcus* species

Key: -= Negative += Positive S = color of slope B = color of butt G= Gas production H_2S= Hydrogen sulphide production (blackening)
R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

Table 4 showed the result of the cultural morphology and microscopic characteristics of the fungal isolates. *Aspergillus, Mucor, Fusarium, Penicillium* and *Rhizopus* species were isolated from the dried okra samples. Dilip *et al.* (2013) reported the presence of eleven major fungal isolates such as; *Aspergillus* species, *Penicillium* species, *Rhizopus* species and *Alternaria* species from dried okra. Amadi *et al.* (2014) reported that *Rhizopus stolonifer, Fusarium oxysporum, Rhizoctonia solani, Penicillium oxalicum, Botryodiplodia theobromae* and *Aspergillus flavus* were the major fungi causing spoilage of fresh okra fruits in Awka South LGA, Anambra State, Nigeria. Arise *et al.* (2012) reported the isolation of *Aspergillus niger*, *Aspergills tamari*, *Fusarium compactum* and *Rhizopus nigricans* from traditional sun dried okra. The results of this study are similar to their study.

Table 4: Cultural morphology and microscopic characteristics of the fungal isolates from the dried okra samples

Cultural morphology	Microscopy	Possible fungi
Whitish cottony broom-like colonies with greenish centre	Septate hyphae with spores	*Penicillium* species
Whitish, enlarged	Septate hyphae	*Aspergillus* species

fluffy with black centre colonies		
Whitish cottony like colonies that covered the plate	Non-septate hyphae with spores	*Rhizopus* species
Whitish cottony broom-like colonies	Septate hyphae with spores	*Fusarium* species
Whitish, fluffy, enlarged colonies with grey center	Non-branched hyphae	*Mucor* species

IV. CONCLUSION

The results of this study have shown that dried produce such as okra could be contaminated with pathogenic microorganisms that could cause ill-health to humans. The isolation of *Staphylococcus* species from the produce is of public health concern as this organism is known to cause Staphylococcal food-poisoning. Considering the nutritional importance of this produce and the need to sustain its availability, modern and reliable methods of drying, packaging and storage should be adopted so as to prevent contamination with pathogenic microorganisms and gain their benefits all year round

V. RECOMMENDATION

To ensure that the purpose of drying okra is effectively achieved and the nutritional benefits of okra adequately harnessed, hygienic conditions should be maintained by the sellers. Proper washing and cooking of these produce should be practiced so as to reduce their microbial loads. Processors of these produce should be educated on the dangers that are associated with poor drying and handling during storage and sale.

Conflict of Interest

The authors have not declared any conflict of interest regarding funding and publication of this work.

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