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MICROBIAL ANALYSIS OF SUN-DRIED OKRA SAMPLES FROM SOME AKOKO AREAS OF ONDO STATE, NIGERIA

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ABSTRACT

This study investigated the microbial safety of sundried okra fruits from markets in some Akoko towns of Ondo State, Nigeria. The pH of the dried market samples fell between 5.22 and 6.16 while the water activity was between 0.513 and 0.572. Significant differences were also observed in their viscosity. Samples from Ipe-Akoko and the control had the highest and the lowest moisture contents respectively while the lowest and highest crude ash contents were from the same samples. Crude protein contents of the dried samples ranged from 16. 56% to 21.53% while the fresh sample contained 23.03%. Little significant differences were observed in the crude fibre contents of the market samples. The control sample contained 0.73% crude fat contents while the market samples ranged between 2.76% and 4.35%.

The carbohydrate contents showed little significant differences among each other but significantly different from the fresh sample. Bacteria isolated from the samples were *Bacillus subtilis. Staphylococcus aureus, Micrococcus cereus, Clostridium* spp, *Pseudomonas aeruginosa, Escherichia coli. Klebsiella pneumuniae, Streptococcus faecalis, Enterobacter aerogenes* and *Lactobacillus plantarum* while the fungi include *Rhizopus stolonifer, Aspergillus niger, A. fumigatus, A. flavus, Penicillium, Mucor pusillus* and *Candida crusei.* The total bacterial and the fungal loads of the dry samples ranged from 4.42 log sfu/g to 6.65 log cfu/g, and 3.22 log cfu/g and 4.83 log cfu/g while coliform counts and lactic acid bacterial counts fell between 0.91 log cfu/g and 3.74 log cfu/g, and between 2.72 log cfu/g and 3.6 log cfu/g. This study suggested that dried okra is nutritious but proper hygienic conditions moist be maintained during processing or using controlled drying methods and should be cooked to avoid the risk of ingesting pathogenic microorganisms.

KEYWORDS: Okra, Sun-Drying, Microorganisms, Hygiene, Moisture

INTRODUCTION

Vegetables form a valuable part of human diet in some regions of the world. They contribute to the nutritive values of foods in many developing countries. They are a good source of vitamins, minerals and dietary fibre; and water to aid digestion. Vegetables are rich in minerals such as potassium, sodium, calcium, iron, zinc and phosphorus (Ijeomah, *et al*., 2012). They play an important role in maintaining general health due to the presence of minerals and vitamins in them (Fayemi, 1999).

Most vegetables are perishable due to their high moisture content. They are either consumed within few days or preserved for later consumption. Drying is one of the techniques used for post harvest management of foods especially in developing countries where cold storage is inadequately practised. Drying may be achieved by sun or using hot-air oven. Drying preserves food by reducing water activity of the food to a level insufficient for enzyme activity or the growth of microorganisms thereby preventing decay and spoilage (Ofor and Ibeawuchi, 2010). Drying enhances storage stability,

reduces bulkiness of foods and cost of packaging. More than 20% of the world perishable crops are dried to increase shelf-life and promote food security (2003).

Okra (*Abelmoschus esculentus*) is an important vegetable crop grown in tropical, sub-tropical and warm temperate regions around the world. The edible portion is the immature fruit. The fruit becomes fibrous and not suitable for consumption when fully mature. The most important okra producing countries include India, Nigeria, Pakistan, Ghana and Egypt (De Lannoy, 2001; Varmudy, 2011).

It is known as "Ila" in "Yoruba", "Kubewa" in "Hausa" and "Okwale" in "Igbo" tribes of Nigeria (1974; Tindall, 1983). Okra is a prominent fruit and leafy vegetable grown for domestic consumption of the highly nutritious immature leaves and fruits in Nigeria (Farinde *et al*., 2007). Whole, fresh okra pods also make excellent pickles. Okra could also be fried to make its slippery characteristics less pronounced.

Okra mucilage is suitable for medicinal and industrial applications. It has medically found application as a plasma replacement or blood volume expander (Siemonsma and Kouame, 2004). Industrially, okra mucilage is usually used in to glace certain papers and also useful in confectionery among other uses. Okra provides an important source of vitamins, calcium, potassium and other mineral matters which are often lacking in the diet of developing countries. It medicinal value has also been reported in curing ulcers and relief from hemorrhoids (Siemonsma and Kouame, 2004).

Okra is one of the perishable commodities due to its high moisture content. The main traditional method of post-harvest preservation of the vegetable is by sun-drying in Ondo State of Nigeria. After slicing fruits into small chips, they are indiscriminately sun-dried on roof tops, concrete constructions, mats, along roadsides and in courtyards for about three weeks depending on the intensity of sunlight. This unhygienic art inevitably poses the risk of exposing the commodity to direct microbial contamination or indirectly from dust, flies, rodents and even human handlers (Ofor and Ibeawuchi, 2010). The aim of this study is to assess the microbial safety of sun-dried okra fruits in sold in Akoko areas of Ondo State.

MATERIALS AND METHODS

Collection of Samples

Sundried okra fruits were purchased from five different communities in Akoko Regions of Ondo State. Communities were Akungba-Akoko (Ibaka market), Ikare-Akoko (Obada Market), Oka-Akoko (Ajoke market), Ipe-Akoko (Ipe market) and Supare Market. All the samples were kept in cellophane bags and brought to Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko, Nigeria for analyses.

Preparation of Control Sample

The control sample was prepared as follows. Five hundred grams of fresh and healthy okra fruits were washed with 2000 ml of sterile distilled water. They were drained and cut into slices of thickness of about 3 mm with a sterilized knife. The sliced okra fruits were sterilized by soaking in 1000 ml 3% sodium metabisulphide solution for 3 minutes. The sample was then rinsed with sterile distilled water and drained using a plastic sieve sterilized with 85% ethanol. The sliced okra fruits were spread over stainless steel trays and oven-dried at 65^{o}C for 96 hours.

Determination of pH

The pH of each sample was determined with the aid of pH meter by dispensing ground 10 g of the dried okra sample into 10 ml of sterile distilled water and shaken properly. An electrode was then inserted into the mixture and stirred properly to ensure stable reading.

Determination of Water Activity (aw) and Viscosity

Water activity was determined using Hydro lab Multi-channel Humidity and Water Activity Analyzer (Huntington, USA).

For the determination of viscosity, 10 gm of okra powder was mixed with 100 ml of distilled water. The viscosity of the slurry at ambient temperature (30 ± 2°C) using Brookfield Viscometer (LV-8, England) using appropriate spindle as described by the AOAC (2005) method was determined.

Enumeration of Microorganisms

Ten grams of the ground sample was added to 90.0 ml of 0.1 % peptone solution as diluent. From the appropriate 10-fold dilutions, total bacteria were enumerated on Standard Methods Agar incubated at 37^{o}C for24 hours.

Lactic acid bacteria were enumerated on DeMan, Rogosa and Sharpe Agar (Difco,) incubated at 30°C in an anaerobic jar with anaerocult for 4 days. Coliform bacteria were enumerated on violet red bile agar (Acumedia) incubated at 37°C for 24 hours, and confirmed in Brilliant Green Bile Lactose Broth (Oxoid) incubated at 37°C for 24 hours.

Identification of Bacteria

Inocula were aseptically transferred from each slide into plates of respective media using streak plate technique. Bacterial plates were incubated at 37^{o}C for 24 hours while fungal plates at 25^{o}C for 72 hours. A 24 hour old culture was prepared from each plate for identification purposes. Bacteria isolates were identified based on their cultural characteristics, Gram staining reaction and various identification tests Isolates were identified according to Holt *et al*. (1994). Lactic acid bacteria were also identified by assaying in API 50 CHL galleries (BioMerieux).

Identification of Moulds

Mould isolates were cultured by three point inoculation on CYA and MEA at 25°C for 5 days. The young cultures of the isolates were stained with lactophenol-blue and identified to the genus level by colony and cell morphology and biochemical tests according to Alexopoulos, and Mims, (1979).

Proximate Analysis

Proximate composition of the okra samples were carried out according to the AOAC (2005) method for moisture content, ash, fiber and carbohydrate content. Crude protein was determined using Kjeidahl method and fat content was done using soxhlet's apparatus.

Statistical Analysis

Data obtained were analyzed by ANOVA and significant differences between means were compared using Duncan (Duncan 1955) multiple range test with the aid of SAS/STAT program (SAS 1998).

RESULTS

All the dried okra samples were weakly acidic with the highest pH value among the market samples in Ipe-Akoko sample while the least value (6.19) was from Oka-Akoko sample (5.22). There was no significant difference in the pH of dried okra sample from Akungba-Akoko and the control sample. was n The highest water activity in sundried okra samples was found in Supare-Akoko sample (0.572) followed by Ipe sample (0.566) while Iwaro-Oka sample had the lowest water activity (0.513) obtained in dried okra sample. Higher significant differences were observed in the water activities among the samples. The highest and the least water activities were found in fresh okra (0.794) and the control sample (3.12) respectively. The viscosity of each sample was significantly different from one another. The dried okra sample from Akungba community had the highest viscosity value of 1180 BU while the lowest value was found in the control sample (740 BU) and were highly significantly different from each other. The viscosity of the fresh and the control samples were 1330 BU and 740 BU respectively Table 1.

The highest crude ash, carbohydrate and fibre contents were determined in the control sample Table 2. Among the dried market samples, the highest and lowest moisture contents were determined in samples from Ipe-Akoko (15.43%) and Akungba-Akoko (10.97%). Oka-Akoko sample had the highest moisture content (10.73%) while the lowest content was found in Iwaro-Akoko sample (8.35%). Ash contents of the dried market samples were significantly different and ranged between 8.35% and 10.73% while the fresh and the control sample contained 10.24% and 11.53% respectively. The highest significant crude protein content was found in fresh okra sample (23.03%) followed by the control (21.34%). Crude protein contents of the dried okra market sample was highest in Oka-Akoko sample (20.19) followed by Supare-Akoko sample (19.04%) while the lowest content was found in Iwaro-Oka sample (16.56%). Little significant differences were observed in crude fibre contents of all the samples with the highest from Supare-Akoko sample (6.49%) followed by Akungba-Akoko sample (6.48%) while the lowest content was found in Oka-Akoko sample (5.26%). Crude fat contents were also significantly different and ranged from 2.73% to 4.35% in dried market samples. However, the crude fat content of the control sample was very significantly small (0.73%). The crude carbohydrate contents of the dried samples ranged between 46.02% and 51.31% while the fresh sample contained 15.89%.

However, microorganisms were not isolated from the control sample while various fungi and bacteria were isolated from dried and the fresh samples Table 3. Dried okra from Ipe had the highest total bacterial count (6.65 log cfu/g) total coliform count (3.56 log cfu/g). The highest fungal and lactic acid bacterial counts of (4.83 log cfu/g) and (3.76 log cfu/g) were observed in Supare sample respectively. However sample from Akungba the lowest total bacterial counts, fungal count, coliform count and lactic acid bacterial count with respective values of 4.42 log cfu/g, 3.22 log cfu/g, 0.91 log cfu/g And 2.72 log cfu/g.

Bacillus subtilis was predominant in Oka- Akoko, Supare- Akoko, Iwaro- Akoko and Ipe- Akoko and the fresh samples. *Staphylococcus aureus* and *Micrococcus cereus* were isolated in all the market samples including the fresh sample Table 4. *Escherichia coli* was isolated from Supare-Akoko and Iwaro-Akoko samples while *Klebsiella pneumuniae* was found in dried okra samples from Oka-Akoko, Supare-Akoko and Ipe-Akoko. *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Lactobacillus plantarum* were only isolated in Supare-Akoko and Ipe-Akoko respectively. Five genera of fungi were isolated from all the samples Table 5. They include *Rhizopus stolonifer*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium*, *Mucor pusillus* and *Candida crusei.*

Aspergillus flavus and *Penicillium italicum* were isolated in all the died okra samples except Iwaro-Akoko and Akungba-Akoko samples respectively. *Rhizopus stolonifer* and *Candida crusei* were only isolated from dried okra samples from Oka-Akoko and Iwaro-Akoko as well as the fresh okra pods.

Table 1: pH and Total Titratable Acidity Okra Samples

Sample	pH	Water Activity	Viscosity(BU)
Fresh	5.14±0.2^{e}	0.794±0.02^{a}	1430±25^{a}
Control	5.45±0.1^{c}	0.312±0.02^{f}	740±18^{e}
Akungba	5.47±0.2^{c}	0.515±0.01^{e}	840±21^{d}
Oka	5.22±0.2^{de}	0.526±0.04^{d}	840±16^{d}
Supare	5.34±0.3^{d}	0.572±0.02^{b}	1000±26^{c}
Iwaro	5.74±0.2^{b}	0.513±0.03^{de}	1180±24^{b}
Ipe	6.19±0.1^{a}	0.566±0.02^{c}	1120±21^{bc}

Values with the same superscript letter(s) down a column are not statistically significantly (P>0.05) different

Table 2: Proximate Analysis of Okra Samples

Sample	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	Fat (%)	Carbohydrate (%)
Fresh	40.96±2.0^{a}	10.24±1.0^{b}	23.03±2.1^{a}	6.42±0.1^{b}	3.46±0.1^{d}	15.89±1.2^{d}
LAB	8.67±0.8^{e}	11.53±0.8^{a}	21.34±1.5^{b}	6.38±0.2^{b}	0.73±0.1^{f}	51.31±2.3^{a}
Akungba	10.97±0.7^{cd}	9.88±0.4^{d}	18.47±0.6^{e}	6.48±0.3^{a}	4.35±0.3^{a}	49.85±4.2^{ab}
Oka	12.63±0.8^{c}	10.73±0.8^{b}	20.19±1.2^{c}	5.26±0.1^{d}	3.95±0.2^{b}	47.24±1.8^{bc}
Supare	14.65±0.4^{b}	10.07±1.0^{c}	19.04±0.8^{d}	6.49±0.2^{a}	3.73±0.2^{c}	46.02±2.4^{c}
Iwaro	12.91±0.3^{c}	8.35±0.6^{e}	16.56±1.0^{g}	5.65±0.1^{c}	3.86±0.2^{b}	52.67±2.8^{a}
Ipe	15.43±0.6^{b}	8.47±0.4^{e}	17.75±1.2^{f}	5.87±0.2^{c}	2.76±0.4^{e}	49.72±2.2^{ab}

Values with the same superscript letter(s) down a column are not statistically significantly (P>0.05) different

Table 3: Microbial Counts of the Dried Okra Samples

Sample	Total Bacteria (Log cfu/g)	Fungi (Log sfu/g)	Coliform (Log cfu/g)	Lab (Log cfu/g)
Fresh	6.18±0.48^{b}	4.42±0.78^{b}	2.42±0.26^{b}	3.12±0.62^{b}
Control	ND	ND	ND	ND
Akungba	4.42±0.68^{e}	3.22±0.82^{f}	0.91±0.24^{d}	2.73±0.12^{d}
Oka	4.95±0.43^{d}	3.71±0.14^{de}	1.12±0.03^{c}	3.23±0.21^{b}
Supare	6.12±0.26^{b}	4.83±0.24^{a}	0.56±0.01e	3.76±0.18^{a}
Iwaro	5.17±0.56^{c}	3.53±0.23^{e}	0.93±0.01^{d}	2.83±0.06^{c}
Ipe	6.65±0.16^{a}	4.27±0.26^{c}	3.56±0.01^{a}	3.74±0.08^{a}

Values with the same superscript letter(s) down a column are not statistically significantly (P>0.05) different

Table 4: Bacteria Isolated from the Dried Okra Samples

Sample	Bacillus Subtilis	Staphylococcus Aureus	Micrococcus Cereus	Clostridium spp	Pseudomonas Aeruginosa	Escherichia coli	Klebsiella Pneumoniae	Streptococcus Faecalis	Enterobacter Aerogenes	Lactobacillus Plantarum
Fresh	+	+	+	-	-	-		-	-	-
Control	-	-	-	-	-	-	-	-	-	-
Akungba	-	+	+	-	-	-	-	-	-	+
Oka	+	+	+	-	-	-	+	-	-	-

Table 4: Contd.,

Supare	+	+	+	+	-	+	+	+	-	-
Iwaro	+	+	+	+	-	+	-	-	+	-
Ipe	+	+	+	+	+	-	+	-	-	-

Table 5: Moulds Isolated from the Dried Okra Fruits

Sample	Rhizopus Stolonifer	Aspergillus Niger	Aspergillus Fumigatus	Aspergillus Flavus	Penicillium	Mucor Pusillus	Candida Crusei
Fresh	+	-	-	+	-	+	+
Control	-	-	-	-	-	-	-
Akungba	-	+	-	+	-	-	-
Oka	+	-	+	+	+	-	+
Supare	+	-	-	+	+	+	+
Iwaro	-	+	+	-	+	+	-
Ipe	-	+	+	+	+	+	-

DISCUSSIONS

The results of pH values reveal that dried okra samples are slightly acidic and little variations were observed in all the samples. This was in agreement with Kolawole *et al*. (2011) and Matazu and Suleiman (2002) that the internal pH of vegetables fall between 4.5 and 6.4. Burkill (1997) reported that *Hibiscus sabdariffa* contains 13% of malic and citric acids on dry weight basis. The author also detected ascorbic, saponic and tartaric acids in the sample (*Hibiscus sabdariffa*). Very little significant differences were observed in water activity of all the dried okra samples. However, the lowest water activity of the control sample could be due to the temperature of drying which is higher than the ambient temperature of this region (28±2^{o}C). Water activity is an important intrinsic factor that determines the spoilage of foods.

The moisture contents of the samples were directly corresponded with their respective viscosity. This was in agreement with the report of Potter and Hotchkiss (1996) who reported that the rheological properties of starch and gums in food system may be altered by heat and the hydrophilic properties of such foods may also be affected. The variation in the moisture contents in all the market samples could be due to the temperature and relative humidity of their respective market locations as well as the length of sun-drying. Higher microbial counts observed in dried okra samples from Supare-Akoko and Ipe-Akoko could be attributed to higher moisture contents observed in samples from these communities. Foods with high moisture contents are prone to easy microbial spoilage and subsequent short shelf life (Ijeomah *et al.*, 2012). Moderate moisture content of ≤ 12mg/g is preferred for shelf stability of food on long storage. High moisture content in fresh okra implies that the vegetable has low storage capacity and are therefore easily perishable (Effiong *et al*., 2009).

The higher protein content observed in fresh okra sample than the control and the market samples had been reported in some vegetables (Sobowale *et al*., 2010). However, the protein contents of the dried okra samples were higher than some of the commonly consumed vegetables and cereals in Nigeria (Ihekoronye and Ngoddy, 1985; Mepha *et al*., 2004; Sobowale *et al.*, 2010). The dried okra can be considered as a vegetable rich in protein compared to *Talinum triagulare*, *Amaranthus hybridus* and *Celosia argentia* (Oguntona., 1998). The low fat contents of the dried okra in the control sample could be attributed to the controlled processing temperature (Mepha *et al*., 2004). Lower fat in any given food reduces chances of rancidity (Oguche, 2012). Cholesterol had been reported to be present in fruits and vegetables (Akubo *et al*., 2009)

The crude fibre content of the sample ranged from 5.26 to 6.48%. Faghohun *et al*. (2011) observed crude fibre ranging between 5.75% and 7.78% in sundried melon seeds. Dietary fibre confers laxative effect in the gastrointestinal tract, thereby shortening transit time of food in the tract, and increasing water-holding capacity of feaces. Therefore dietary fibre helps to alleviate disorders such as constipation, diverticulatics, irritability bowel syndrome, gall stone and colorectal cancer (Shailong and Ugwuona, 2011). The ash contents of the dried okra from the markets as well as the control were present in appreciable amounts but higher than some other legumes. Minerals play major role in many biochemical reactions where they function as co-enzyme and aid physiological functioning of major metabolic processes in the body (Bryant *et al*., 1988: Arise *et al*. (2012).

Microbial species isolated from the dried okra samples may be due to contamination during the drying process or through the handlers. Commodities which are exposed to sun during drying might become highly contaminated because of the longer drying time required. Kolawole *et al.* (2011) observed that foods sun dried over a long period of time are highly contaminated and yield poor quality.

The variations in microbial composition and distribution among all the samples could be attributed the environment where they were sun-dried and the sample sources. The highest microbial counts obtained from Supare-Akoko and Ipe-Akoko samples might be an indication of poor hygiene practice during preliminary processing. Besides, samples from these communities had the highest water activity. It had been reported that samples with higher water activity were more liable to microbial attack than those with lower water activity. Microbial growth can occur during drying in those internal tissues which still contain sufficient moisture Kolawole *et al.* (2011).

Staphylococcus aureus and *Micrococcus* were isolated from all the samples while *Bacillus* was not isolated from Akungba-Akoko alone. *Staphylococcus aureus* and *Micrococcus* had been reported to be normal flora of human beings while *Bacillus* had been identified as normal flora of many cereals and legumes. The presence and abundance of *Bacillus* in some of the samples may not be surprising as these organisms are indigenous to soil environment (Atlas and Bartha, 2007). Similar findings were reported by Ezeronye and Ubalua (2005). Kolawole *et al* (2011) reported that some common microbial contaminants of food include *Staphylococcus aureus*, *Micrococcus* and *Bacillus*.

The presence of *E. coli* and *Streptococcus faecalis* may be as a result of recently fecal contamination of the sample from the handler or from the processing water (Atlas and Bartha, 2007).

The occurrence of coliforms in very high numbers in most of the dried vegetables is an indication of poor handling of the vegetables during processing (Ezeronye and Ubalua, 2005). Factors which could be responsible for the high counts include wetting the plants with contaminated water in the field, fertilizing the vegetable with human or animal wastes, vegetables not being properly washed and preferably sanitized before drying; drying on exposed surfaces and packing and transporting in plastic containers or sacks not adequately clean (Kudjawu, *et al*., 2011).

Most of the fungi isolated from the dried okra samples from these communities had been reported to be associated with pre-harvest and post-harvest contamination of dried fruits and vegetables (Fagbohun *et al*., 2011). A few outbreaks of human disease as a result of eating these vegetables contaminated by waste water have been reported (Epstein *et al.*, 1982; Rabah *et al*., 2010). These fungi could be from the air, soil, storage house and or improper handling of the products. Their presence in foods is of great public health concern (Tsado *et. al.*, 2013). Pathogens existing in soils or water can be the sources of both pre- and post-harvest contamination of several vegetables (Mapanda *et al*., 2005).

Aspergillus fumigatus has been reported to be toxigenic and produce mycotoxins (Bankole and Adebanjo, 2003). Kpodo *et al*. (1996) reported the presence of aflatoxin producing Aspergillus *flavus* and *Aspergillus parasiticus* and citrinin-producing *Penicillium citrinum* in maize samples in Ghana. *A. flavis*, which has been implicated to be carcinogenic and responsible for respiratory infections in immuno-compromised patients, hypersensitivity or allergic reactions (Amaike and Keller, 2011). *Rhizopus stolonifer* was equally isolated in this study has also been implicated in several human ailments (Schipper, 1984). This organism is most commonly found growing on soft fruits such as bananas, oranges and carrots.

CONCLUSIONS

In conclusion, research work shows that sun-dried okra pods were relatively rich in protein compared to some sun-dried vegetables. When sun-dried under unhygienic conditions, they are not fit and safe for human consumption as they can serve as vectors for diverse pathogenic microorganisms. Therefore, it is essential to ensure basic hygienic practice during preservation and preparation of dried vegetables meant for human consumption.

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