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The microbiota of dried traditional vegetables produced in the Sudan Savannah and Guinea Savannah agro-ecological zones of Ghana

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Abstract: Traditional vegetables are an important article of diet of the ethnic groups from the northern parts of Ghana. Such vegetables are preserved by sundrying and consumed throughout the year. These are mostly leafy vegetables and include *Hibiscus sabdariffa* (sorrel), *Bomtax costatum* (kapok), *Ceratotheca sesamoides*, *Adansonia digitata*, and *Hibiscus esculentus* (okro), respectively called by the local names *shure* or *sobolo*, *daala*, *yaudo*, *kuuka*, and okro (common name). The dominant microbiota of ten common dried traditional vegetables were investigated by enumerating total bacteria, yeasts and moulds, lactic acid bacteria, *Bacillus* species and coliform bacteria. Isolates were characterized by colony and cell morphology, and by biochemical tests. The water activities of all the dried vegetables were between 0.513 and 0.539. *Shuri* and *bisap* had low pH values, between 2.6 and 2.71, whilst the other vegetables had relatively higher pH values ranging from 5.21 to 6.39. Moulds and *Bacillus* spp. dominated the biota of all the dried vegetables although lactic acid bacteria and coliforms were also isolated in most of these products. Bacterial counts of the dried vegetables were between 10^3 and 10^7 CFU/g. *Aspergillus* spp, *Rhizopus* spp, *Eurotium* spp, *Penicillium* spp and *Aureobasidium* spp. were the major genera of moulds identified in the dried vegetables. The dominant *Bacillus* species in all samples was *Bacillus subtilis* except in the okro pods and leaves where *Bacillus cereus* was dominant. The dominant lactic acid bacteria were *Lactobacillus plantarum* and pediococci. *Escherichia coli* was not detected in any of the dried vegetables, however, *Enterobacter aerogenes* was detected in most of the samples. Because of the high microbial levels in the dried vegetables, it is recommended that handling procedures be improved by primarily sanitizing the fresh vegetables prior to drying in solar dryers.

Keywords: microbiota, microflora, dried, vegetables

Introduction

Traditional vegetables are an important component of the diet of the ethnic groups from the northern parts of Ghana. These plants are part of the natural flora of the Sudan Savannah and Guinea Savannah agro-ecological zones and most are indigenous to West Africa. They include *Hibiscus sabdariffa* (sorrel), *Bomtax costatum* (kapok), *Ceratotheca sesamoides*, *Adansonia digitata*, *Corchorus olitorius* and, *Hibiscus esculentus* (okro, both leaves and pods) respectively called by the local names *shure* or *sobolo*, *daala, yaudo, kuuka, ayoyo* and okro (common name). They are produced in northern Ghana but are available in the dried form in most parts of the country. Though cooked as fresh leaves or pods, the bulk is preserved by sundrying and used in the dried form throughout the year. In southern Ghana (comprising the Coastal Savannah, Transitional and Forest Zones) the main traditional vegetables used are garden eggs (*Solanum aethiopicum*) and the leaves of the cocoyam plant (*Xanthosoma mafaffa*). Common non-traditional vegetables are also used throughout Ghana and include lettuce, cabbage, carrots, salad leaves, cucumber and french beans.

The traditional vegetables are mainly used in the preparation of soups and stews apart from the dried calyx and petals of *Hibiscus sabdariffa* which are used for the preparation of non-alcoholic beverages. Apart from their food uses, most of the traditional vegetables are reported to have important medicinal properties. According to Burkill (1997) *Hibiscus sabdariffa* is used in infusions, diuretic, cholagogic, cough relief, and for treatment of hypotension, toothache, biliousness, as a purgative, an aphrodisiac and in childbirth. The bark, leaves and roots of kapok

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are used in the treatment of yellow-fever, headache, blennorrhoea and diarrhoea (Burkill, 1985). Okro pods serve as anti-bacterial agents in transfusion, and post-partum haemorrhage in pregnant women (Burkill, 1994). Kuuka or baobab leaves are used for the treatment of kidney and bladder diseases, asthma, diarrhoea, urinary tract diseases, and as a blood cleanser, prophylactic, worm expeller, amongst others, whilst *yaudo* is used to cure exhaustion, scabies, scurf dysentery, and for difficult child-birth (Burkill, 1985). Ayoyo (*Corchorus olitorius*) is used to counter malnutrition, as a purgative, for fever and heart troubles (Burkill, 2000).

The small-holder farmers who produce the traditional vegetables preserve them by sundrying but generally do not adhere to the basic principles of Good Hygienic Practices. Under these circumstances it is suspected that dried vegetables may harbour high microbial loads and this work was carried out to assess the microbiological quality of dried traditional vegetables sold on the open markets in Ghana.

Materials and Methods

A total of 80 samples of the different traditional vegetables were purchased from three open markets in Accra and one at Wa in the Upper West Region.

Determination of pH and water activity (a_w)

Ten (10) g of sample was homogenized in a blender with 10 ml of distilled water and the pH determined using Jenway Research pH meter (model 3330, Gransmore Green, England). Water activity was determined using Hydrolab Multi-channel Humidity and Water Activity Analyzer, (Huntington, USA).

Enumeration of microorganisms

Ten (10) g of homogenized sample was added to 90.0 ml of 0.1 % peptone solution as diluent. From the appropriated 10-fold dilutions, total bacteria was enumerated on Standard Methods Agar (SMA, Acumedia Manufacturers, Inc., Lansing, Michigan, USA) incubated at 30°C for 2 d; moulds and yeasts on Dichloran 18% Glycerol Agar (DG18, HiMedia Laboratories Put. LTD., India) incubated at 25°C for 5 d; *Bacillus* spp. on Dextrose Tryptone Bromcresol Purple Agar (DTBCP, Difco, Lansing, Michigan, USA) incubated at 37°C for 2 d. For *Bacillus* spores, the 10^{-1} dilution was heated at 100°C for 5 min to destroy vegetative cells and other non-sporeformers before further serial dilutions. Lactic acid bacteria was enumerated on DeMan, Rogosa and Sharpe Agar (MRS, Difco, Beckon Dickinson and Company Sparks, MD, USA) incubated at 30°C in an anaerobic jar with anaerocult (Oxoid, Basingstoke, Hamsphire, England) for 4 d. Coliform bacteria were enumerated on Violet Red Bile Agar (VRBA, Acumedia Manufacturers, Inc., Lansing, Michigan, USA) incubated at 37°C for 24 h, and confirmed in Brilliant Green Bile Lactose Broth (Oxoid, Basingstoke, Hamsphire, England) incubated at 37°C for 24 h (NMKL 44, 2004). The presence of *E. coli* was confirmed using EC Broth (Oxoid, Basingstoke, Hamsphire, England) at 44°C for 24 h (NMKL 125, 2005).

Isolation and identification of microorganisms

Ten to fifteen isolates were taken from the highest dilution plates and continually streaked on Agar to obtain pure colonies. Isolates of *Bacillus* spp., coliforms and lactic acid bacteria were initially examined by Gram stain, catalase and oxidase tests.

Identification of Bacillus *species*

Gram posititve, catalase positive rods mostly bearing phase-bright spores were assumed to be *Bacillus* spp. and further examined according to Claus and Berkerley (1986) and Parry et al. (1983) by growth at pH 5.7 and 6.8; in 7% and 10 % (w/v) sodium chloride; liquefaction of gelatin; starch hydrolysis; decomposition of casein. Tests for acid production from 18 different carbohydrates were carried out as described by Obilie et al (2003). The eighteen carbohydrates tested were: D(+)glucose, D(-) mannitol, L(+)arabinose, D(+)xylose, amygdalin, cellobiose, fructose, galactose, lactose, maltose, melezitose, melibiose, raffinose, ribose, salicin, sorbitol, sucrose, xylose and trehalose. Isolates were further characterized by assaying in API 50 CHB galleries (BioMerieux, SA, Marcy-l'Etoile, France) and strains tentatively identified by referring to the API reference table.

Identification of Lactic acid bacteria

Gram positive catalase negative isolates from MRS were characterized by their morphological and biochemical characteristics according to Kandler and Weiss (1986) and Salminen and Wright (1993). They were tested for growth at 15°C and 45°C; at pH 4.4 and 9.6; in 6.5% and 18% sodium chloride (w/v); acid production from MRS broth with Durham tubes in which glucose was replaced with gluconate as a sole carbon source; and gas production from glucose. Isolates were tentatively identified by testing for acid production from 18 carbohydrates as described by Obilie et al (2004), and also by assaying in API 50 CHL galleries (BioMerieux, SA, France).

Identification of moulds

Mould isolates were cultured by three point inoculation on CYA and MEA at 25°C for 7 d and

Table 1. Water activity and pH of dried traditional vegetables produced in Ghana

Dried Vegetables	Aw*	pH
Shuri (*Hibiscus sabdariffa*)	0.539 ± 0.004*	2.60 ± 0.34
Bisap (*Hibiscus sabdariffa)*	0.513 ± 0.001	2.72 ± 0.48
Daala (*Bomtax costatum*)	0.513 ± 0.001	5.21 ± 0.13
Yaudo (*Ceratotheca sesamoides*)	0.534 ± 0.007	6.18 ± 0.54
Kuuka (*Adansonia digitata*)	0.534 ± 0.025	6.05 ± 0.19
Ayoyo (*Corchorus olitorius*)	nd	nd
Okro (*Hibiscus esculentum*)	0.528 ± 0.005	5.86 ± 0.13
Okro leaves (*Hibiscus esculentum*)	0.515 ± 0.003	5.57 ± 0.13
Powdered okro (*Hibiscus esculentum*)	0.534 ± 0.025	6.05 ± 0.19

*Mean and standard deviation of 8 samples of each vegetable analyzed.

Table 2. Microbial population in log10 CFU/g of dried traditional vegetables

Vegetables	Total Bacteria	Moulds and Yeasts	*Bacillus* species	Lactic Acid Bacteria	Coliforms
Shuri (sorrel)	6.78 ± 0.87	6.74 ± 0.58	6.22 ± 0.79	2.20 ± 1.22	0.91 ± 0.78
Bisap/Sobolo	5.00 ± 0.96	4.06 ± 1.40	3.71 ± 0.38	5.05 ± 2.71	0.34 ± 0.64
Daala (kapok)	7.41 ± 0.90	6.43 ± 0.67	6.63 ± 0.79	6.33 ± 0.96	5.16 ± 0.42
Yaudo	7.45 ± 0.70	6.49 ± 0.69	6.44 ± 0.64	5.82 ± 0.76	6.85 ± 1.24
Kuuka (baobab leaves)	7.06 ± 0.86	6.10 ± 0.54	6.43 ± 0.93	5.39 ± 1.72	5.98 ± 1.44
Ayoyo (bush okro)	5.49	7.66	6.34	5.70	6.34
Okro leaves	7.11 ± 1.12	4.91 ± 1.03	4.99 ± 0.58	2.13 ± 1.36	2.25 ± 1.20
Okro	7.30 ± 0.81	4.46 ± 0.70	6.03 ± 1.12	5.75 ± 0.39	6.67 ± 0.46
Powdered okro	7.82 ± 0.46	5.16 ± 0.83	6.77 ± 1.07	6.14 ± 1.10	7.01 ± 0.42

*Mean and standard deviation of 8 samples of each vegetable analyzed

Table 3. Genera of moulds isolated from dried traditional vegetables in Ghana

Vegetables	*Aspergillus* species	*Penicillium* species	*Rhizopus* species	*Eurotium* Species	*Aureobasidium* Species
Shuri	+	-	-	-	-
Sobolo	+	+	-	-	+
Daala	+	-	-	-	-
Yaudo	+	-	+	-	-
Kuuka leaves	+	+	-	-	-
Okro leaves	+	-	+	+	+
Okro	+	-	+	+	+
Powdered okro	+	-	+	+	+

+ = isolated and - = not isolated

Table 4. Biochemical profile of coliforms isolated from dried traditional vegetables in Ghana

	Isolates Number									
Test	1	2	3	4	5	6	7	8	9	10
Voges-Proskauer	-	-	+	+	+	+	-	+	+	+
Nitrate reductase	-	-	+	+	+	+	-	+	+	+
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-
Hydrogen sulfide	-	-	-	-	-	-	-	-	-	-
Indole	-	-	+	+	+	+	-	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase	-	-	+	+	+	+	-	+	+	+
Malonate utilization	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis	+	+	+	+	+	+	-	+	+	+
B-Galactosidase	+	+	+	+	+	+	-	+	+	+
Arabinose fermentation	+	+	+	+	+	+	-	+	+	+
Adonitol fermentation	-	-	+	+	+	+	-	+	+	+
Inositol fermentation	-	-	+	+	+	+	-	+	+	+
Sorbitol fermentation	-	-	+	+	+	+	-	+	+	+
Species identified*	K	K	E	E	E	E	K	E	E	E

*K = Kluvera species and E = Enterobacter aerogenes

identified to the genus level by colony and cell morphology according to Pitt and Hocking (1997).

Confirmation of coliforms

Coliforms were identified by assaying in MICRO-ID® (Remel, Lenxa, KS).

Results and Discussions

Water activity and pH of dried vegetables

Very little differences were observed in the water activity of the various dried traditional vegetables produced in the Sudan Savannah and Guinea Savannah Zones of Ghana (Table 1). These are agro-ecological zones characterized by high ambient temperatures of about 30°C and relatively dry conditions with relatively humidity ranging between 40-75% during the day. All the dried vegetables analyzed in the present work had been sun dried under these favourable ambient conditions and had produced stable dried products. However at these low water activity levels it was expected that some types of microorganisms such as moulds could survive in the products.

The pH values recorded showed that *shuri* and *bisap* were highly acidic products with mean pH values of between 2.6 and 2.71 whilst the other vegetables had relatively higher pH values ranging between 5.21 and 6.39. *Shuri* consists of the dried calyx, petals, and leaves of the yellow variety of *Hibiscus sabdariffa*, whilst *bisap* consists of the dried calyx of the red variety of the same plant species. It is therefore not surprising that these flowers had lower pH values than the true leafy vegetables. According to Burkill (1997) *Hibiscus sabdariffa* contains 13% of malic and citric acids on dry weight basis and is also rich in ascorbic, saponic and tartaric acids.

Microbial population of dried vegetables

Despite the low water activity of the dried vegetables high microbial counts were recorded in all the samples (Table 2). The concentration of total bacteria in all the samples were in the order of 105 to 10^7 CFU/g with the *Hibiscus sabdariffa* products recording slightly lower populations. This could be attributed to the lower pH of these products. Mould and yeast counts were at concentrations of 10^4 to 10^6 CFU/g and *Bacillus* species at 10^3 to 10^6 CFU/g. Wider variations were recorded in the population of lactic acid bacteria and coliforms in the different dried vegetables. LAB counts ranged from concentrations of 10^2 to 10^6 CFU/g and coliforms from less than 10 CFU/g to 10^7 CFU/g. In all, apart from the population of lactic acid bacteria, the lowest microbial counts were observed in bisap and this could be attributed to

the lower pH of the dried calyx. Though both *shuri* and *bisap* are derived from *Hibiscus sabdariffa*, *shuri* contains leaves in addition to the petals and calyx, whilst *bisap* contains only the dried calyx. The dried leaves in *shuri* could have harboured higher microbial counts.

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. Several factors could account for this including high counts in the fresh vegetables before sundrying; vegetables not being properly washed and preferably sanitized in salt or vinegar solution, etc, before drying; drying on exposed surfaces such as concrete floors and roof tops rather than in solar tent dryers; packing and transporting in plastic sacks not adequately clean.

Microbial species on dried vegetables

Moulds

Moulds were identified to the genus level based on colonial and cellular morphology. Only five genera of moulds were found in all the different dried vegetables. These were *Aspergillus* spp., *Rhizopus* spp., *Eurotium* spp., *Penicillium* spp and *Aureobasidium* spp. As shown in Table 3 *Aspergillus* species were found in all the dried vegetables. *Rhizopus*, *Eurotium* and *Aurebasidium* species were only found in the three okro products apart from *Aureobasidium* spp. in *bisap*. The presence of some of the genera of moulds on these dried vegetables should be a matter of public health concern since some species of *Aspergillus* and *Penicillium* produce mycotoxins. In 1996, Kpodo et al. reported the presence of aflatoxin producing *Aspergillus flavus* and *Aspergillus parasiticus* and citrinin-producing *Penicillium citrinum* in maize samples in Ghana.

Bacillus species

All representative isolates of Gram positive and catalase positive rods on Dextrose Tryptone Bromcresol Purple Agar hydrolysed casein, starch and gelatin; grew at pH 5.7, and 6.8; and in 7% and 10% NaCl. Eight out of ten representative isolates were not able to grow at neither 10, 50 nor 65°C. They were able to produce acid from D-glucose, L-arabinose, D-xylose and D-mannitol. They fermented amygdaline, arabinose, cellubiose, fructose, glucose, mannitol, D-mannose, melizitosse, melibiose, raffinose, ribose, salicin, sorbitol, sucrose, and trehalose but not galactose, gluconate and lactose. In the API 50 CHB kit, most of these isolates fermented ribose, alpha methyl-D-glucoside, amygdalin, esculin, salicin, L-arabinose, cellobiose, maltose, saccharose, trehalose, inuline, amidon, glycogen, glycerol, D-xylose, D-glucose, D-fructose, D-mannose, inositol, mannitol, sorbitol, arbutin, D-turanose, melibiose, raffinose, glycogen and beta gentioniose. These isolates were identified as *Bacillus subtilis* by reference to the Bergey's Manual and the API database. The other two representative isolates were identified as *Bacillus cereus* based on the results of the biochemical tests including the pattern of carbohydrate fermentation in the API kit.

The *Bacillus* population of *sure, sobolo, daala, yowudo*, and *kuuka* were dominated by *B. subtilis* whereas the okro products (okro leaves, okro pods and okro powder) were dominated by *B. cereus*. The predominance of *Bacillus subtilis* in the *Bacillus* population of the dried vegetables is not surprising since *B. subtilis* has been isolated in several foods in Ghana and is even reported to be responsible for the spontaneous fermentation of soybeans and African locust beans into *dawadawa* and texture modification during the fermentation of cassava meal into *agbelima* and *akyeke* (Amoa-Awua and Jakobsen 1995; Obilie et al., 2003; Dakwa et al. 2005; Terlarbie et al., 2006; Pakouda et al., 2009)

Lactic acid bacteria

All Gram-positive catalase-negative cultures isolated from the dried vegetables on MRS were presumed to be LAB and were mostly facultative heterofermentative rods because of their ability to produce CO_2 from glucose but not gluconate. Representative isolates of these cultures were found to be non-oxidative; grew at pH 4.4 and 9.6 but not at 10°C and 45°C or in 6.5% and 18% NaCl and were confirmed as *Lactobacillus* species according to Kandler and Weiss (1986) and Axelsson (1993). The results of the carbohydrate fermentation profiles showed that the dominant species was *Lactobacillus plantarum* (results not shown). *L. plantarum* dominated the LAB population of all the dried vegetables examined and this was not surprising because it appears to be ubiquitous in the Ghanaian environment. *L. plantarum* is reported to be dominant or an important component of the lactic acid bacteria population responsible for the spontaneous fermentation of several Ghanaian indigenous foods and others including agbelima, gari, nixtamalized maize dough, palmwine, maize dough, and cocoa (Halm et al., 1993; Amoa-Awua et al., 1996; Obilie et al., 2004; Sefa-Dedeh et al., 2004; 2004; Amoa-Awua et al ., 2005; Kumi, 2005; Nielsen et al., 2006).

Coliforms

Isolates on Violet Red Bile Agar which were purple-red in colour, Gram negative, non-

sporeforming rods and fermented lactose with formation of gas within 48 h at 35°C were identified as coliforms (Prescott et al., 1996). Isolates from seven representative samples were identified as *Enterobacter aerogenes* using the MICROID and API®20E databases (Table 4). The remaining three representative isolates were tentatively identified as *Kluyvera* spp. based on biochemical profiles in the API 20E database. *Enterobacter aerogenes* was detected in all the dried traditional vegetables whilst *Kluyvera* spp. was isolated from *shuri*, and *sobolo*. Despite the high levels of coliforms in the dried vegetables (10 CFU/g to 10^7 CFU/g), *E. coli* was not detected in any of the samples.

Conclusion

The microbiota in dried traditional vegetables sold on the open markets in parts of Ghana are dominated by aerobic mesophiles including spore forming bacteria mainly *Bacillus* spp., lactic acid bacteria, coliforms and moulds including *Aspergillus* spp., *Rhizopus* spp., *Eurotium* spp., *Penicillium* spp. and *Aureobasidium* spp. These occur in high numbers and there is the need to sanitize the vegetables before drying, improve the drying environment and employ Good Hygienic Practices in order to reduce the high microbial counts in the dried vegetables.

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