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Microbiological and Nutritional Quality of Hawked Kunun (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria

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Abstract: The microbiological and nutritional quality of freshly processed and hawked kunun drinks in South Western Nigeria was investigated at Ibadan, Nigeria. The microbes found associated with both the hawked and the laboratory prepared kunun samples are *Lactobacillus plantarum*, *Bacillus sutilis*, *B. cereus*, *Streptococus feaceaum*, *S. lactis*, *Staphylococcus aureus*, *Micrococcus acidiophilis*, *Escherishai coli*, *Pseudomonas aureginosa*, *Saccharomysces cerevisiae*, *Candida mycoderma*, *Aspergillus niger*, *Penicillium oxalicum* and *Fusarium oxysporum*. However, the freshly processed kunun drinks harbored no coliform bacteria. The crude protein content of the hawked kunun drinks was found higher than that of the laboratory processed kunun samples, while the P^{H} of the Kunun zaki drinks were highest in the laboratory processed samples. However, there were no significant differences between the carbohydrates contents of the laboratory processed kunun drinks sample and that of the hawked kunun drinks

Key words: Kunun zaki, coliform, hawked kunun drinks

Introduction

Food is essential ingredients for sustenance of life either of plants or animals. Its demand can therefore, not be overemphasized. In Countries like Nigeria, people depends mostly on indigenous technology for food preparations especially food of plant origin. Some of the food that originates from plant includes beverages such as Zobo and Kunun drinks.

Kunun is the non-alcoholic fermented beverages widely consumed in the Northern parts of Nigeria. This non-alcoholic beverage is however becoming more widely accepted in several other parts of Nigeria, owing to its refreshing qualities. Beside the fact that the two dominants religious group, Christians and Muslims use it as a substitute for alcoholic ones and the fact that it is very nutritious and medicinal (Bestshart, 1982). Kunun is consumed anytime of the day by both adult and children as breakfast drink food complement, It is a refreshing drink usually used to entertain visitors appetizer and is commonly served at social gathering. While Onuorah *et al*. (1987) reported Kunun as being regarded as after meal drinks or refreshing drinks in rural and urban centers. Although there are various types of Kunun processed and consumed in Nigeria which include Kunun zaki, Kunun gyada, Kunun akamu, Kunun tsamiya, Kunun baule, Kunun jiko, Amshau and Kunun gayamba. However, kunun zaki was most commonly consumed.

Kunun-zaki processed from sorghum grains contains 11.6% protein, 3.3% fat, 1.9% ash and 76.8% carbohydrate and arrays of amino acid (Lichtenwalner *et al*., 1979). Its processing is mostly done by women using simple household equipment and utensil. The processed Kunun is usually packed for sale either in cellophane sealed packs bottle or in bulk in large container and distributed under ambient temperature or cooled in a refrigerator where available.

According to Odunfa and Adeyeye (1985) the traditional processing of Kunu involves the steeping of millet grains, wet mill with spices (ginger, cloves pepper), wet sieving and partial gelatinisation of the slurry, followed by the addition of sugar and bottling. Brief fermentation usually occurs during kunun processing. This briefly fermentation which usually occurs during steeping of the grains in water over a 8-48hrs period is known to involve mainly lactic acid bacteria and yeast (Odunfa and Adeyeye, 1985).

The preparation of this beverage has become technology in many homes in the rural communities and more recently in the urban areas where more women have developed the skill and commercial production has helped to alleviate poverty among the people.

In Nigeria, many women have been able to set up small scale commercial production of kunun due to support from the government through the poverty alleviation scheme.

However, due to the involvement of several people including unemployed school leavers in the production of kunun for commercial purposes, it's processing is highly prone to microbial contamination. A large number of lactic acid bacteria, coliforms, molds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content for fermentation processes undesirable (Odunfa, 1988; Ojokoh *et al*., 2002; Amusa *et al*., 2005).

In developing nation like Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequate

knowledge of food processing and handling practices, there is likely to a high risk of chemical and microbial contamination.

In most Nigeria cities, the sales and consumption of this locally made beverage is high due to the high cost of other non-alcoholic drinks. However, this drink is usually hawked in the motor parks, school premises and market places. This research was conducted to investigate the microbiological and nutritional qualities of this hawked non-alcoholic drink called Kunun in south western Nigeria.

Materials and Methods

Hawked Kunun drinks samples were purchased from four locations in each state comprises of Apata, Challenge, mokola, Dugbe in Oyo State, Okelewo, Sabo, Ita Eko and Lafenwa in Ogun state, Alekuodo, Ayetoro, Oja Oba and Fagbewesa in Osun State and Oshodi, New garage, Bariga and Ojota in Lagos State all in southwestern Nigeria.

Five samples were purchased in each location, properly labeled and placed in plastic containers. These samples were respectively brought to the microbiology laboratory at the Institute of Agricultural Research and Training, Obafemi Awolowo University (IAR and T/OAU) Moor Plantation, Ibadan, Nigeria for microbiological analysis, while the biochemical analysis was carried out at the Livestock laboratory of the same Institute. The experiment was conducted between July and Oct. 2004 and repeated in 2005. A Kunun drink sample was also prepared in the laboratory using Sorghum obtained from IAR and T research farm. This drink sample was also subjected to the same analysis.

Isolation of microbe associated with Kunun zaki drinks: Ten fold dilutions of each kunun samples were made using peptone water. Appropriate dilutions were made and 0.1 mL of the diluted samples were pour plated in triplicate plates on Plate Count Agar (PCA) for viable count, Eosin Methylene Blue (EMB) for *Escherichia coli* count, Manitol slat Agar (MSA) for Staphylococcus count and Briliant bile broth (BGBB) for coliform test. Sabourand Dextrose Agar, with Chloramphenicol (250mg $100mL^{-1}$) was used for fungi, while for yeast count he medium was adjusted to P^H 3.5 with tartaric acid. All plates were incubated for 48hours at 30°C except for sabourand Dextrose Agar that were incubated at 26°C for 6 days Colonial counts were made using digital illuminated colony counter (Gallen kamp model) Pure cultures of each isolates were obtained by streaking the specific colonies on suitable media and incubated appropriately, these were then maintained in an agar slants in McCarthney bottles.

Identification of the microbial isolates: Identification was on the basis of presence and characteristics of typical structures such as conidia and hypha (Barnett and Hunter, 1972). Isolation and identification of bacteria in both freshly prepared and hawked Kunun drinks were done using methods described by Harrigan and McCance (1976) ICSMF (1988), Colins and Lyne (1984), Adegoke *et al.* (1993).

The associated fungi were identified with reference to Frazier and Westhoff (1978), while the yeast were identified using the methods of Beech *et al.* (1986) and Lodder (1970). The identity of the microbes were further confirmed by comparison with existing cultures already identified by the Mycological Institute, Kew, London Obtained from the Institute of Agric. Research and Training, Moor plantation Ibadan, Nigeria. The P^H of the samples were determined using P^H meter (Tritrimeter U9N model) The moisture content and the titratable acidity were determined as described by Egan *et al.* (1981).

Other analysis carried out on the Kunun drink samples include the p^H, the % ash, % moisture content, % total solid, protein, total titrable acidity and ether extract. (AOAC) 1990.

The data collected were subjected to the analysis of variance and mean separation was performed using Statistical Analysis Software (Software SAS, 1993).

Results

The highest crude protein % was found in the hawked Kunun zaki samples obtained from Okelewo in Ogun State area while the least was found in the Fagbewesa in Osun State (Table 3). The P^H of hawked Kunun zaki drinks were highest in the laboratory processed kunun samples while the least was found in hawked kunun drink samples obtained from Alekuodo in Osun State metropolis respectively (Table 3). The carbohydrate content of the hawked Kunun drink samples ranges from 85.30 to 83.04% while that of the laboratory prepared sample was 83.0% (Table 3). There was no significant differences between the carbohydrates content of the laboratory processed kunun drinks sample and that of the hawked kunun drinks. The Titratable acidity (TTA) was found to be 0.29% in the laboratory prepared sample while it ranges between 0.30% and 0.63% in the hawked samples.

Result of the experiment indicated that the highest crude protein content was found in the hawked Kunun zaki samples obtained from Okelewo in Ogun State area while the least was found in the Fagbewesa in Osun State (Table 1). The P^H of hawked Kunun zaki drinks were highest in the laboratory processed kunun samples while the least was found in hawked kunun drink samples obtained from Alekuodo in Osun State metropolis respectively (Table 1). The carbohydrate content of the hawked Kunun drink samples ranges

Table 1: The Nutritional qualities of Hawked Kunun-zaki in selected locations in southwestern Nigeria

States	Location	P^H	TTA	%CP	%EE	%ASH	%TS	%MC	%CHO
OYO	Apata	5.65[b]	0.62[a]	2.68[b]	0.40[a]	0.20[b]	12.45[ab]	86.25[a]	84.75[ab]
	Challenge	5.60[b]	0.43[b]	2.70[a]	0.41[a]	0.21[ab]	12.12[b]	87.49[a]	84.79[ab]
	Mokola	5.75[a]	0.60[a]	2.65[bc]	0.39[a]	0.18[c]	12.64[b]	87.37[a]	83.84[a]
	Dugbe	5.43[c]	0.30[c]	2.63[c]	0.40[a]	0.22[a]	13.45[a]	86.00[a]	83.21[a]
OGUN	Okelewo	5.72[a]	0.43[b]	2.81[a]	0.42[c]	0.23[b]	13.34[a]	84.65[b]	85.30[a]
	Sabo	5.45[c]	0.30[c]	2.74[c]	0.61[a]	0.24[b]	12.24[c]	87.97[a]	84.56[a]
	Ita Eko	5.47[c]	0.31[c]	2.77[b]	0.60[a]	0.26[a]	12.35[c]	87.91[a]	84.47[ab]
	Lafenwa	5.64[b]	0.60[a]	2.80[a]	0.58[ab]	0.27[a]	12.68[b]	87.33[a]	84.76[ab]
OSUN	Alekuodo	5.23[d]	0.43[b]	2.63[c]	0.64[a]	0.27[b]	13.83[a]	86.17[a]	83.22[a]
	Ayetoro	5.42[c]	0.44[b]	2.72[a]	0.57[c]	0.30[ab]	12.84[c]	87.16[a]	84.29[a]
	Oja-Oba	5.51[b]	0.44[b]	2.68[b]	0.58[c]	0.35[a]	12.14[d]	87.86[a]	84.48[a]
	Fagbewesa	5.65[a]	0.61[a]	2.60[d]	0.58[c]	0.33[a]	13.38[b]	86.36[a]	83.64[a]
LAGOS	Oshodi	5.34[a]	0.62[a]	2.70[b]	0.63[a]	0.17[c]	12.07[cd]	88.93[a]	84.58[a]
	Newgarage	5.36[a]	0.63[a]	2.68[c]	0.54[c]	0.18[c]	12.25[c]	87.94[a]	84.36[a]
	Bariga	5.35[a]	0.43[b]	2.75[a]	0.57[b]	0.16[c]	13.27[b]	86.73[a]	83.42[a]
	Ojota	5.37[a]	0.43[b]	2.68[c]	0.58[b]	0.14[d]	13.58[a]	85.95[ab]	83.04[a]
	Control	5.65[a]	0.29[c]	3.83[a]	0.55[c]	0.16[c]	13.55[a]	86.30[a]	83.00[a]

Table 2: The incidence of Bacteria found associated with hawked kunun zaki in major locations in south western Nigeria

Location	*Bacillus subtilis*	*Bacillus cereus*	*Lactobacillus* plantarum	*Streptococcus feacium*	*Staphylococcus aureus*	*Micrococcus acidiophillus*	*Escherishia coli*	*Pseudomonas aureginosa*	*Streptococcus lactis*
Apata	+	-	+	+	+	+	-	-	+
Challenge	+	+	+	+	+	+	+	+	+
Mokola	+	+	+	+	+	-	-	+	+
Dugbe	+	+	+	-	+	+	-	-	+
Okelewo	+	+	+	+	+	+	-	-	+
Sabo	-	-	+	+	+	+	-	-	+
Ita Eko	-	-	+	+	+	-	+	+	+
Lafenwa	+	-	+	+	+	+	-	+	+
Alekuodo	+	+	+	-	-	-	-	-	+
Ayetoro	+	-	+	-	+	+	+	-	+
Oja-Oba	+	-	+	+	+	+	-	+	+
Fagbewesa	+	-	+	-	+	+	-	+	+
Oshodi	+	+	+	+	-	+	-	+	+
Newgarage	-	-	+	+	+	+	+	-	+
Bariga	+	-	+	+	+	+	-	-	+
Ojota	+	-	+	+	+	+	+	-	+
Control	+	-	+	-	-	+	-	-	+

+ = present- = absent

Table 3: The incidence of moulds and yeasts found associated with hawked kunun zaki in major locations in south western Nigeria

Location	*Aspergillus. niger*	*Penicillium oxalicum*	*Fusarium oxysporum*	*Candida mycoderma*	*Saccharomyces cerevisiae*
Apata	+	-	+	-	+
Challenge	-	+	-	+	+
Mokola	-	-	+	+	+
Dugbe	+	-	-	-	+
Okelewo	-	-	+	+	+
Sabo	+	+	+	-	+
Ita Eko	-	+	-	+	+
Lafenwa	+	-	+	-	+
Alekuodo	-	-	-	+	+
Ayetoro	-	-	+	-	+
Oja-Oba	+	+	+	+	+
Fagbewesa	-	+	-	+	+
Oshodi	+	-	+	-	+
Newgarage	+	+	+	+	+
Bariga	-	+	-	-	+
Ojota	-	+	+	+	+
Control	-	-	-	+	+

from 85.30 to 83.04% while that of the laboratory prepared sample was 83.0% (Table 1). The Titratable acidity (TTA) was found to be 0.29% in the laboratory prepared sample while it ranges between 0.30% and 0.63% in the hawked samples.

Ten different microbes were found associated with the Hawked (marketed) Kunun zaki in south western Nigeria, while the laboratory processed samples harboured six microbes (Table 2 and 3). These microbes include *Lactobacillus plantarum*, *Bacillus*

Table 4: The total microbial load of hawked Kunun-zaki in major locations in southwestern Nigeria

State	Mean total viable count (cfu $mL^{-1}\times10^6$)	Mean total coliform count (Cfu $mL^{-1}\times10^6$)	Mean yeast count (cfu $mL^{-1}\times10^6$)	Lactic acid bacterial count (cfu $mL^{-1}\times10^6$)
Oyo	6.8±0.24	5.0±0.17	22.21±0.21	57.25±0.31
Ogun	7.8±0.27	2.2±0.15	23.23±0.34	49.65±0.27
Osun	8.8±0.21	5.6±0.21	21.86±0.41	53.32±0.12
Lagos	6.3±0.19	5.2±0.31	20.54±0.22	54.2±0.31
Control	2.2±0.31	Nill	21.4±0.12	47.32±0.11

+ = present- = absent

sutilis, *B. cereus*, *Streptococus feaceaum*, *S. lactis*, *Staphylococcus aureus*, *Micrococcus acidiophilis*, *Escherishia coli*, *Pseudomonas aureginosa Saccharomysces cerevisiae*, *Candida mycodema*, *Apergillus niger*, *Penicillium sp and Fusarium oxysporum*. However, the incidence of the associated microbes was higher in the marketed Kunun zaki compared to the laboratory-processed samples. In both samples *Saccharomyces cerevisae* and *L. plantarum* had the highest rate of occurrence followed by *M. acidiophillus*, *S. lactis* and *Bacillus subtilis*, while the least was *E. coli*.

The highest mean bacterial count was recorded on samples obtained from Abeokuta in Ogun state (7.8×10^7 cfu mL^{-1}) while the least mean bacterial count of 2.2×10^7 cfu mL^{-1} was found in the laboratory processed samples (Table 4). The coliform count ranges from 5.2×10^6 cfu mL^{-1} on hawked kunun samples from Lagos to 2.2×10^6 cfu mL^{-1} obtained from Abeokuta in Ogun state. While no coliform was found associated with the laboratory processed samples. The highest yeast count was obtained from the hawked Kunun samples from Ogun State with 23.23×10^6 cfu mL^{-1} while samples from Lagos harbored the least yeast population (Table 4).

Discussion

Result of the experiment indicated that the highest crude protein content was found in the hawked Kunun zaki samples compared to the laboratory prepared samples. Reasons for this might have been as a result of some of the additives added to the processed kunun samples. However, the protein contents of these Kunun drinks were very low probably because most of it might have been loss during processing. According to Hamad and Fields (1979), much of the protein in cereals is usually located in the testa and germ which are usually sifted off during processing; However, Lichtenwalner *et al.* (1979) had also reported that the protein content of sughum based kunun gruel was 11.6%.

The P^H Kunun zaki drinks were highest in the laboratory processed kunun samples. Reasons for this finding might not be connected with the fact that fermentation might have taken place in the hawked kunun samples since there is usually a time lag between the time of processing and the time of hawking especially if the sale of the drink is not fast. There was no significant differences between the carbohydrates content of the laboratory processed kunun drinks sample and that of the hawked kunun drinks. Reasons for the above might be due to the fact that kunun drinks all over the southwest is prepared from virtually same variety of Sorghum which has similar carbohydrate content.

The microbes found associated with both the hawked and the laboratory prepared kunun samples comprises of bacteria which includes, *Lactobacillus* plantarum,, *Bacillus sutilis*, *B. cereus*, *Streptococus feaceaum*, *S. lactis*, *Staphylococcus aureus*, *Micrococcus acidiophilis*, *Escherishai coli*, *Pseudomonas aureginosa*, the mould which are, *Apergillus niger*, *Fusarium oxysporum* and *Penicillium oxalicum* and the yeast which includes *Candida mycoderma* and *Saccharomyces cerevisiae*. The presence of some of these organisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates such as sugars which often led to the production of acids after fermentation. Odunfa and Adeyeye (1985) reported that *L. plantarum* was the predominant organism in the fermentation responsible for lactic acid production. While, *S. lactis and Micrococcus acidiophilis* are known to be involved in fermentation of agricultural produce. The presence and the activities of these fermenters might be responsible for the souring of taste usually observed if not consumes within six hours of processing Rhodes and Fletcher (1966) found that *Bacillus* and *Lactobacillus* species were readily found in foods of low acid content like juices and beverages where they produce organic acids.

Bacillus species are spore formers whose spores could survive high temperatures of processing. The thermoduric nature of the spores of these microbes ensures survival at pasteurization temperatures and hence their presence in the Kunun samples that have been subjected to heat treatment during processing.

Staphylococcus and *Psuedomonas* species were possible contaminants from handlers and utensils used especially after the processing, as they are mesophiles though some *Pseudomonads* are spoilage organisms at refrigerated temperatures. The presence of *Pseudomonas spp.* and *Bacillus subtilis* in the marketed Kunun drink might be responsible for changes in taste which normally occurs if not consumed within few hours of production. The ropiness associated with the fermented drink has been associated with the presence of both *Pseudomonas spp.* and *Bacillus subtilis* (Adegoke *et al.*, 1993).

Some of these associated microbes have been implicated in food poisoning outbreak of some food materials (Sartory and Howard, 1992). The presence of *Escherichia coli* in food is an indication of faecal contamination of product. However, Odunfa (1988) reported that *Staphylococcus* spp levels of 10^8 mL^{-1} is considered potential hazardous to consumers. According to Sartory and Howard (1992), the presence of *E. coli* in water indicates faecal contamination and most of the coliforms found associated with the hawked Kunun are known to be causative agents of food borne gastroenteritis and bacterial diarrhoea diseases (Jiwa *et al*., 1981; Onuorah *et al*., 1987).

The presence of *Aspergillus niger*, *Penicillium oxalicums* and *Fusarium oxysporum* in the kunun samples might not be too surprising as they are known common spoilage organisms of carbohydrate foods as well as storage micro flora of many cereals including sorghum (Rhodes and Fletcher, 1966).

These fungi also have high survival rate of their spores hence their presence in these food drinks could have emanated from the air as air spora since most of the hawked kunun drinks are not usually well covered. The presence of *S. cerevisae and C mycoderma* in both the hawked and freshly prepared kunun samples is probably because they played a significant role in the flavor development (Odunfa and Adeyeye, 1985). Osuntogun and Aboaba (2004), had earlier reported the association of *Penicillium spp*, *Aspergillus spp Lactobacillus spp* and *Streptococcus sp* from Kunun drink samples. However, Ojokoh *et al*. (2002) isolated *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus flavus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsiella* sp from fermented zobo drinks.

The presence of coliform bacterial in the hawked kunun drinks in Nigeria is a source of concern because the teaming populace relies on these drinks as alternative to the bottled soft drinks whose price is becoming unaffordable.

It is therefore suggested that kunun drinks should be properly processed to avoid microbial contamination. While treated municipal water or clean water should be used during for processing and dilution of the processed drinks to avoid contamination with enteropathogenic bacteria. Since spices have been reported to inhibit microbial growth (Zaika *et al*., 1983; Adegoke and Skura, 1994). Oboh and Elusiyan (2004) had reported that fortification of zobo drinks with pineapple juice and lemon grass greatly enhanced the inhibition of the growth of contaminating pathogenic bacteria like *P. aeruginosa*. Adebesin *et al*. (2001) reported that addition of some spices to groundnut products reduced the microbial load significantly. Hence the addition of spices to the processed kunun drinks is highly advocated. The processing environment should be hygienic, while the packaging materials should be sterilize and additives such as sugar, ginger and other coloring materials should be sterilized. Health education training should be organized regularly for the processors by the health workers on the importance of cleanness of their environment.

Food products that must be developed to improve the nutritional status of the populace in the developing world must be relatively cheap and nutritionally standardized. Hence, kunun zaki drink which is highly relished by both young and adult and also reported to have therapeutic properties (Bestshart, 1982) beside being one of the popular weaning food in African countries is therefore being recommended for industrial production. It should however be pasteurized, bottled and probably chilled. If properly processed and packaged it will not only alleviate the longing for fluid intake in warm tropical climates but would also provide a cheaper and more nutritive drink than the sugar laden fizzy (soft) drinks in the market.

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