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Antibiotic sensitivity pattern of bacterial isolates and physico-chemical composition of maize flour sold in major markets in Benin City, Midwestern Nigeria

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SUMMARY. Samples of maize flour sold in selected markets in Benin City, Midwestern Nigeria were evaluated for antibiotic sensitivity pattern, bacteriological and physico-chemical qualities. The adoption of pour plate technique revealed a relatively high bacterial count in order of 10 - 10^3. Nil *Salmonella* counts were recorded in this study. *Bacillus* spp. was the most predominant (41.6 %) and the least predominant among the bacterial isolates was *Pseudomonas aeruginosa* (7.9 %). The various isolated bacteria showed variable patterns to the evaluated antibiotics, with zones of inhibition ranging from 0 mm to 20 mm. pH value ranged from 6.3 to 6.5; percentage moisture content ranged from 11.87% to 12.31%. There was a slight variation in the titratable acidity of the samples (2.10 to 2.56). Percentage fat content ranged from 5.10 % to 5.32 %, while the percentage protein and ash contents had a range of 8.45 % to 9.0 % and 0.85 % to 1.28 % respectively. This study revealed that maize flour from Benin metropolis markets harbored high bacterial counts with an array of antibiotic resistant bacteria. From a public health point of view, the bacterial quality of this relish food item sold in Benin City markets is indeed alarming and as such stringent measures should be adopted to manage the quality and curtail its possible role increasing the incidence of antibiotic resistance among population. However, this flour type was also observed to possess good basic dietary nutritional requirement (pH, moisture content, protein, fat and ash).

Keywords: antibiotic-sensitivity-pattern, bioteriological, maize-flour, markets, physico-chemical.

Introduction

Flour has been described as a microbiologically safe product due to its low water activity property (ICMSF, 1998); however, this property excludes the growth and survival of pathogenic bacteria that contaminate flour (Berghofer *et al*., 2003). Deibel and

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Swanson (2001) reported that cereals and cereal products is a notable food resource for the world's population and also a substrate for the proliferation of several spoilage micro-organisms under improper storage conditions. Nutritious traditional cereal foods are well sort after delicacies and they play very important role in diets in the South-Western geopolitical zone of Nigeria in particular and cereal producing zones of Africa in general.

Nutritionally, flour is known to contain a high proportion of carbohydrates, and relatively low in minerals, fats and proteins (Effiuvwevwere and Akoma, 1997; Batool *et al*., 2012), with a percentage moisture content ranging from 11 – 14 % (Batool *et al*., 2012) but the stipulated limit is 15% (WFP, 2012).

Maize flour is produced by the removal of the outer skin of the seed kernels. The removal of the outer skin of the grain during milling reduces the microbial load, ash protein and fat content (Fandohan *et al*., 2006). This has a profound effect on the physico-chemical and microbiological quality of the flour. Flour with low ash content (biomass), like extra super maize meal (refined) and cake flour have reduced fat and protein content and concomitantly low microbial load (Sperber, 2007).

The main aim of this study was to evaluate the antibiotic sensitivity pattern of maize flour borne bacterial isolates and ascertain the physicochemical qualities of maize flour sold in some major markets in Benin City, Edo State, Nigeria.

Materials and methods

Sources and collection of samples

A total of one hundred (100) samples were collected for this study from five (5) selected markets with Geographical Position System (GPS), namely; Uselu (N06.37359°, E005.61517°), Oka (N06.29031°, E005. 66377°), Ekiosa (N06.32025°, E005.63661°), Ekiuwa (N06.35192°, E005.61398°) and New Benin Market (N06.35168°, E005.63047°), located in Benin City, Edo State, Nigeria (Figure 1). Adopting standard aseptic and safety precautions, twenty (20) samples were collected per market (approximately 500g/product) using sterile polyethene bag. They were transferred to the laboratory for analysis within 24 hrs.

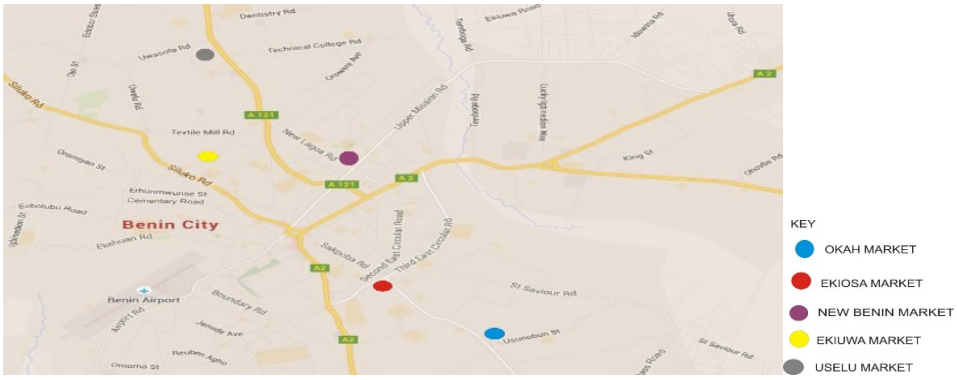

Figure 1. Map of studied area showing sampling markets (Credit: Google)

Enumeration and cultural methods

Twenty grams of each maize flour sample was aseptically weighed and homogenized with 180ml of sterilized 0.1% peptone water to produce a 10^1 homogenate. Subsequent serial dilutions up to 10^6, were aseptically made from the initial homogenate. The total heterotrophic bacterial counts were determined by the pour plate technique using 1.0ml of appropriate serially diluted samples in nutrient agar (Oxoid) and incubated at 30°C for 48 hrs. For total coliform plate counting, 1.0ml of the serially diluted samples were plated on MacConkey Agar (Oxoid) and incubated at 37°C for 24 hrs. Total Staphylococcal plate counts were determined using mannitol salt agar (Oxoid) at 37°C for 72hrs. Total *Salmonella* counts were determined by homogenizing 25 g of each maize sample into 225 ml lactose broth for 2 mins and then incubated for 24 hrs at 37 °C. This was followed by subculturing of 1 ml of the overnight incubated culture into selenite F broth (Oxoid) and was incubated for 24 hrs at 37 °c. After which 1 ml of pre-enriched culture was re-suspended into deoxycholate citrate agar (DCA) (Oxoid) for 24 hrs at 37°C. Triplicate plates of appropriate dilutions were made. The means of colony counts were used to compute colony forming units per gram (cfu/g).

Purification and identification of isolates

Bacterial purification was done by sub-culturing the various isolates onto nutrient agar plates and Gram-stained (Cheesbrough, 2000). Phenotypic profiling of both Gram-positive and Gram-negative bacteria was undertaken using API 50CHB and API 20E strips (BioMerieux, Marsielle, France). Additional tests of spore stain and oxidase were also performed.

Antibiotic sensitivity assay

Antibiotic sensitivity patterns of the isolated bacteria were determined by disc diffusion method (Cheesbrough, 2000). The test bacterial suspension were inoculated unto Muller-Hinton agar and followed by application of the discs (Oxoid) impregnated with different antibiotics. Antibiotic disc contained the following antibiotics: Amoxicillin (25µg), Streptomycin (10 µg), Ceftriaxone (30 µg), Ofloxacin (5 µg), Gentamicin (10µg), Cotrimoxazole (25 µg), Pefloxacin (5 µg), Ciprofloxacin (10 µg), Erythromycin (10µg), Chloramphenicol (30µg).The diameter of the zone of inhibition of each antibiotic disc was measured and recorded (mm).

Determination of physicochemical qualities

pH, titratable acidity, moisture, protein, fat and ash content were analyzed according to AOAC (1998).

Results and discussion

Generally, the results indicated a high total viable count (Table 1A). The mean range of bacterial counts was in the order of 10 - 10^3. The mean total heterotrophic bacterial counts ranged from 5.0×10^3 cfu/g to 7.1×10^3 cfu/g. The mean total coliform counts ranged from 3.0×10^2 cfu/g to 4.2×10^2 cfu/g, while the mean staphylococcal counts ranged from 3.0×10^2 cfu/g to 4.1×10^2 cfu/g. Nil *Salmonella* counts were recorded. Ntuli *et al*. (2013) reported that flour is known to be a safe commodity due to its low water activity, but the current study revealed the presence of microbial contaminants in the retail maize flour which are of public health concern. The high bio-load and variety of microorganisms detected and isolated from these market maize flours may be attributed to the poor sanitary and handling practices following production. These production practices may include spreading on the floor, measurement with the aid of bare hands, coughing, sneezing, exposure of the flour to moisture during retailing and others as strong contribution factors (Imarhiagbe and Emoghene, 2006). The result from this study is in agreement with previous works done by Ntuli *et al*. (2013), Aydin *et al*. (2009) and Berghoefer *et al*. (2003). Total bacterial counts and coliform counts are important parameters which are indicative of the hygienic properties of the food. *Salmonella* spp. in food items is known to account for more than 50 % of all food poisoning cases (WFP, 2012). *Salmonella* spp. causes diseases such as typhoid, paratyphoid and food poisoning (Prescott *et al*., 2002).

Table 1A.

Microbial load of maize flour in selected Benin City Markets

Markets	Total Heterotrophic Count (x 10^3 cfu/g dry weight of flour)	Total Coliform plate Count (x 10^3 cfu/g dry weight of flour)	Total Staphylococcal Count (x 10^3 cfu/g dry weight of flour)	Total *Salmonella* Count (x 10^3 cfu/g dry weight of flour)
Uselu	7.1	4.2	4.1	0.0
Oka	6.5	3.1	3.0	0.0
Ekiosa	5.2	3.0	3.0	0.0
Ekiuwa	5.0	3.0	4.0	0.0
New Benin	6.7	3.7	3.6	0.0

Table 1B revealed the percentage frequency of occurrence of bacterial isolates obtained from the maize flour samples. The result showed that *Bacillus* spp. was the most predominant (41.6 %) followed by *Enterobacter aerogenes* (16.9 %), *Micrococcus* sp. (13.5 %), *Staphylococcus* spp. (11.2 %), *Staphylococcus aureus* (9.0 %) and the least predominant among the bacterial isolates was *Pseudomonas aeruginosa* (7.9 %). Most of the bacteria isolated in this study are enteric organisms and also found in common proportion in soil, hence this food type and other cereal products are prone to microorganisms during pre- and post-harvesting stages. The presence of high counts of *Bacillus* spp., *Staphylococcus* spp., may be related to their ability to withstand dryness

and other harsh environmental conditions such as low water activity (Prescott *et al,* 2002). Some of these organisms may have found their way into this food sample through cross contamination by handlers. Certain strains of *Bacillus* have been known to cause food poisoning (Prescott *et al*., 2002).

Table 1B.

Percentage frequency of the maize flour bacterial isolates

Bacterial isolate	No of isolates	Percentage frequency of occurrence (%)
Bacillus sp.	22	22.5
Bacillus subtilis	19	19
Enterobacter aerogenes	17	16.9
Micrococcus sp.	13	13.5
Staphylococcus aureus	9	9.0
Staphylococcus sp.	11	11.2
Pseudomonas aeruginosa	7	7.9
	n=98	**100**

The measured antibiotic inhibitory zones (mm) revealed variable antibiogram patterns of the tested isolates (Table 2). It was also observed that all the bacterial isolates were sensitive to chloramphenicol with a zone of inhibition ranging from 12 mm to 20 mm, and there were no zone of inhibition around cotrimoxazole (which indicate possible resistance to the antibiotics). Omogbai and Ikenebomeh (2013) reported the increased incidences of bacterial resistance to antibiotics are indicative of the prevailing trend of antibiotic abuse and misuse by the society. This phenomenon is basically due to the fact that individuals purchase and consume antibiotics without necessary medical advice. Bacterial groups co-habiting a common environment may express a similar antibiotics pattern if they share in a common pool of R-factor plasmids (Spanggard *et al.,* 1993).

Table 2.

Diameter (mm) of zones of inhibition of antibiotic

Isolate	AMX 25µg	STR 10µg	CRO 30µg	OFL 5µg	CHL 30µg	GEN 10µg	COT 25µg	PFX 5µg	CPX 10µg	ERY 5µg
Bacillus sp.	0	0	0	0	13	10	0	0	0	0
B. subtilis	0	0	0	0	13	0	0	0	0	0
Enterobacter aerogenes	15	11	0	14	18	18	0	12	8	0
Micrococcus sp.	0	12	0	12	12	0	0	12	0	0
Staph aereus	0	0	0	0	13	0	0	0	0	0
Staph sp.	0	0	0	0	12	0	0	0	0	10
P. aeruginosa	0	0	13	8	20	0	0	12	12	0

KEY: AMX (amoxicillin), STR (streptomycin), CRO (ceftriaxone), OFL (ofloxacin), CHL (chloramphenicol), GEN (gentamicin), COT (cotrimoxazole), PFX pefloxacin), CPX(ciprofloxacin), ERY(erythromycin). B (*Bacillus*), *Staph aereus* (*Staphylococcus aereus), P. aeruginosa (Pseudomonas aeruginosa*)

The physico-chemical qualities of any food items are principal determinants of consumer acceptability and safety. Table 3 showed the physico-chemical composition of maize flour from the selected markets in Benin City. The pH value ranged from 6.3 to 6.5; the percentage moisture content also range from 11.87 to 12.31.The shelf life and microbial growth are basically influenced by factors such as pH and moisture (Batool *et al*., 2012). Thus, pH and moisture are important parameter to be considered in the evaluation of the quality and acceptability of a food item such as maize flour. Also, high moisture content is undesirable in food and it potentates microbial growth (Kordylas, 1990). The percentage moisture content of the samples was relatively high (ranging from 11.87 to 12.31 %). The high count recorded in all samples may be indicative of favorable pH and moisture condition for growth and survival. Flour is susceptible to spoilage especially when stored in moist conditions; especially when stored over a longer period of time (Victor *et al*., 2013). Slight variations were also detected in the titratable acidity of the samples (2.10 to 2.56). Omonigho and Ugbo (1998) reported that increase in titratable acidity might be due to the increased microbial activities of the micro-organisms. Percentage fat content ranged from 5.10 to 5.32, while the percentage protein and ash contents ranged from 8.45 to 9.0 and 0.85 to 1.28 respectively. WFP (2012) had proposed 8.0 % protein content as minimum requirement for maize flour quality. Thus, the relative high protein contents (8.45 to 9.0 %) observed in this study is an indication that maize flours from this region are of good quality. It had been ascertained that ash content in maize flour infers the level of husks (Ekinci and Unal, 2003), and the flour's mineral composition (Victor *et al*., 2013). It was observed that percentage ash content of all analyzed maize flour samples were below the threshold of recommended limit of 3.0 % (WFP, 2012).

Table 3.

Physico-chemical qualities of maize flour sold in Benin City markets

Markets	pH	Titratable Acidity (0.1m NaOH)	Moisture Content	Fat	Protein	Ash
			(%)			
Uselu	6.5	2.43	12.10	5.18	8.72	1.28
Oka	6.5	2.10	12.31	5.32	9.0	1.14
Ekiosa	6.3	2.43	12.00	5.10	8.82	1.21
Ekiuwa	6.4	2.47	11.87	5.29	8.80	0.85
New Benin	6.3	2.56	12.00	5.18	8.45	1.20

NB: Overall mean value

Conclusions

This study revealed that maize flour from Benin metropolis markets harbored high counts of bacteria with array of antibiotic resistant bacteria. When considered from the public health point of view, the bacterial quality of this relish food item sold

in Benin metropolis markets is alarming and as such stringent measures should be adopted to manage the quality and curtail its possible role in increasing the incidence of antibiotic resistance among population. However, this flour type was also observed to possess good basic dietary nutritional requirement (pH, content of moisture, protein, fat and ash).

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