NUTRITIONAL AND MICROBIOLOGICAL ASSESSMENT OF GLUTEN FREE ROASTED MAIZE VENDED AT AROMA AWKA, ANAMBRA STATE

[1]Ibekwe Ifeyinwa M., [2]Chukwumbah Emmanuel C. and [3]Ndife Rachael C.
[1]Department of Nutrition and Dietetic, Anambra State polytechnic Mgbakwu,
[2,3]Department of Science Laboratory Technology, Microbiology Federal Polytechnic Oko
[1]ibekwe@gmail.com 08037571982
[2]chukwumbahemmanuelc@gmail.com 08165604637

Abstract
Maize (Zea mays L.) is one of the most gluten free consumed cereal grains all over the world. The nutritional and microbial composition of gluten free yellow maize (Zea mays) obtained from five different vendors at Aroma, Awka, Nigeria, was determined in this study. The maize samples were collected from the vendors using simple random sampling and analyzed for proximate and microbial composition by standard procedures. The yellow maize samples from showed moisture (5.20 -7.90%), Ash (1.30 to 3.80%), crude protein (5.00 - 6.60%), crude fiber (1.45 to 3.11%) and lipid content (2.00 to 3.91%). Carbohydrate content was found to be high among the samples (74.79 to 84.75 %). The various samples were also found to be contaminated with high microbial load. The total aerobic count of the maize sample ranged from 1.3 x 10^5 to 9.1 x 10^5 cfu/g Vendor (4) and vendor (5) showed higher bacterial count ranging from 4.8 x 10^5 to 9.1 x 10^5cfu/g. The total fungi count range from 1.2 x 10^5 to 8.4 x 10^5cfu/g. The total coliform count ranged from 0to 6.4 x 10^5, Feacal coliforms were observed ranging from 0 to 3.3 x 10^5. Staphylococcuss count ranged from 1.1 x 10^5 to 3.0 x 10^5cfu/g Salmonella spp was isolated in eight samples and Pseudomonas aeruginosa was isolated in three samples. The microbial genera isolated and identified were Staphylococcus aureus, Bacillus subtilis, Escherichia coliSalmonella spp. Pseudomonas aeruginosa, Aspergillus niger, Rhizopus stoloniter, Aspergillus fumigatus and Saccharomyces cerevisae. It is therefore concluded and recommended that, roasted yellow maize are high in nutritive value and could be recommended to celiac patients. Hence good sanitary, processing, packaging and hygienic practices should be employed to reduce the microbial contamination of roasted maize.

Keywords: Nutritional, Microbial, Gluten free, Maize (*Zea mays*)

INTRODUCTION
Gluten free diets are mainly food with zero percent content of food gluten. Gluten is a glycoprotein composed by two components which are gliadin and glutenin. The glutenins occur in two forms, the high- and low-molecular-weight fractions, while the gliadins exist as three structural forms, α-, ω-, and γ-gliadins (Shewry*et al*., 2002; Shewry and

Halford., 2002). Gluten a complex protein is found mainly in cereals like wheat, barley, rye and triticale but absent in maize, soy bean and rice (Raghu et. al., 2017). Gluten is also a nutritional term used to refer to certain cereal prolamins, i.e., the ethanol-soluble proteins of wheat, rye, barley, their cross bred grains, and possibly oats (Wrigley *et al*., 2006; El-Chammas, and Danner 2007; Wieser, 2006; Capriles, and Arêas, 2014). The gluten-free diet encompasses food groups that are naturally devoid of gluten such as fresh fruit, vegetables, seafood, meat, poultry, legumes, nuts and most dairy products (Theethira*et al*., 2015). Gluten-free diet is the only medically accepted treatment for celiac disease (Raghu *et al*., 2017).

Maize (Zea mays L.) is one of the gluten free most consumed cereal grains all over the world, along with rice. Maize (Zea mays L.) is one of the leading cereal grains worldwide, along with wheat and rice (FAOSTAT, 2014). Maize is a major cereal crop for both livestock feed and human nutrition, with its high content of carbohydrates, fats, proteins, some of the important vitamins and minerals, maize acquired a well-deserved reputation as a poor man's nutricereal (RidhiKataria, 2014). A maize kernel composition for the nutritional value of yellow dent corn on a dry matter basis is 71.7% starch, 9.5% protein, 4.3% oil, 1.4% ash, and 2.6% sugar (Watson, 2003). Maize grains are a rich source of starch (72%), ash (17%), protein (10.4%), fiber (2.5%), oil (4.8%), vitamins and minerals (Farhad*et al*., 2009). Nutritionally, the maize kernel, like that of other cereal grains, includes pericarp (6%), endosperm (82%) and germ (12%) (Watson, 1987). The main structural component of the endosperm of maize includes starch, a complex carbohydrate that constitutes an average of 71% of the maize grain and is a source of concentrated energy, with bulk of the proteins in a mature maize kernel in the endosperm and germ (Prasanna *et al*., 2001). However, the germ protein is superior in both quantity and quality (Prasanna *et al*., 2001).

The maize grain accounts for about 15 to 56% of the total daily calories in diets of people in about 25 developing countries, particularly in Africa including Nigeria and Latin America (FAO Agrostat, 1992). The maize, more often than not, is cooked, roasted or sun dried for storage to be processed into desired product such as corn pap, corn flour, pop-corn, canned sweetened corn syrup and other indigenous delicacies.

Roasted maize is a ready to eat snack or food processed by preheating with or without the husk, over arranged rack placed in the middle of an oven preheated below 350°F for 5 to 10 minutes. This improves the flavour and also contributes to browning or caramelisation of the maize kernel. In Nigeria, the enormous yield recorded in maize production annually as a result of mechanized farming and irrigation system have led to maize availability for export, consumption, processing and storage. In southern eastern Nigeria, in Awka metropolis the numerous vendors of processed roasted maize have emerge due to high consumption and profit often recorded.

Due to the reported unhygienic practice observed among local food processors, such food is always contaminated by microorganisms and often poses significant health challenges to the consumers (Badau*et al*. 2018). Carelessness in food practices such as the use of unclean utensils and equipment, raw materials selection, selling of foods in open places are potential threats and so increase risk of public health (Badau*et al*. 2018). Hence, due to high consumption of this maize, this work was aimed to investigate the nutritional and microbial content of gluten free roasted maize vended at Aroma Junction Awka, Anambra State.

Materials and Methods

2.1. Study Area

The study area is Aroma Junction Awka along the (Enugu-Onitsha federal highway), in the Awka South local government area of Anambra State, Nigeria.

2.2 Source of Sample

A total number of 15 maize samples were purchased from five different vendors, along the Enugu-Onitsha highway. The samples were sampled in twelve different sterile containers and labelled. They were taken immediately to the laboratory for analysis.

2.3 Proximate analysis of the seed of Maize (*Zea mays* L)

The Maize (Zea mays L) samples were evaluated as described by the Association of Official Analytical Chemist (AOAC, 2000) as all the chemicals used in this study were of analytical grade. The roasted maize seedlings were analysed for moisture, ash, crude protein, crude fibre, fat or lipid and carbohydrate.

2.4. Media used and Preparation

The media used for the isolation was Nutrient agar (NA), Sabourand Dextrose Agar, MacConkey Agar, Salmonella Shigella Agar, Mannitol Salt Agar, Centrimide agar and Eosine Methylene Blue Agar. This media was prepared according to the manufacturer's instruction.

2.5 Microbial Technique

Using a sterile forceps, ten gram of each sample of maize was weighed and dispensed into 90ml of sterile distilled water as a diluent. Serial dilution was conducted by adding 1ml from tube one into the second test tube and mixed carefully by shaking it gently. After performing the serial dilution, 1ml of 10^2 dilution of the solution was pipette into an empty petri dish. The prepared culture media was allowed to cool at 45°C after sterilization and about 20ml of Nutrient agar, Sabourand dextrose agar, MacConkey agar, EMB agar, Centrimide agar and Salmonella Shigella Agar were poured into a well labelled petri dish containing 1ml of the aliquot of each sample. The plates were swirled

to mix them thoroughly and left to solidify. The plates were incubated at 37°C for 24 hours. The cultures that developed were counted and sub-cultured to obtain pure cultures which were stored on agar slants (Chessbrough, 2005).

2.6. Identification of Microbiological Isolates

Each of the bacterial colonies on the agar plates was sub-cultured and the pure culture obtained. The isolates were identified by cultural, morphological and biochemical characterization carrying out tests which include Gram staining, and biochemical tests (Ndife and Chukwumbah, 2021). For morphological characteristics, a small portion of the discrete colonies on each plate was smeared on a microscope slide with a drop of distilled water added. The smear was gently fixed by heat and immersion oil was dropped on the surfaces and then viewed under x 100 objective lens of the microscope (Chessbrough, 2005). Fungal identification was based on macroscopic and microscopic features of colonies as previously described (Cheesbrough, 2006). The colony counts were expressed as colony forming units per gram (cfu/g) of the sample.

2.7. Biochemical Analysis

The biochemical test carried out included Gram staining reaction, Catalase test, Coagulase test, Indole test, Oxidase test, Citrate utilization test, motility, glucose, sucrose, lactose and lactophenol test for the identification of fungi.

RESULTS:

Table: 1. Nutritional evaluation of roasted yellow maize seeds

Samples	**Moisture** (%)	**Ash** (%)	**Crude fibre** (%)	**Crude protein** (%)	**Crude fat** (%)	**Carbohydrate** (%)
A01	7.80	1.30	1.48	6.00	2.90	80.52
A02	5.50	1.30	1.45	5.00	2.00	84.75
A03	7.90	3.80	1.48	5.30	3.65	77.87
A04	5.20	3.30	2.31	6.60	3.91	78.68
A05	6.00	3.30	3.11	6.30	3.50	77.79

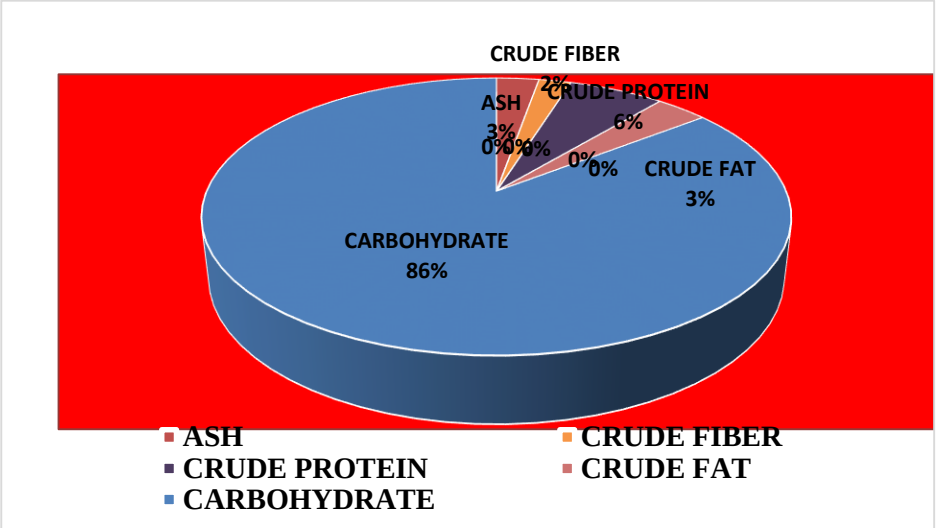

FIG. 1 AVERAGE NUTRITIONAL COMPOSITION OF A YELLOW MAIZE

TABLE: 2. MICROBIOLOGICAL ANALYSIS OF ROASTED MAIZE SAMPLES

Sample	Total Aerobic Bacteria (cfu/g)	TY/MC Total yeast and mold count (cfu/g)	Total coliform count (cfu/g)	Feacal coliform count	*Staphylococcus Spp* count	*Salmonella Spp*	*Pseudomonas Spp*
Vendor 1							
A01	3.1×10^5	1.2×10^5	1.6×10^5	1.3×10^5	1.1×10^5	Present	Absent
B01	3.3×10^5	2.0×10^5	1.3×10^5	1.2×10^5	1.2×10^5	Present	Absent
C01	3.2×10^5	2.3×10^5	1.4×10^5	1.3×10^5	1.1×10^5	Absent	Absent
Vendor 2							
A02	1.9×10^5	1.3×10^5	5×10^4	0	1.0×10^5	Absent	Absent
B02	1.3×10^5	1.3×10^5	3×10^4	0	1.0×10^5	Absent	Absent
C02	2.0×10^5	1.0×10^5	2×10^4	0	1.6×10^5	Absent	Absent
Vendor 3							
A03	2.3×10^5	6.8×10^5	0	1.3×10^5	1.6×10^5	Absent	present
B03	5.5×10^5	3.3×10^5	3.7×10^5	2.3×10^5	1.3×10^5	Present	Absent
C03	2.9×10^5	2.0×10^5	1.2×10^5	1.3×10^5	1.8×10^5	Present	Absent
Vendor 4							
A04	5.6×10^5	3.3×10^5	3.3×10^5	1.0×10^5	1.5×10^5	Absent	Absent
B04	4.8×10^5	3.2×10^5	1.8×10^5	1.2×10^5	2.1×10^5	Present	Present
C04	5.3×10^5	3.1×10^5	1.4×10^5	3.3×10^5	1.3×10^5	Absent	Absent
Vendor 5							
A05	8.3×10^5	6.3×10^5	3.4×10^5	2.3×10^5	3.0×10^5	Present	Absent
B05	9.1×10^5	7.3×10^5	6.4×10^5	2.3×10^5	2.3×10^5	Present	Present
C05	7.5×10^5	8.4×10^5	1.4×10^5	3.3×10^5	2.9×10^5	Present	Absent

Key: cfu/g:- Colony forming unit per gram

TABLE: 3 Biochemical test of bacterial isolates

ISO	Gram	Cat.	Cit.	Coa.	Oxi	Ind.	Mot.	Glu.	Lac	Suc.	Probable org.
1	Rod -	+	+	-	+	-	+	-	-	-	*Pseudomonas aeruginosa*
2	Rod -	-	+	-	-	-	+	+	+	+	*Salmonella spp*
3	Rod -	+	-	-	-	+	+	+	+	+	*Escherichia coli*
4	Rod +	+	+	-	+	-	+	-	-	-	*Bacillus Spp*
5	Cocci+	-	+	+	-	-	-	+	+	+	*Staphylococcus aureus*

KEY: Iso: Isolate, GR: Gram Reactions, Cat: Catalase, Cit: Citrate, Coa: Coagulase, Oxi: Oxidase, Ind: Indole, Mot: Motility, , G: Glucose, L: Lactose, S: Sucrose, Probable Org.: *Staphylococcus aureus, Bacillus Spp, , E. coli: Escherichia coli, Pseudomonas aeruginosa and Salmonella spp,* +Positive, -: Negative, EMB: Eosin methelene blue agar, SSA: Salmonella Shigella agar, Centrimide agar and Mannitol salt Agar.

TABLE4: Frequency Occurrence of Probable Bacterial Isolates

Probable Organisms	Frequency (%)
Escherichia coli	20
Salmonella spp	21
Bacillus subtilis	14
Pseudomonas aeruginosa	5
Staphylococcus aureus	40

TABLE5: Frequency Occurrence of Probable Fungi Isolates

Probable Organisms	Frequency (%)
Aspergillus niger	15
Aspergillus fumigates	10
Rhizopus stolonifer	25
Saccharomyces cerevisiae	50

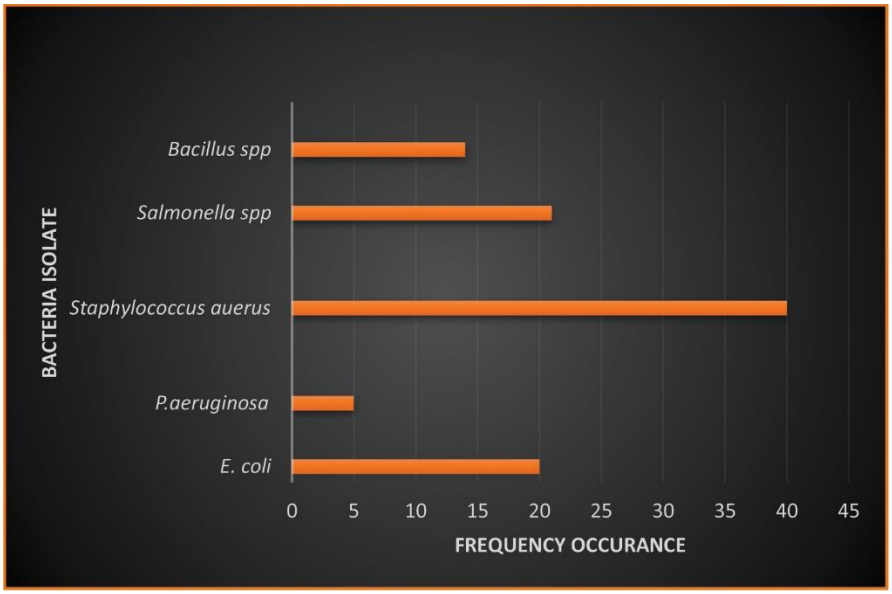

FIG. 2 Frequency occurrence of probable Bacterial isolates

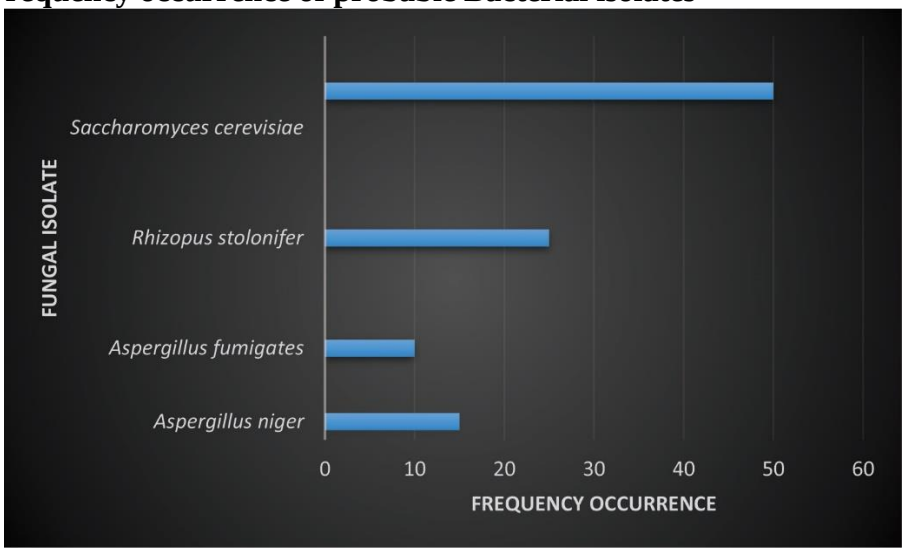

FIG.3 Frequency occurrence of probable fungi isolates

DISCUSSION

Table 1 shows nutritional composition of the five sampled out of (twelve) yellow dent collected maize samples from five different vendors. The moisture content ranged from 5.20 to 7.90 %. The low moisture contents generally are an indication of high shelf life especially for foods that are properly packaged against external condition (Makanjuola *et al*., 2018). Crude protein ranged from 5.0 to 6.6% of which basic function is to supply

adequate amount required (Pugalenthi *et al*., 2004). Crude fibre ranged from 1.45 to 3.11, fibre helps in the maintenance of human health and has been known to reduce cholesterol level in the body. High fiber foods expands the inside wall of the colon, causing the passage of waste, thus making it an effective anti-constipation. Fibre also reduces the risk of various cancers, bowel diseases and improves general health and well-being of individual (Makanjuola *et al*., 2018). Ash content ranged from 1.30 to 3.80%, the high ash content of the maize sampled is an indication that maize has high organic content of food minerals.

However, crude fat ranged from 2.00 to 3.91. Due to the low value in fats the maize grains can be recommended to patient who desire to lose weight. Carbohydrate ranged from 74.79 to 84.75%, the high carbohydrate contents can be considered as a potential source of food energy for both young and aged individual who desire high calorie foods. The result of the nutritive assessment of maize reported in this work is in conformity with the values reported by Adeniyi and Ariwoola (2019) for sampled Maize (*Zea mays* L.) varieties grown in South-Western, Nigeria. The percentage carbohydrate content of 74.79 to 84.75 recorded in this work is in accordance with the results reported by Ape *et al*. (2016), and Okonkwo and Agharandu (2017). Similar range of value reported by Robert *et al*., (2020) for maize were carbohydrate 75.48, protein 7.10, fibre 6.69, lipid 4.18 ash 1.79 and moisture 11.51. Correspondingly, Edema (2005) reported 8.96 crude protein; 4.09 Fat, 1.33 crude fibre; 1.48 ash and moisture 7.15, for nutrition value of maize flour. However, drastic decrease in lipid or fat and crude fibre content cold be linked to the temperature which the maize was subjected during roasting and the effect of various processing technique employed.

The result of the microbial evaluation showed that the sampled maize was heavily contaminated with high microbial count. as shown in (Table 2). The total aerobic count of the maize sample ranged from 1.3 x 10^5 to 9.1 x 10^5 cfu/g while vendor 4 and vendor 5 show high bacterial count ranging from 4.8 x 10^5 to 9.1 x 10^5cfu/g. The total yeast and mould count range from 1.2 x 10^5 to 8.4 x 10^5. The total coliform count ranged from 0 ^{to} 6.4 x 10^5cfu/g. Feacal coliforms were observed and ranged from 0 to 3.3 x 10^5cfu/g. While *Staphylococcuss* count ranged from 1.1 x 10^5 to 3.0 x 10^5cfu/g. Presence of salmonella spp were seen in eight samples and pseudomonas aeruginosa was isolated in three samples.

The yellow maize samples in this study were found to harbour a total of eight bacterial species. These were *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Salmonella spp* (Table 3 & 4). Four fungi were isolated and they were *Aspergillus niger*, *Rhizopus stoloniter*, *Aspergillus fumigatus* and *Saccharomyces cerevisae* (Tables 5).

Tables 3 and 4 showed the percentage frequency of occurrence of bacterial. *Staphylococcus aureus* was the most frequently occurring bacterial isolate (40%) while the least occurring was *Bacillus subtilis* (14%). The highest occurring fungal isolate was *Saccharomyces cerevisiae* (50%) as the least was *Aspergillus fumigates* (10%) (Table 3). The maize samples purchased from different vendors investigated were not of acceptable microbiological quality. The International Commission for Microbiological Specification for Foods (ICMSF,1996) states that ready-to-eat foods with plate counts between $0 - 10^3$ is acceptable, between $10^4 - \leq 10^5$ is tolerable and 10^6 and above is unacceptable. The high level of contamination of maize samples could be associated to the fact that it is food that is eaten without extreme heat processing after prior roasting; similarly, poor processing method, poor hygiene practice, improper and unhygienic handling of the product, bad sanitation operations and use of old school or newspaper in packaging are major source of roasted maize contamination. The result of the research work is in agreement with Andrzej *et al*., (2020) that Estimated of microbiological contamination of maize seeds using isothermal calorimetry and quantitative real-time polymerase chain reaction (qPCR) they reported that The results of this investigation clearly pointed out a longer activity of microbiota metabolism in samples with a high content of fungi (including yeast) *Lactobacillus spp.* and *Fusarium Pseudomonas spp.*, toxicogenic *Penicillium spp. Aspergillus* spp. and sporulating bacteria of the genus *Bacillus spp*. The results of this study are supported by the work of Yeboah-Manu, et.al. (2010), Madueke, *et al*. (2014), and Monday, et.al. (2014), who had sampled several ready to eat food and snack sold in open market and had identified and isolated similar microorganisms which include *Bacillus cereus, Staphylococcus aureus, Klebsiella spp*. and *pseudomonas spp.* The manner in which the roasted maize is displayed in the open market after roasting to attract buyers and the poor handling technique exhibited by the consumers who may or may not buy after handling and touching the maize husk also have high influence level of contamination as observed in the sampled maize. The vendor rushing towards bus commuters on transit in order to sell their product without good packaging also attributed to the maize contamination, with these pathogens being of public concern. Furthermore, the culture of using old school note books and newspapers as packaging materials, have also contributed to high bacteria load and presence of fungi of the maize. The implications of using old, unkempt newspapers and old notes from students showed that microorganisms found in the snacks are sourced from the vendors whereas analysis of using sterile polythene bags sharply contrasts from the results of using the vendor packaging materials (Ayoade*et al*., 2017). This is in agreement with Greig *et al*. (2007) that reported that mishandling and disregard to hygienic measures on the part of the food vendors have been reported to introduce contaminant and pathogens that survive and multiply in sufficient numbers to cause illness in the consumer.

The high fungi load observed among the samples maybe attributed to open environment where this vendor sell their product. This is in line with Aboloma (2008) that reported the environmental factors due to dust and open air; therefore, environmental contaminants have also been implicated as food borne pathogens. Due to the high carbohydrate level and frequent exposure to the environment, it justifies the high fungal count reported in this research. However, the aflatoxin and mycotoxin released by this fungal species *Aspergillus niger*, *Rhizopus stoloniter*, *Aspergillus fumigatus* when consumed could lead to serious food poisoning and illness, as mycotoxins can have damaging effects when ingested, especially by immunocompromised individuals (Izah, *et al*., 2015). The highest frequency exhibited by *Staphylococcusauerus*could be as a result of contaminations from personnel who vend the maize or the previous consumers who wanted to buy the maize but didn't succeed, through dirty hands of the vendor or customers, and clothes that comes in contact with the roasted maize. *Staphvlococcusauerus*is common organisms found in all individuals and expelled from the respiratory tract, nose, mouth, clothing, hand and skin (Aboloma, 2008). Therefore, the chance of the microorganisms getting into the maize is high when the vendors and the customers sneeze, blow their noses or blow air into the polythene bags to open them (The University of Utah, 2011) and (Acheampong, 2015) among others. *Salmonella* is known as a pathogen that causes typhoid, fever and food poisoning (Ekperigin and Nagaraja, 2009) while Pseudomonas sp is an opportunistic pathogen that cause bacteremia and gastrointestinal infections (Kim *et al*., 2009).

Conclusion

The study concluded that roasted yellow maize is high in carbohydrate, crude protein and fibre. The absence of gluten in maize also suggests it nutritive and dietetic therapy in treatment for celiac diseases. Hence, the presence of microbial pathogen which is attributed to poor food handling, personal hygiene, packaging materials (old newspapers) for the roasted maize and the environment coupled with vendors and consumers' poor attitudes towards food safety are sources of pathogenic microorganisms. Educating the food handlers or food vendors on food safety practices and a close and monitoring supervision of ready-to-eat foods sold in the community should be carried out by relevant authorities like NAFDAC to prevent outbreak of possible food borne illness.

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