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Effect of Processing on the Microbial Load of Maize Meal Products Sold Withing Enugu Metropolis

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Abstract: Studies were carried out to assess that effect of processing on the microbial load of maize meal products sold within Enugu metropolis using appropriate microbial and biochemical techniques. Eight pathogenic organisms (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Streptococcus* spp., *Aspergillus niger*, *Bacillus cereus and Candida albican*) were isolated in the processed (Akamu, Agidi and Kunu Zaki) and unprocessed maize samples. The pH levels of Kunu Zaki, Akamu, Agidi and unprocessed maize samples were 5.6, 5.1 and 4.9, respectively. The percentage mean bacterial counts in the samples were 43.32, 34.35, 15.00, 3.70 and 3.53% for *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus* spp. and Salmonella enteritidis, respectively. The percentage fungal contamination of the processed and unprocessed samples were 78.49, 14.22 and 7.29% for *Bacillus cereus*, *Aspergillus niger* and *Candida albican*, respectively. The mean bacterial and fungal counts in the processed and unprocessed sample were within tolerable limits of human consumption. The samples were contaminated with the isolated pathogenic organisms in the following order: Kunu Zaki>Akamu>Agidi>unprocessed maize.

INTRODUCTION

Maize (*Zea mays l. Poaceae*) is the most important cereal in the world after wheat and rice with regard to cultivation areas and total production (Osagie and Eka, 1998). The global production of maize is estimated to be about 300 million tons per year with 50% of the world production from America alone (Purseglove, 1992). In Nigeria, its production is quite common in all parts of the country from the North to the South with an annual production of about 11.2 million metric tons (CBN., 2010).

Raw, sweet maize kernels are composed of 76% water, 19% carbohydrates, 3% proteins and 1% fat (Olaofe, 1988). Maize is prepared and consumed in a multitude of ways which vary from region to region or from one ethnic group to the other.

For instance, the traditional method of preparing akamu involves soaking maize in water for 2-3 days followed by wet milling and sieving to remove bran and hulls (Banigo and Muller, 1972). The residue is retained on the sieve and later discarded as an animal feed. The filtrate which is the slurry is used in making pap or Akamu. Pap is the most important traditional food for weaning infants and the major breakfast cereal for adults (Odunfa, 2005). The pap is prepared by dissolving the slurry with water and subsequent addition of hot water with stirring to form a semi-liquid porridge. It's colour depends on the colour of the cereal used.

Agidi is prepared much like pap (Akamu) with the raw-slurry filtrate as the base material. In case of Agidi, the raw-slurry paste is mixed with oil (by choice), crayfish, biscuit bone, maggi, pepper and salt and put inside plantain husk and cooked until it forms a thick, solid gel-like porridge (Ijabadeniyi, 2007). Agidi is eaten with pepper stew and fried fish etc. Kunuzaki is an alcoholic beverage that is very popular in the Northern part of Nigeria but is being consumed now in all parts of the country. It is acceptable to people of all works of life and is being served at home and public places as food appetizer, refreshing drink and complementary food for infants (Rosemary *et al*., 2015). It forms the major source of protein for many Nigerians, especially, the rural populace who could not afford imported milk products.

Depending on the cereal availability, sorghum, maize, millet, guineacorn or rice are commonly used for kunu preparation (Odunfa and Adeyele, 1985). The process of preparation of kunu zaki involves fermenting cereal grains for 2-3 days and wet milling with spices (ginger, clove, black pepper, ehuru) and sweet potato (optional).

It is then followed by sieving and potential gelatinization of the slurry, sugar addition, cooling and bottling (Adeyemi and Umar, 1994). Fermented maize is widely utilized as food in African countries and in fact, cereals account for as much as 77% of total caloric consumption (Osungbaro, 2009).

Fermented foods are of great significance because they provide and preserve vast quantities of nutritious foods in a wide diversity of flavours, aromas and textures which enrich the human diet. Fermented foods have been with us since, humans arrived on earth and then will be with us far into the future as they are the source of alcoholic foods/beverages, vinegar, pickled vegetables, sausages, cheese, yoghurt, vegetable protein amino acid/peptide sauces and pastes with heat like flavours, leavened and sour-dough breads (Steinkraus, 1997). Studies have shown that fermented foods have become a major cause of diarrheal diseases through contamination with pathogenic bacteria (Simango and Rukure, 1992). Recent studies have shown that high microbial contamination screened in traditionally fermented foods were as a result of unhygienic practices arising from the processing and post-processing of the products, poor storage condition and use of dirty water (Olasupo *et al*., 2002).

The microbial contamination were equally likely to come from containers, bags, measuring and transport devices used, quality of water for washing and fermenting of the cereals and cassava products, sneezing, talking, coughing, dust raised by passers-by and vehicle, dirty environment and other unhygienic habits of the chains of handlers (William and Dennis, 1998). Bacteria and fungi genera such as *Aspergillus niger*, *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli* and *Klebsiella pneumoniae* have been implicated in food poisoning and other food borne diseases such as diarrhea, tuberculosis, typhoid fever and cholera. Consequently, studies were carried out to assess the effect of processing on the microbial load of maize meal products consumed within Enugu metropolis, Enugu state, Nigeria.

MATERIALS AND METHODS

Collection of samples: Forty samples (ten for each) were purchased from market outlets within Enugu metropolis. They were separately packed in polythene bags and containers and immediately taken to the laboratory where it was stored in a refrigerator prior to analysis.

Preparation of media: The media for culturing was aseptically prepared according to the established procedures and autoclaved at 121°C for 15 min.

Serial dilution and culturing: About 1 g of crushed unprocessed, cassava samples bought from the market outlets within Enugu metropolis was added into a beaker containing 10 mL of distilled water in a ratio of 1:10 and was mixed thoroughly until the solution homogenized. A ten-fold serial solution was carried out as previously described. The same procedure was repeated for abacha, garri and akpu samples obtained from the markets within the metropolis.

Bacteria were grown in nutrient agar at 37°C for 24 h. Pure cultures of different isolates were obtained and stored in a nutrient broth slant. For fungi isolates the in ocula were grown in potato dextro agar for 96 h at room temperature. Cultural and morphological characterization of the bacteria and fungi isolates were determined accordingly to Harriga and Mclance.

Biochemical test: Biochemical testing for identification of fungal and bacterial isolates were carried out by conducting citrate methyl red, coagulate, indole, catalase and glucose test procedures.

Gram staining: Using a sterile loop, a light suspension of organism in sterilized water was prepared in a clean microscope slide. The film was air-dried and heat-fixed by passing the slide twice through a gas flame. The slide was then allowed to cool. The slide was placed on a staining rack flooded with crystal violet solution and left for 30 sec before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 30 sec before washing off with running tap water. To decolourlize, 50:50% acetone-alcohol was run over the film and washed off immediately with distilled water. The film was flooded with safranin solution and left for 1 min before washing off with distilled water. A drop of immersion oil was then placed on the film and was examined under the microscope using the x100 oil inversion lens. Dark purple colour indicated gram positive reaction and pink colour indicated gram negative reaction.

Determination of pH: The pH of the sample were determined following the method described by (AOAC). About 2 g of each samples were homogenized in 10 mL of distilled water and the pH of the suspension determined using a reference glass electrode pH meter.

RESULT AND DISCUSSION

Table 1 shows that the mean pH levels of the unprocessed maize grains, Kunu Zaki, Agidi and Akamu samples were 4.9, 5.6, 5.9 and 5.1, respectively. Figure 1 shows that the pH levels of the samples decreased in the following order; Agidi>Kunu Zaki>Akamu>unprocessed maize. The results of the pH levels of the processed and unprocessed samples shows that the different duration and stages of fermentation adopted by the processors, the quality of water used in processing the samples and the variety of ingredients added accounted for the varied pH levels of the processed and unprocessed samples.

Table 1: pH levels of the processed and unprocessed maize meal products

Samples	pH
Unprocessed maize	4.9
Kunu Zaki	5.6
Agidi	5.9
Akamu	5.1

It was observed that the pH levels of the processed samples shows loss in acidity compared to the unprocessed samples with agidi being the least acidic while the unprocessed maize samples was the most acidic. Omemu *et al*. (2018) reported similar pH levels between 4.07-6.07 in maize in their styles of the microbiological assessment of maize co-fermented with pigeon pea as obtained from this study.

Fermentation of maize samples results to the production of lactic acid and other organic acids by organisms responsible for fermentation which during the dewatering stages of the processing of the maize grains into finished products subsequently decreases its acidity.

Table 2 shows that eight pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Streptococcus* spp., *Aspergillu sniger*, *Bacillus cereus and Candida albican*) were isolated from unprocessed maize samples and

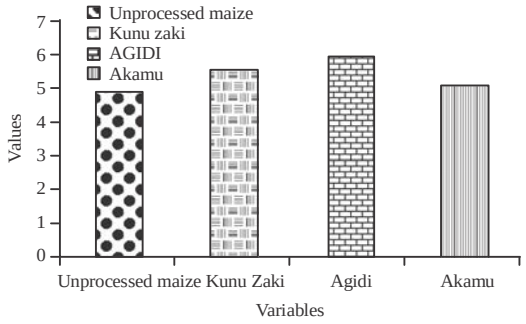

Fig. 1: Bar chart representation of the pH levels of the processed and unprocessed maize meal products sold within Enugu metropolis

Table 2: Biochemical characteristics of bacterial and fungal isolates

Cultural characteristics	Cellular morphology	Gram staining	Glucose	Indole	Coagulate	Catalase	Citrate	Methyl red	Most probable identity
Black with yellow reverse	Rods slightly rough with cylindrical cell	-	-	-	+	-	NA	-	*Aspergillu sniger*
Pink, smooth, flat and irregular	Rods in single pairs and clusters	-	-	-	+	+	+	-	*Klebsiella pneumoniae*
Creamy and irregular	Rods scattered	+	-	-	-	+	-	+	*Salmonella enteritidis*
Pink, round into smoothing shining surface	Rods in clusters spores present and flagellated	+	+	+	NA	+	+	-	*Bacillius cereus*
Cream, flat with a dull wrinkled surface	Slightly rough with cylindrical cells	- +	+	+	-	+	-		*Candida albican*
Cream, circular raised	Cocci raised in clusters	+	+	-	+	+	-	-	*Streptococcus* spp.
Red-coloured with a smooth serrated edge	Rods straight	-	+	+	NA	+	+	-	*Escherichia coli*
Yellowish-orange	Cocci in pairs	+	+	-	-	+	+	-	*Staphylococcus* and slimy *aureus*

Table 3: Mean bacteria counts in the processed and unprocessed maize meal products (Cfu/g)

	Mean bacterial count (Cfu/g)				
Samples	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella enteritidis*	*Klebsiella pneumoniae*	*Streptococcus* spp.
Unprocessed maize	33	47	NA	28	NA
Kunu Zaki	$2.3^4\times10^3$	$4.8^2\times10^3$	1.76×10^2	4.32×10^2	$2.9^4\times10^2$
Agidi	1.22×10^2	3.51×10^2	2.40×10^2	3.11×10^2	1.88×10^2
Akamu	3.07×10^3	1.80×10^3	1.56×10^3	1.66×10^3	1.34×10^2
WHO	$\leq10^3$				

Table 4: Mean fungal counts in the processed and unprocessed samples (Cfu/g)

	Fungi count		
Samples	*Aspergilliu sniger*	*Bacillus cereus*	*Candida albican*
Unprocessed maize	NA	60	NA
Kunu Zaki	1.90×10^2	6.07×10^3	2.11×10^2
Agidi	1.51×10^3	$4.66.\times10^3$	7.04×10^2
Akamu	2.97×10^2	2.34×10^3	1.09×10^2
WHO permissible limits	$\leq10^3$		

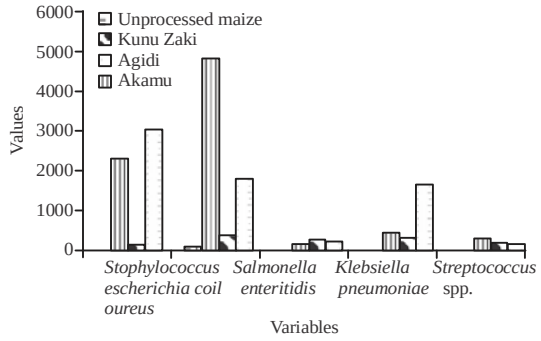

Fig 2: Bar chart represented of the mean bacterial counts in the processed and unprocessed sample

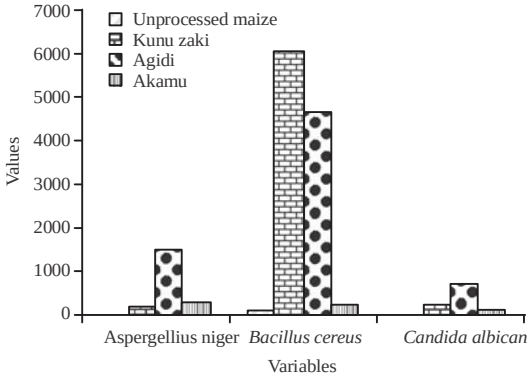

Fig. 3: Bar chart representation of the mean fungal counts in the processed and unprocessed samples (Cfu/g)

processed ones (Kunu Zaki, Akamu and Agidi) sold within Enugu metropolis. The bacterial and fungal molds isolated in this study are commonly present as contaminants in fermented food products and do not appear to play any significant role in the fermentation processes. According to Mbata *et al*. (2009) the sources of these microorganisms could be from human skin, cooking utensils, processing equipment, the environment and water.

The presence of these pathogenic bacteria, especially, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* and *Salmonella enteritidis* indicates unhygienic standards, excessive personnel handling and poor quality water during processing, post processing handling, storage condition and marketing.

Table 3 shows that the mean bacterial count in the unprocessed samples sold within Enugu metropolis were 33, 47 and 28 Cfu/g for *Staphylococcus aureus*, *Escherichia coli and Klebsiella pneumoniae* (Fig. 2 and 3). The mean counts of the bacteria isolate in the Kunu Zaki samples were 2.34×10^3, 4.82×10^3, 1.72×10^2 4.32×10^2 and 2.94×10^2 Cfu/g for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae* and *Streptococcus* spp., respectively.

The mean counts of the bacterial isolates in the agidi samples were 1.22×10^2, 3.51×10^2, 2.40×10^2, 3.11×10^2 and 1.88×10^2 Cfu/g for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Klebsiella pneumoniae and Streptococcus*, respectively.

Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, *Klebsiella pneumoniae and Streptococcus* spp. were isolated in the Akamu samples sold within Enugu metropolis with mean bacterial counts of 3.07510^2, 1.80×10^3, 1.56×10^2, 1.66×10^3 and 1.34×10^2 Cfu/g, respectively. Table 4 shows that the only fungal isolates, *Aspergillus niger* in the unprocessed samples had mean count counts of 60 Cfu/g.

The mean fungal counts in the kunu zaki samples were 1.90×10^2, 6.07×10^3 and 2.11×10^2 Cfu/g for *Aspergillus niger*, *Bacillus cereus* and *Candida albican*, respectively. The mean fungal counts in the agidi samples were 1.5×10^3, 4.66×10^3 and 7.04×10^2 Cfu/g for *Aspergillus niger*, *Bacillus cereus* and *Candida albican*,

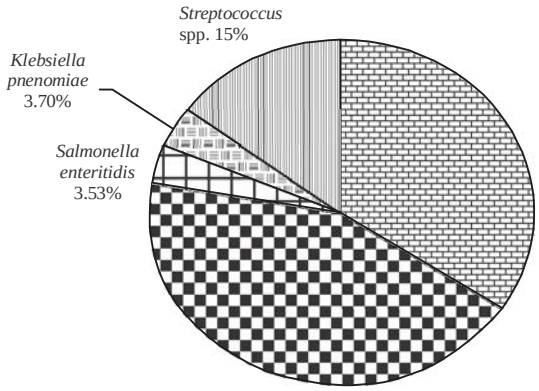

Fig. 4: Pie chart representation of the percentage mean bacterial contamination of the processed and unprocessed maize meal samples

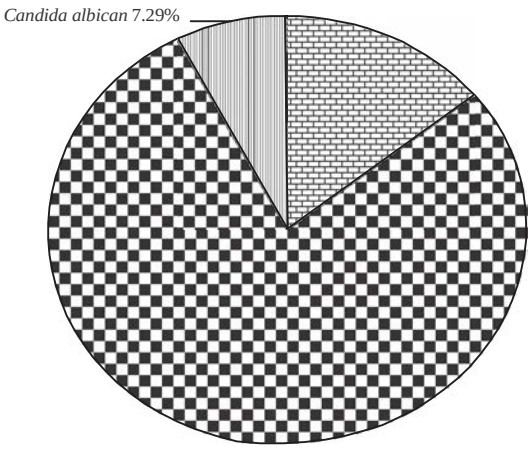

Fig. 5: Pie chart representation of the percentage means fungal contamination of the processed and unprocessed maize samples

respectively. The mean fungal count in that akamu samples were 2.9×10^2, 2.34×10^2 and 1.09×10^2 Cfu/g for *Aspergillus niger, Bacillus cereus and Candida albican*, respectively.

Escherichia coli had the highest mean bacterial counts in the unprocessed maize, Kunu Zaki and Akamu samples while *Staphylococcus aureus* had the highest mean counts in the agidi samples sold within the metropolis. For the fungal isolates that highest mean counts was observed for *Bacillus cereus* in the unprocessed maize, kunu zaki and akamu sample's while the highest mean counts recorded for Aspergillusniger in the agidi samples.

The result of this study shows that the bacterial and fungal contaminations (especially, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus cereus* and *Aspergillusnige*) in the processed samples were above acceptable limits in edible foods (Fig. 4 and 5).

The presence of the investigated pathogenic organisms in the processed samples that had some form of cooking at the processing stages suggests heavy post-processing microbial contamination. The studies of Guthrine (2003) confirms that the most important sources of this organisms in foods are the nasal canals infected hands, faecal matter, dust, insects and aural animals and this constitute health hazards. In addition, the spores of most of the isolated pathogenic organisms have the ability to withstand high temperature and usually produce enterotoxins which are not easily destroyed.

According to Uraih and Izuagbe (1990) foods are usually contaminated with pathogenic organisms at the processing stages because these organisms usually produce endospores which makes them to be extraordinarily resistant to environment stresses such as heat, ultra-violet radiation, chemical disinfectants and dessication.

The pathogenic organisms isolated in the processed and unprocessed samples sold within Enugu metropolis have been implicated in a wide variety of food borne illnesses such as blood diarrhea, gastroenteritis, typhoid fever and a host diseases the lungs, liver and other intestinal organs.

Ijabadeniyi (2007) obtained a higher count of 1.6×10^6 Cfu/g for *Staphylococcus aureus* in his study on microorganisms associated with ogi traditionally produced for three varieties of maize than what was gotten in this study. Obtained a comparable percentage contamination of 30.8% for *Escherichia coli* in kunu zaki sold at Gariki, Enugu state, Nigeria to that reported in this study.

CONCLUSION

Eight pathogenic organisms (*Staphylococcus aureus, Escherichia coli, Salmonella enteritidis, Klebsiella pneumoniae, Streptococcus* spp., *Aspergillusniger, Bacillus cereus and Candida albican*) were isolated from processed and unprocessed maize meal products sold within Enugu metropolis with mean counts within tolerable limits of human consumption. The bulk of these microbial contaminations were in the processed maize meal products (Kunu Zaki, Agidi and Akamu). Since, these pathogenic organisms have been implicated in many deadly food borne diseases, their presence in the investigated food products should be of a health concern to consumers. More worrisome is the fact that these processed meal products have become daily snack and breakfast for adults and for weaning infants in the region. Improvement in personal hygiene use of clean water and packaging container,

better storage conditions and careful post-processing handling would greatly reduce the microbial load in the food products.

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