



Research Paper

Effect of processing on the microbial load of cassava meal products sold within Enugu metropolis

Accepted 15th June, 2019

ABSTRACT

Studies were carried out to assess the effect of processing on the microbial load of cassava meal products sold within Enugu metropolis using appropriate analytical techniques and standard biochemical procedures. Eight pathogenic organisms (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus* spp., *Salmonella enteritidis*, *Aspergillus niger*, *Bacillus cereus* and *Candida albican*) were isolated from the processed and unprocessed cassava meal samples. The pH of the processed samples was between 4.9 and 5.4, thus promoted the growth of the pathogenic organisms. The percentage bacterial counts in the processed and unprocessed samples were 44.3, 43.65, 7.45, 2.70 and 1.99% for *S. aureus*, *S. enteritidis*, *K. pneumoniae* and *Streptococcus* spp., respectively. The percentage fungal contaminations of the processed and unprocessed samples were 67.04, 27.58 and 5.37% for *A. niger*, *B. cereus* and *C. albican* respectively. The processed and unprocessed samples harboured the isolated pathogenic organism in the following order: akpu>garri>abacha>unprocessed cassava. The mean bacterial and fungal counts in the processed and unprocessed samples were within tolerance limits.

Okeke Onyeka¹, Ezech Ernest², Ozuah A. C.³ and Ezejiofor C. C.⁴

¹Plastic Production Unit, Scientific Equipment Development Institute, Enugu, Nigeria.

²Chemical Engineering Department, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

³Integrated Science Department, Nwafor-Orizu College of Education, Anambra State, Nigeria.

⁴Microbiology Department, Caritas University, Enugu, Nigeria.

*Corresponding author. E-mail: onyekaoke207@yahoo.com

Key words: Bacteria, fungi, contamination, disease, cassava, abacha and garri.

INTRODUCTION

Cassava (*Manihot esculenta*) is a dicotyledonous plant and widely grown root crop in tropical regions of Africa, Latin America and Asia (Ihenkeronye and Ngoddy, 2005). It is the most important root crop in Nigeria in terms of food security, employment creation and income generation for crop producing households (Ugwu and Ukan, 2002). It supplies about 70% of the daily calories of over 50 million people in Nigeria (Oluwole et al., 2004).

Nigeria is the largest producer of cassava in the world with about 45 million metric tonnes and its cassava transformation is the most advanced in Africa (Egesi et al., 2006). Cassava is grown throughout the tropics and could be regarded as the most important root crop in terms of area cultivated and total production. It is one of the most drought tolerant crops, capable of growing on marginal soils (Adebayo-Oyetumo et al., 2003). The cassava root is long and tapered with a firm homogenous flesh encased

in a detachable rind of about 1 mm thick, rough and brown on the outside (Ihenkeronye and Ngoddy, 2005). Fresh cassava roots cannot be stored for long because they rot within days of harvest since they are bulky with about 75% moisture content, therefore, it must be processed into various forms in order to increase the shelf-life of the products, facilitate transportation and marketing, reduce cyanide content and improve portability (Onwuka and Ogbogu, 2007; Emurotu et al., 2012).

Traditionally, cassava roots are processed by various methods into numerous products and utilized in various ways according to local customs and preferences. Cassava processing procedures vary depending on the products; from simple processing (peel, boil and eat) to complicated procedures for pressing into garri for example, which involve many steps namely, peeling, grating, processing, fermenting, sifting and frying (Iwuoha and Eke, 1996). Palm

oil is added to the cassava mash to give the garri an aesthetic value and source of vitamin A.

Garri is classified based on texture, length of fermentation, region or place where it is produced and colour imparted by the addition/non-addition of palm oil (Olopade et al., 2004). It has high swelling capability and can absorb up to four times its volume in water (Jekayinga and Oladije, 2007).

Another traditionally processed cassava root product is abacha. Abacha is processed by first harvesting and peeling the cassava tuber and will be quickly followed by cooking the tubers for about three hours to soften. The cooked cassava will be sliced into small pieces and soaked in water for twelve (12) hours. After this period, it will be washed thoroughly with water until it becomes edible (Otusola, 1991; Iwuoha and Eke, 1996). Other abacha recipes can be prepared by dutifully following all other steps previously described and then spreading the edible wet abacha on a clean sack under the sun to dry.

Another traditionally processed cassava product is akpu or fufu and is an acid fermented cassava product produced through submerged fermentation of peeled cassava in Nigeria, West Africa countries and other parts of the world (Inetianbor et al., 2017). It is usually prepared as a stiff porridge using boiling water, prior to being consumed with soup. The processing involves peeling, cutting, submerged fermentation, mashing and dewatering. The mashed cassava in a clean sieve bag can be cooked in ball-shapes and pounded into gelatinized pastes (Okolie et al., 1992).

One of the constraints in the commercialization of traditionally processed cassava products is that the quality and unhygienic nature of the products varies from one processor to the other and even from one processing batch to the other by the processor (Oyewole and Sanni, 1995). Traditional processing of cassava products is generally unhygienic. Unhygienic conditions during production (for instance, lack of protective clothing, lack of hand washing, fermenting with unclean water, washing with dirty water, washing in dirty sieve sacs, drying on dirty surfaces) and storage at unfavourable conditions often results in bacteria and mould contamination which could result to major health concerns to humans and livestock (Manjula et al., 2009).

In Nigeria, the processing, sale and distribution and marketing of cassava products such as abacha, garri and akpu in local markets are associated with unwholesome and unhygienic practices which may lead to microbial contamination due to transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites which increases microbial load of these products. According to Arasi and Adebayo (2000), unhygienic handling and poor sanitary measures that are very obvious has been observed between the last stages of production of traditionally processed cassava products and the time it's being displayed in the markets, where the main patronage of many consumers handling this products constitute

serious health implications as many chances have been given to contamination by organisms of epidemiological importance. Microbiological quality of food indicates the amount of microbial contaminants it has, a high level of contamination indicates the level of food storage and its handling and is more likely to transmit diseases (Monday et al., 2014). Bacterial count in prepared food is a key factor in assessing the quality and safety of food. Food and water in particular have been described as vehicle for the transmission of microbial diseases (WHO, 2011).

Safe food is a basic human right despite the fact that many foods are frequently contaminated with naturally occurring pathogenic microorganisms which cannot be detected organoleptically (seen, smelled or tested) but can cause disease including death especially if the way they are conserved during processing and exposition for sales provides condition for those microorganisms to grow and reach considerable levels of contamination (WHO, 2002). Food borne illness is a major international health problem and an important cause of reduced economic growth (Frenzen et al., 2005). The problems of food safety in industrialized world differ considerably from those faced by developing countries, as traditional methods are used for processing and packaging of fresh produce while in developed countries, high standard is employed (Akinsanya et al., 2013). The global incidence of food borne diseases is difficult to estimate but it has been reported that in 2010 alone about 2.7 million people died from food borne diseases alone (WHO, 2013). Bacteria and fungi such as *Salmonella* spp., *Staphylococcus aureus*, *E. coli*, *Aspergillus* spp. and *Bacillus cereus* can cause food poisoning diseases such as tuberculosis, typhoid fever, cholera, stomach pain, diarrhea and vomiting and sometimes death (Odetunde et al., 2014). Since it has been generally proven that food borne disease could easily be contacted through the way food is handled from processing to consumption stage, the present study was carried out to assess the effect of processing on the microbial load of cassava meal products (abacha, akpu and garri) sold in market outlets within Enugu metropolis, Enugu State, Nigeria.

MATERIALS AND METHODS

Collection of samples

Eighty samples (twenty for each) were purchased from market outlets within Enugu metropolis. They were separately packed in polythene bags and immediately taken to the laboratory for 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

One gram of crushed unprocessed cassava samples bought from market outlets within Enugu metropolis were 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 (Inetianbor et al., 2014). 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 (Cheesbrough, 2006). For fungi isolates, the inocula were grown in potato dextro agar for 96 h at room temperature (Fawole and Oso, 2004). Cultural and morphological characterizations of the bacteria and fungi isolates were determined accordingly to Harriga and McCance (2006).

Biochemical test

Biochemical testing for identification of fungal and bacterial isolates were carried out by conducting citrate, methyl red, coagulate, indole, catalase and glucose tests (Cheesbrough, 2006).

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 s before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 30 s before washing off with running tap water. To decolourize, 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 samples was determined following the method described by AOAC (2000). About 2 g of each sample were homogenized in 10 ml of distilled water and the pH of the suspension determined using a reference glass electrode pH meter.

RESULTS AND DISCUSSION

Table 1 and Figure 1 show that the pH levels of the unprocessed cassava, abacha, garri and akpu were 4.3, 4.9, 5.1 and 5.4, respectively. The varying periods of fermenting the cassava tubers into finished products could have significantly contributed to the varying pH levels of the processed and unprocessed samples. Research has shown that among the several factors which encourage, prevent or limit the growth of microbes in food substances, the most important are low pH, hygienic practices and storage temperature (Jay, 1987). The low pH of food substances greatly limits the number and type of microbes that can survive and grow on it.

Table 2 shows that five bacteria and three fungal isolates were identified in the processed (abacha, garri and akpu) and unprocessed cassava meal products sold within Enugu metropolis. The identity of the probable bacterial and fungal isolates considering their cultural and morphological characteristics were, *Aspergillus niger*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *B. cereus*, *Candida albican*, *Streptococcus* spp., *E. coli* and *S. aureus*.

The bacterial and fungal isolates identified in this study are commonly present as contaminants in the processed cassava meal products and do not appear to play any significant role in the fermentation processes. The sources of these microorganisms could be human skin, cooking utensils processing equipment, the environment and water (Omemu and Faniran, 2011). Odetunde et al. (2014) observed that pathogenic organisms in foods may indicate that such foods were exposed to conditions favourable for their introduction and growth. Guinner et al. (1996) reported that fungal and bacterial contamination of foods result to discolouration, giving rise to unpleasant taste and odour.

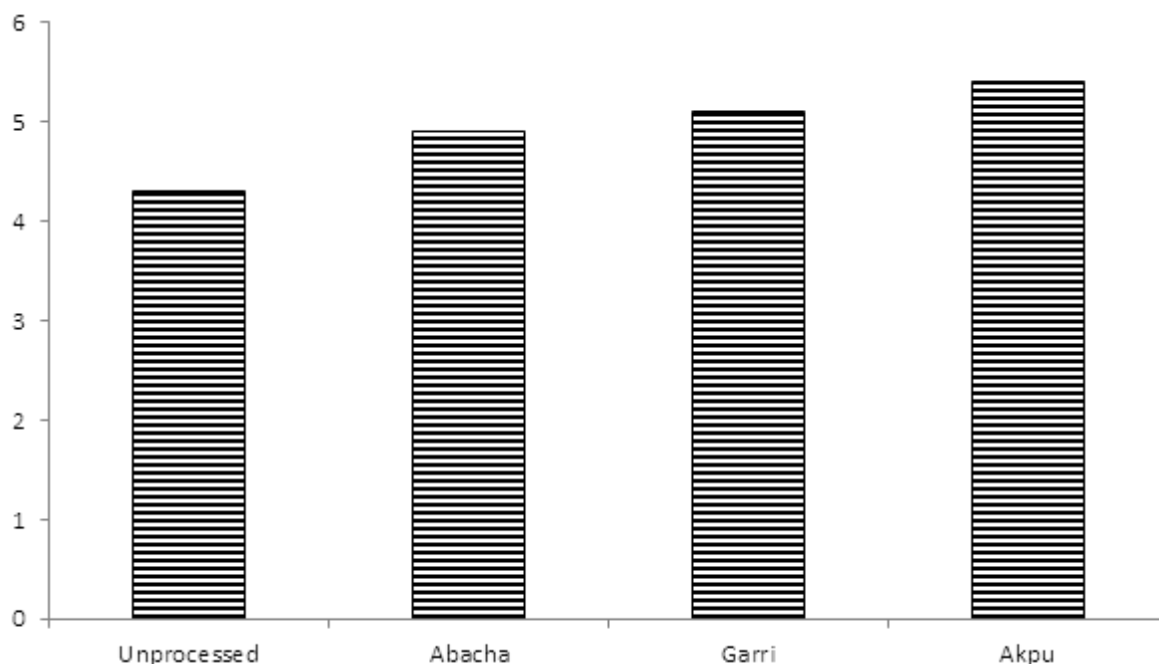
According to Ogiehor and Ikenehomeh (2005), the microbial contamination of traditionally processed food products were likely from dirty containers bags, measuring and transport devices, dirty water, sneezing, coughing, talking dust raised by passers-by and vehicles, dirty environment and other unhygienic habits of the processors. The presence of *E. coli*, *S. aureus*, *B. cereus*, *Streptococcus* spp. and *S. enteritidis* in food products have been attributed to come from the mouth, nose and skin of the food handlers and from faecal materials with the implication that such food products might not be safe for human consumption.

Table 3 and Figure 2 show that the mean counts of *S. aureus*, *E. coli*, *S. enteritidis* and *Streptococcus* spp. in the unprocessed samples were 21, 32, 16 and 7 cfu/g, respectively. The mean counts of *S. aureus*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *Streptococcus* spp. in the abacha samples were 1.81×10^2 , 1.091×10^3 , 2.10×10^3 , 0.88×10^2 and 1.53×10^2 cfu/g, respectively.

The mean bacterial isolates in the garri samples sold within Enugu metropolis were 2.46×10^3 , 3.17×10^3 , 0.66×10^2 , 1.76×10^2 and 0.35×10^2 cfu/g for *S. aureus*, *E. coli*,

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

Sample	pH
Unprocessed	4.3
Abacha	4.9
Garri	5.1
Akpu	5.4

**Figure 1:** Bar chart representation of the pH levels of the processed and unprocessed cassava meal products.

S. enteritidis, *K. pneumoniae* and *Streptococcus* spp., respectively. *S. aureus*, *E. coli*, *S. enteritidis*, *K. pneumoniae* and *Streptococcus* spp. were isolate in the akpu samples sold within Enugu metropolis with mean counts of 573×10^3 , 422×10^3 , 1.14×10^3 , 2.55×10^2 and 1.70×10^2 cfu/g, respectively.

Table 4 and Figure 3 show that the mean fungal counts in the unprocessed cassava samples were 13 and 9 cfu/g for *A. niger* and *C. albican*, respectively. *A. niger* *B. cereus* and *C. albican* were isolated in the abacha samples sold within Enugu metropolis with mean counts of 0.54×10^2 , 1.01×10^2 and 0.98×10^2 cfu/g respectively. The mean fungal counts in garri samples were 1.97×10^2 , 0.69×10^3 and 0.80×10^3 cfu/g for *A. niger*, *B. cereus* and *C. albican*, respectively. *A. niger*, *B. cereus* and *C. albican* were isolated in the akpu samples sold within Enugu metropolis with mean fungal counts of 3.64×10^3 , 0.81×10^3 and 1.24×10^2 cfu/g, respectively. The bacteria with the highest mean counts in the processed cassava meal sample was *S. aureus* in the akpu samples, while the bacterial with least mean count was *Streptococcus* spp. in the garri samples (Figure

4). *A. niger* had the highest mean counts in the akpu samples sold within Enugu metropolis, while *C. albican* had the lowest fungal mean count in unprocessed cassava (Figure 5). Hence, the major microbial contaminants of the processed samples were *S. aureus*, *E. coli*, *S. enteritidis*, *A. niger* and *B. cereus*.

The results of this study showed that the mean counts of *S. aureus*, *E. coli*, *S. enteritidis* and *A. niger* were found to be above the recommended acceptable limits in the processed cassava samples. Olapade et al. (2014) obtained similar coliform count of between 6.0×10^2 and 3.0×10^3 cfu/g for *S. aureus*, *Klebsiella* spp., and *A. niger* in garri sold in Ota, Ogun State, Nigeria to the results in this study.

Munday et al. (2014) obtained a higher mean bacteria count of between 4.5×10^4 and 8.7×10^4 Cfu/g for *E. coli*, *S. aureus*, *Salmonella* spp. and *Klebsiella* spp. isolated in ready to eat foods (rice and moi-moi) sold by food vendors in Federal Polytechnic Bali, Taraba State, Nigeria than what was obtained in this study.

Isolation of the eight pathogenic organisms in the processed cassava meal products sold within Enugu

Table 2: Bio Chemical 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	-	<i>Aspergillus niger</i>
Pink, smooth, flat and irregular	Rods in single pairs and clusters	-	-	-	+	+	+	-	<i>Klebsiella pneumoniae</i>
Creamy and irregular	Rods scattered	+	-	-	-	+	-	+	<i>Salmonella enteritidis</i>
Pink, round into smoothing shining surface	Rods in clusters spores present and flagellated	+	+	+	NA	+	+	-	<i>Bacillus cereus</i>
Cream, flat with a dull wrinkled surface	Slightly rough with cylindrical cells	-	+	+	-	+	-	+	<i>Candida albican</i>
Cream, circular raised	Cocci raised in clusters	+	+	-	+	+	-	-	<i>Streptococcus spp.</i>
Red-coloured with a smooth serrated edge	Rods straight	-	+	+	NA	+	+	-	<i>Escherichia coli</i>
Yellowish-orange and slimy	Cocci in pairs	+	+	-	-	+	+	-	<i>Staphylococcus aureus</i>

Table 3: Mean bacterial counts of the processed and unprocessed samples (Cfu/g)

Sample	Mean bacterial count (Cfu/g)				
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella enteritidis</i>	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus spp.</i>
Unprocessed cassava	21	32	16	NA	7
Abacha	1.8×10^2	1.09×10^3	2.1×10^2	0.88×10^2	1.53×10^2
Garri	2.4×10^3	3.17×10^3	0.66×10^2	1.76×10^2	0.35×10^2
Akpu	5.73×10^3	4.22×10^3	1.14×10^3	2.55×10^2	1.74×10^2
WHO permissible limits	$\leq 10^3$				

metropolis corroborated the findings by Ukah and Imere (2017) in their study on the impact of processing environment on the microbial quality of

garri processing in Benin city, Edo State.

It is instructive to note that although the mean bacterial and fungal counts in the processed and

unprocessed cassava meal samples were within tolerated limits (10^4 to 10^5 cfu/g), continued unhygienic processing of the food products can

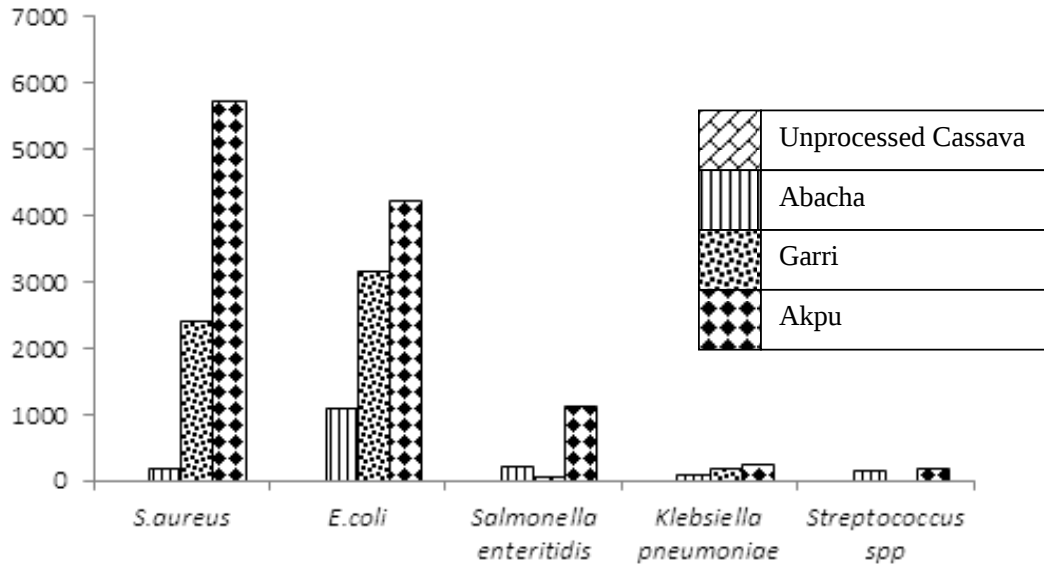


Figure 2: Bar chart representation of the mean fungal counts in the processed and unprocessed samples.

Table 4: Mean fungal counts of the processed and unprocessed samples (cfu/g)

Sample	Mean fungal count (cfu/g)		
	<i>Aspergillus niger</i>	<i>Bacillus cereus</i>	<i>Candida albican</i>
Unprocessed Cassava	13	NA	9
Abacha	0.54×10^2	1.01×10^2	0.97×10^2
Garri	1.97×10^2	0.69×10^3	0.80×10^2
Akpu	3.64×10^3	0.81×10^3	1.34×10^3
WHO Permissible Limits	$\leq 10^3$	$\leq 10^3$	$\leq 10^3$

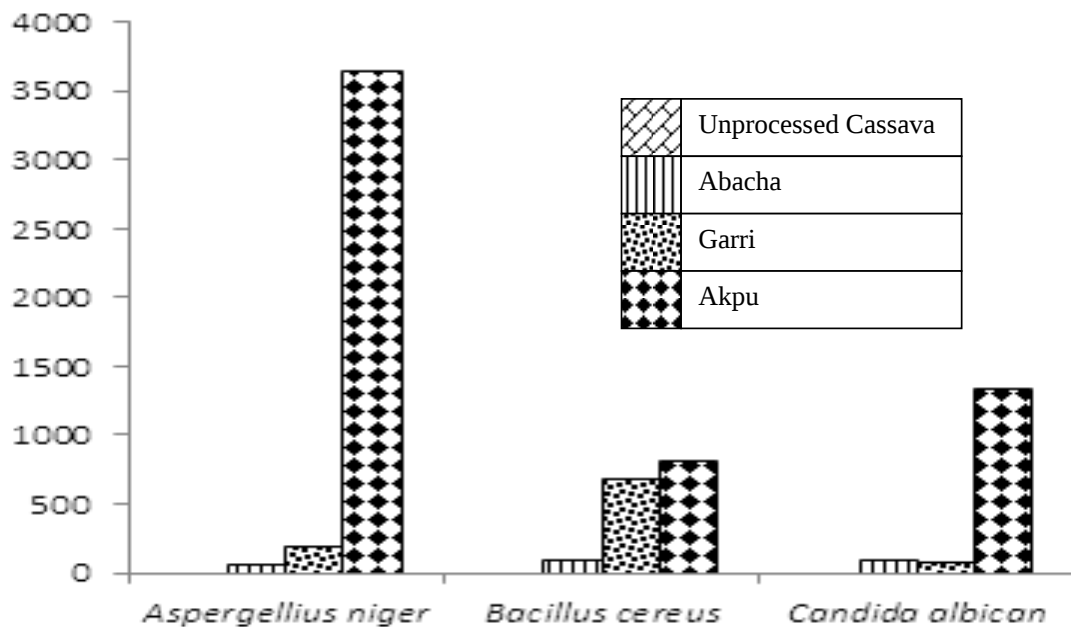


Figure 3: Bar chart representation of the mean fungal counts in the processed and unprocessed samples.

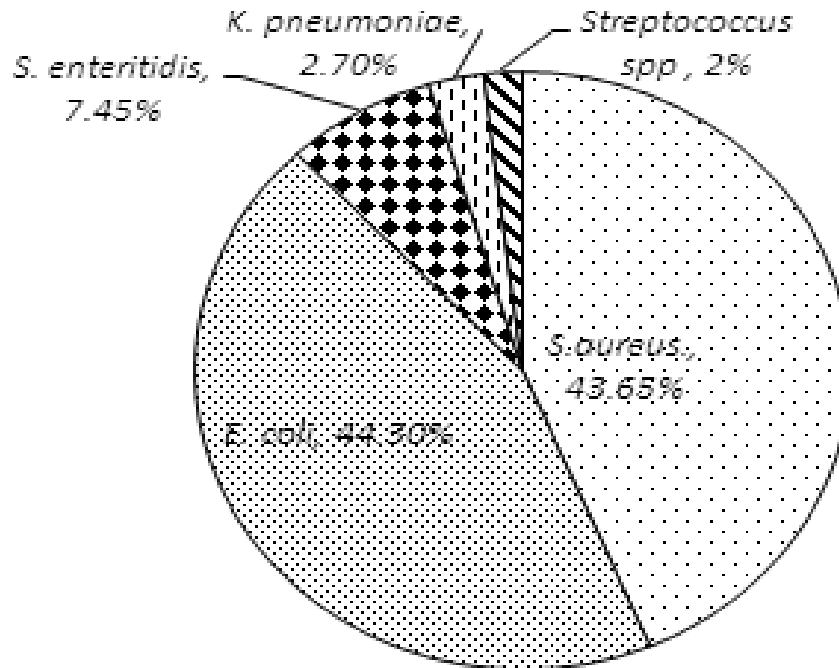


Figure 4: Pie chart representation of the percentage mean bacterial contamination of the processed and unprocessed samples.

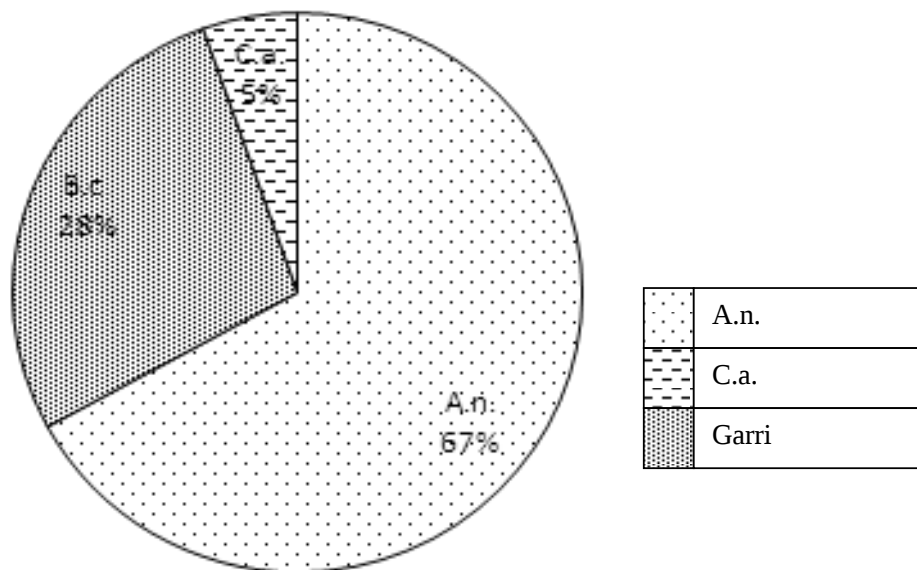


Figure 5: Pie chart representation of the percentage mean fungal contamination of the processed and unprocessed samples.

push the mean counts of these pathogenic organisms to unacceptable limits.

WHO (2005) stated that pathogenic organisms such as *S. aureus*, *E. coli*, *S. enteritidis* and *K. pneumoniae* have been known to cause gastro intestinal infections, sporadic and epidemic diarrhea, food poisoning, typhoid fever and food intoxication among other food borne disease.

Conclusion

Processed and unprocessed cassava meal products sold within Enugu Metropolis were found to have microbial contamination with pathogenic organisms (*S. aureus*, *E. coli*, *S. enteritidis*, *Streptococcus spp.*, *K. pneumoniae*, *A. niger*, *B. cereus* and *C. albican*).

The mean counts of these pathogenic organisms were within tolerable limits in the processed and unprocessed samples. *E. coli* had the highest percentage mean bacteria count of 44.3% in the samples, while the least was *Streptococcus* spp. (1.92%).

The highest percentage mean fungal counts was in *A. niger* isolates (67.04%) while the lowest percentage mean fungal counts was *Candida albicans* isolates (5.3%) in the samples.

The pH ranges of the processed samples especially akpu and garri samples encouraged the growth of the isolated pathogenic organisms in the samples.

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Cite this article as:

Onyeka O, Ernest E, Ozuah AC, Ezejiofor CC (2019). Effect of processing on the microbial load of cassava meal products sold within Enugu metropolis. *Acad. J. Environ. Sci.* 7(6): 062-069.

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