Microbiological Characterization and Antibiotic Resistance Profile of Bacterial Isolates Obtained from Refrigerated Melon Soup (Egusi)

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ABSTRACT

This study was carried out to investigate the microbiological and antibiotic resistance profile of bacterial isolates from refrigerated melon soup. Samples for bacteriological analysis were collected from freshly prepared melon (Egusi) soup immediately and subsequently after the 3^{rd}, 7^{th}, 4^{th}, and 21^{st} days of refrigeration. The isolated bacteria were then screened for their resistance profile using commercially prepared discs. The result showed no bacteria growth was observed in the freshly prepared soup. However, a total bacteria count of 0.5 $\times 10^3$cfu/ml, 6.0 $\times 10^3$cfu/ml, 4.8 $\times 10^3$cfu/ml, and 5.1 $\times 10^3$cfu/ml were observed after the 3^{rd}, 7^{th}, 14^{th}, and 21^{st} days of refrigeration/ freezing respectively. The bacterial isolates were Staphylococcus sp, Streptococcus sp, and Bacillus sp. The Antibiotics resistance profile of the isolates showed that Bacillus sp. was resistant to Pefloxacin while Staphylococcus sp.and Streptococcus sp. were resistant to Erythromycin and Gentamicin. This study has shown that bacteria can be isolated from refrigerated/ frozen melon (Egusi) soup and they are resistant to commercial antibiotics. This is of a great public health concern and spoilage could set in learning to food infection and/or intoxication.

KEY WORDS: *Egusi, Streptococcus sp, Bacillus sp and Staphylococcus sp*

INTRODUCTION

Soup is a liquid dish normally made by boiling meat, fish, or vegetables, in water then enhancing the flavour with seasoning (Galli, 2013).

Egusi (*Citrulluslanatus*) is a melon plant and grows on the ground with seed covered with a mucilaginous coating (Offong and Achi, 2004). The seeds are small and flat; one end of the seed is round while the other end of the seed is tapered. After harvest, they are left on the ground to ferment. The fermented flesh is then washed off the seeds. The seeds are then dried and the light brown husk/mucilage removed by hand or mechanically. When ready to be used as food recipe, the white/creamy seeds are grinded into powder and used as soup thickener (Offong and Achi, 2004). Melon seeds are used as a major source of ingredients in preparation of a traditional soup called ‘‘egusi’’ soup (Bankole *et al.*, 2005). One major problem that characterizes egusi soup is that it deteriorates quickly, because the soup is rich in nutrient and thus serves as a culture medium for bacterial growth (Bankole *et al.*, 2005). Melon seed, ‘‘egusi’’, is prone to fast spoilage than other soup especially during hot weather which results in food poisoning when consumed. As a result, majority of the populace prefer

other soup than ‘‘egusi’’ soup, despite its cheap and easy method of preparation (Offong and Achi, 2004).

It is important to preserve food properly since bacteria are ubiquitous existing in the soil, air and water and thus can contaminate food that is not properly handled. In the presence of favourable intrinsic and extrinsic factor of food indigenous microorganisms may grow rapidly increasing number to a point where some of them can cause illness. As bacteria grow they can produce toxins that are not destroyed or rendered harmless by most food processing techniques. *Clostridium botulinum* is the most notorious of these bacteria. The toxin is almost inevitably fatal but since that bacterium grows in an anaerobic (oxygen poor) environment, it is mostly associated with canned food or food stored in oil. Other bacteria such as *Staphylococcus aureus* produce a heat stable toxin which is of concern too. *Salmonella* causes illness not through toxin but the bacteria itself, a process known as food infection. These toxins are not produced immediately but take hours or even days to develop. Bacteria or their products in food are harmful and have some serious health implications (Corner *et al.*, 2002).

Conventional wisdom of decade age held that properly refrigerated/frozen food would remain safe but science has learned that two types of bacteria psychrophilic and mesophilic bacteria grow during extended refrigeration/freezing or temperature abuse. These bacteria include, *Bacillus cereus*, enteropathogenic *Escherichia coli*, and *Vibrio* which grow at refrigeration/freezing temperature (Corner *et al.*, 1989). Bacteria grow best at temperature between 40^{f} and 140^{f}, that is $(40^{f}-32)9/5=4.4^{\circ}C$ and $(140^{f}-32)9/5=60^{\circ}C$. they multiply rapidly at these temperatures that are why it is important to keep perishable food refrigerated at temperature below 40˚F (Jay, 1987). In the simplest cause, drug resistant organisms have acquired resistance to first line antibiotics thereby necessitating the use of second line agents. This research was carried to investigate the type of bacteria isolates that can grow in ‘‘egusi’ soup at refrigeration temperature.

MATERIALS AND METHODS

Collection of Samples

The following materials (ingredients) were purchased from Amai market Delta State. Meat, grounded melon seeds bitter leave (*Vernonia amygdalina*). Maggi, Onions, Dry fish, Stock fish, Salt, Tomato (*Solanumly copaslcum*), Fresh pepper and palm oil.

Preparation of Melon Soup

The washed pot was placed on the burning gas. 1 ½ cup of water was poured into the pot, washed dry fish, meat, and stock fish was added inside the pot on fire, then sliced onions and little salts was added with 1 tea spoon of pepper and allowed to boil for 10 minutes to taste.

Put down the pot, pour everything in clean dish, put back the pot on the fire, when dry, pour the palm oil into the pot, don’t allow it to bleach, put the blended (super internet Japan electric blender, 51-889 BD) melon or ‘‘Egusi’’ and tomato in the hot oil and continue to stir for two minutes, then pour the steamed meat, dry fish and the stock fish, then stir if for 30 seconds, put the bitter leaf, cover the pot, allowed it to boil for five minutes, put down the pot from the fire, it is ready to serve (Anslem, 2011). The melon (Egusi soup) was allowed to cool and was dispensed into five (5) sterile containers labelled O (fresh soup) control, A

(after 3 days of refrigeration), B (After 7 days of refrigeration), C (After 14 days of refrigeration) and D (After 21 days of refrigeration). The containers A, B, C, and D were covered and kept in the refrigerator set at 5˚C by supply it with a minimum power of 8hours and maximum power of 24hours.

Sterilization of Materials

The materials used for this study were sterilized using standard techniques.

Glass wares- All glass wares were washed and rinsed with sterile water and sterilized in the hot air oven at 160˚C for 1hour.

Culture media- Nutrient agar and MacConkey agar were sterilized by autoclaving at 121˚C and 15 psi pressure/square unit for 15minutes.

Bench top- inoculation hood and working area were sterilized by disinfecting with antiseptic and covering with 75% ethanol. Sterile disposable hand gloves and face masks were worn and changed after each procedure to ensure aseptic conditions.

Microbiological Analysis of egusi soup

Serial Dilution of the Sample

The freshly prepared soup, which served as the control, and those kept (A-D) in the refrigerator were processed for bacteriological analysis using standard culture techniques. 1g part of each 'egusi' soup sample was used for serial dilution to obtain total heterotrophic microbial count for sample. 9ml of sterile water was placed in the test tube containing one gram each of the samples.

The first dilution of each sample containing one gram of soup and 9ml of sterile water was shaken vigorously to homogenize. A sterile pipette was used to transfer 1ml from it to the second tube and shaken to mix. 1ml was serially transferred from 2nd tube to the 3rd for each sample. The tubes were properly labelled to indicate the sample and dilution. They were covered with sterile plastic caps and used for inoculation as in Chessbrough (2002); Obiajuru and Ozumba (2009).

Determination of Microbial Count

Nine gram of nutrient agar was dissolved in 250ml of water in a conical flask, the flask was autoclaved at 121˚C for 15 minutes. The flask was allowed to cool and a 5ml syringe was then use to transfer 1ml of the soup from the diluted test tube each into 6(six) separate petri dishes labelled T0-10^{-1}, T1-10^{-1}, T3-10^{-2}, T4-10^{-2}, T5-10^{-3}, T6-10^{3}. The nutrient agar was then dispensed into each of the plates, covered, and incubate for 24hours at 35-37˚C, individual bacteria colonies where observed and was counted and recorded as colonies forming units (CFU) (Chessbrough, 2002).

Sub Culturing

Morphologically different colonies were aseptically collected, inoculated onto freshly prepared agar plates (Nutrient and MacConkey agar) and incubated at room temperature for 24 hours to obtain pure isolates. Purified bacterial isolates were inoculated unto nutrient agar slants and incubated at 37 ^{0}C overnight and stored in the refrigerator as stock cultures for further biochemical test.

Characterization and Identification of Isolates

The bacterial isolates from different soup samples were identified using their growth morphological characteristics on different media used, bacteriological identification test (Gram staining and motility test) and biochemical identification tests (catalase, coagulase,

oxidase, indole production, citrate utilization, spore testing, lactose and mannitol test) as in Cheesbrough. (2002), Obiajuru and Ozumba (2009).

Antibiotics Susceptibility Testing

Disc sensitivity testing was performed by modified Kirby-Bauer technique on nutrient agar with the test antibiotics (Robert,2003). Spatula full of nutrient agar was dissolved in 100ml of distilled water in a conical flask, the neck of the flask was covered with cotton wool and then autoclaved at 121˚C for 1.5psi. It was then allowed to cool before being dispensed into petri dishes where it was allowed to solidify for 45min. The wire loop was sterilized by flaming intermittently to red hot over a bunsen flame and then used to inoculate the surface of the nutrient agar with the bacteria growth, after which a forceps sterilize by dipping in ethanol and burning it off over a flame was used to apply the various commercial antibiotics disc (Pefloxacin 10ug, Gentamycin 10ug, Amplclox 30ug, Zinnacef 20ug, Amoxacilln 30ug, Rocephin 30ug, Ciprofloxacin 10ug, Streptomycin 30ug, Septrin 30ug, Erythromycin 10ug). The petri dish was then covered aseptically and incubated at 37 ˚C for 24hours. Zones of inhibitions of bacteria growth indicate bacteria sensitivity to the antibiotics.

Results

Table 1. Total bacterial count from the melon (Egusi) soup

DAYS OF STORAGE	TOTAL BACTERIA COUNT (cfu/ml)
Fresh ‘‘Egusi’’ sample	
Day 1	0
Refrigerated sample	
After 3 days	5.0×10^3
After 7 days	6.2×10^3
After 14 days	4.8×10^3
After 21 days	5.0×10^3

The outcome of microbiological and antibiotics resistance profile of bacteria isolated from refrigerated/frozen melon (egusi) soup.

The total microbial count in unrefrigerated and refrigerated/frozen melon (egusi) soup is shown in Table1, the table represents the bacteria load in the unrefrigerated and refrigerated soup. Soup sample (0) contained no bacteria after isolation, after 3 days’ soup contained 5.0×10^3cfu/ml, after 7 days’ soup contained 6.2×10^3cfu/ml, after 24 days’ soup contained 4.8×10^3cfu/ml and after 21 days contained 5.0×10^3cfu/ml. According to the table the unrefrigerated soup had no bacterium; the number of bacteria however, increased from after 3 days to after 14days and became stabilized after 21 days. Thus the bacterial population was lowest after 3 days and was highest after 14days, indicating an increase in bacteria count with period of storage.

Table 2. Characterization and identification of Bacteria Isolates

Test	Isolate A	Isolate B	Isolate C
Colonial morphology	Milk white round colonies with yellow pigment	Large cream white round colonies with smooth edge	Slightly raised cream round colonies
Gram reaction	+	+	+
Shape	Cocci in clusters	Cocci in chains	Rod
Citrate test	-	-	+
Motility test	-	-	+
Catalase test	+	-	+
Coagulase test	+	-	-
Oxidase test	+	-	+
Spore	-	-	+
Lactose	-	+	+
Indole test	-	-	+
Mannitol test	+	-	+
Organisms isolated	*Staphylococcus aureus*	*Streptococcus sp.*	*Bacillus sp.*

Table 2, shows the three bacteria that were isolated from the analysed Egusi soup samples. The probable identification of the organisms showed *Streptococcus* sp. and *Bacillus* sp. The organisms were categorized based on gram stain, coagulase, catalase, indole, oxidase, citrate, mannitol, motility test and lactose tests.

Table 3: Antibiotic susceptibility Profiling of the Isolates Antibiotics

	Staphylococcus sp.	***Streptococcus sp.***	***Bacillus sp.***
Pefloxacin	+	+	R
Gentamycin	R	R	R
Ampiclox	+	+	+
Zinnacef	+	+	+
Amoxicillin	+	+	+
Rocephin	+	+	+
Ciprofloxacin	+	+	+
Streptomycin	+	+	+
Septrin	+	+	+
Erythromycin	R	R	R

R= RESISTANCE
+= SENSITIVE

Table 3 shows the three bacteria that were isolated from the analysed 'esugi' soup samples. The probable identification of the organisms showed *Staphylococcus aureus* and *Streptococcus Sp.* were resistant to Erythromycin and Gentamycin while *Bacillus Sp.* was resistant to Pefloxacin.

DISCUSSION

Microbiological and antibiotics resistance profile of bacterial isolates from refrigerated melon (egusi) soup prove that bacteria can grow during refrigeration/freezing. This project work further indicates the total bacteria count of the isolates, *Bacillus sp*, *Streptococcus sp*, and *S. aureus*. It was observed that the number of the bacteria increased from after 3days 5.0×10^3cfu/ml, after 7 days 6.2×10^3cfu/ml, reduced after 14days 4.8×10^3cfu/ml and became stabilized, after 21 days 5.0×10^3cfu/ml. This work therefore agrees with (Jay, 2005) who stated that freezing does not kill, prevent or inactivate bacteria but only saw their activities. Refrigeration/freezing has a little effect in slowing bacterial growth; it is not effective in preventing their activity and cannot be considered a method of sterilization. It only extends the shelf life of the food by prolonging the time it takes for bacteria to start proliferating in the food.

The growth pattern of bacteria in the refrigerated food is similar to what is observed in the bacteria growth curve, usually, no immediate increase in cell number occurs and this is called the lag phase. The result in this study is in conformity with previous findings of Cheebrough (2002), which shows that Cell division does not take place right away and there is no net increase in mass. This phase is then followed by the exponential or log phases, microorganisms are growing and dividing at their maximum rate of growth is constant during the exponential phase that is the bacteria are dividing and doubling in number at regular interval (Apostle, 2011). Eventually growth cease, and the number of viable cells remain constant, this is the stationary phase. Finally, the death phase occurs. Detrimental environmental conditions like nutrient deprivation and build-up of toxic waste leads to decline in the number of cells. The death of microbial cells like its growth during the

exponential phase is usually logarithmic, that is a constant proportion of the cell die every hour (Paul, 2013). Antibiotics resistance profile of the isolate showed that Bacillus resistance to pefloxicin while Streptococcus and Staphylococcus was resistance to Erythomycin and Gentamycin. The result of antibiotics resistance and sensitivity of isolates in this study is in line with the former findings of Apostle in (2013) who isolated the different bacterial from refrigerated egusi soup in southern part of Nigeria.

CONCLUSION

A refrigerator/freezer is one of the most important equipment in the kitchen for keeping foods safe. These electric units are so common today. Food safety is a top concern for every commercial kitchen. Duration or period of storage plays a huge role in whether food is safe to eat or needed to be thrown out. Little attention has been paid to refrigeration/freezing. Federal food Inspection Agency has not created adequate awareness on refrigerated/frozen food. Food Inspection Agency should create adequate awareness on effects of extended refrigeration on heath.

Eateries, restaurants and homes should avoid refrigerating soup for more than 3days. Foods should always be prepared in a hygienic environment and by healthy people.

Hands should be washed before handling food materials.

Government should provide the public with a food storage roster to guide their food refrigeration/freezing pattern.

Erythromycin and Gentamycin should not be used in treatment of cases involving *Staphylococcus* and S*treptococcus* or pefloxacin for cases involving *Bacillus*.

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