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Investigation of Bacterial Spoilage of Cooked Food Stored in Freezer during Thawing

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ABSTRACT

The ability of microorganisms to survive in freezer temperatures and cause spoilage of foods during thawing was examined. Cooked foodstuffs used for the study were; vegetable soup, jollof rice, and steamed beef. All were freshly prepared and allowed to cool down before freezing for three (3) days at a temperature of 21ºC after which they were brought out of the freezer and allowed to thawing for three (3) hours at 21ºC. Samples were analyzed for bacteria populations and spoilage pathogens by standard microbiological methods. Total heterotrophic bacterial mean counts for vegetable soup, jollof rice sample, and steamed beef samples were 5.90 x 10^4CFU/g, 6.61x 10^4CFU/g, and 6.43 x 10^4CFU/g respectively. Four bacterial isolates identified and their percentage occurrence from vegetable soup were; *Bacillus cereus* (10%), *Listeria monocytogenes* (10%) *Staphylococcus aureus* (10%) and *Streptococcus* spp (10%). Three isolates from jollof rice were *Bacillus cereus* (10%), *Micrococcus* spp (10%). and *Salmonella* spp (10%) while three isolates from steamed beef were *Pseudomonas* spp (10%)., *Escherichia coli* (10%) and *Listeria monocytogenes* (10%). *Bacillus cereus*, occurred in the soup and jollof rice while *Listeria monocytogenes* occurred in the soup and steamed beef. Percentage occurrences of bacteria in the various samples were vegetable soup, 40%, jollof rice and steamed beef, 30% each. The bacteria isolates in this study are known potential pathogens. Their presence in these foodstuffs samples analyzed is of public health significance. There is need for awareness to be created and caution taken concerning foodstuffs stored in the freezer. When trying to thaw the food, rapid thawing is required or thawing in the refrigerator to avoid further contamination.

Keywords: Cooked food, bacteria, cool temperature, freezer, thawing, spoilage, pathogens.

Introduction

Temperature is an important factor for microbial growth. Each species has its own optimal growth temperature at which it flourishes. Human microbial pathogens usually thrive at body temperature 37ºC. Low temperatures usually inhibit or stop microbial growth and proliferation but often do not kill bacteria. Refrigeration (4ºC) and freezing (-20ºC or less) are commonly used in the food, pharmaceuticals and biotechnology industry (Digirolamo *et al*., 2006; Hamid *et al*., 2014).

Refrigeration preserves food by slowing down the growth and reproduction of microorganisms and the action of enzymes which cause food to rot. The introduction of commercial and domestic refrigerators drastically improved the diets of many in the 1930s by allowing foods, such as fresh fruit, salads and dairy products, to be stored safely for longer periods, particularly during warm weather (Roberto and Richard, 2006; Geraldine and Mary, 2007). It also facilitated transportation of fresh food on long distances.

For longer periods of preservation, freezing temperatures are preferred to refrigeration. Since early times, farmers, fishermen, and trappers have preserved their game and produce in unheated buildings during the winter season (Mossel, 2006; Oscar, 2013). Freezing food slows down decomposition by turning residual moisture into ice, inhibiting the growth of most bacterial species. Fridge temperatures inhibit the proliferation of bacteria better than moulds and yeast.

Freezing temperatures curb the spoiling effect of microorganisms in food, but can also preserve some pathogens unharmed for long periods of time. While it kills some microorganisms by physical trauma, others are sublethally injured by freezing, and may recover to become infectious. Frozen products do not require any added preservatives because microorganisms do not grow when the temperature of the food is below -9.5°C, which is sufficient in itself to prevent food spoilage. Long-term preservation of food may call for food storage at even lower temperatures (Stephen and Phil, 2004; Neelam *et al*., 2005, Karoline *et al*., 2012).

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The bacteriological quality of frozen products depends on the bacteria load of the raw material, contamination during handling and processing and extent of removal of these contaminants during processing. The freezing and storage under frozen condition has detrimental effect on surviving microorganisms and reduction in count is highly variable (Taha *et al*., 2003; Douglas, 2004). Spoilage microorganisms generally grow and cause spoilage when the cooked food product is held for a long time before freezing, frozen at a very slow rate (slow freezing conditions), thawed too slowly or held under thawed condition for a long time (Colin and Da-Wen, 2011). However, the ultimate activity of microorganisms depends on the duration and temperature of holding the product. As most microorganisms are unable to grow below -10ºC or -12ºC, increase in temperature above this limit results in dramatic increase in growth rate. Bacteria grow most rapidly in the range of temperatures between 40 and 140ºF (5ºC and 60ºC) the "danger zone" some doubling in number in as little as 20 minutes (Wendorff, 2001; Chaves *et al*., 2011).

Organisms called psychrotroph, also known as psychrotolerants, prefer cooler environments, from a high temperature of 25ºC, to refrigeration temperature about 4ºC. They are found in many natural environments in temperate climates. They are also responsible for the spoilage of refrigerated food. The organisms retrieved from arctic lakes, such as Lake Whillans, are considered extreme psychrophiles (cold loving). Psychrophiles are microorganisms that can grow at 0ºC and below, have an optimum growth temperature close to 15ºC, and usually do not survive at temperature above 20ºC (Sheryl and Linda, 2001; Silvia and Donald, 2009). They are found in permanently cold environments, such as the deep waters of the oceans. Because they are active at low temperature, psychrotrophs are important decomposers in cold climates.

Freezing is among the most successful techniques used to prolong the storage life of food, with benefits including retention of nutrients and a product that, once thawed, closely resembles the fresh form of food. The preservation properties of freezing are based on the application of temperatures sufficiently low to minimize chemical and biochemical reactions and halt metabolic processes.

Thus, freezing can have a lethal or inhibitory effect on microbiological systems. Freezing of foods and inhibition of microbial growth in frozen foods may actually be considered as coincidental events.

It is well known that freezing temperatures can prevent the growth of microorganisms. However, prolonged shelf life of frozen foods is limited more by adverse chemical (enzymatic and oxidative) and physical (freezer burn) changes than by microbiological concerns (Colin *et al*., 2001; James *et al*., 2009). This does not imply that freezing is not important from the standpoint of microbiological safety and quality.

In fact, apart from irradiation, freezing is probably the method of preservation that is simultaneously least destructive to foods and among the best ways to inhibit microbial growth. Freezing does not kill germs and bacteria; instead, it essentially puts them into hibernation. They are inactive while the food is frozen and will "wake up" as soon as the food thaws, which mean the bacteria, will have the moisture needed to survive. Frozen food has higher risk of causing food poisoning according to the food standard agency (Cheng-Chou and Shu-Jen, 2000; James, 2000). According to the U.S. Department of Agriculture (USDA) guidelines for refrigeration and food safety, there are two kinds of bacteria that can grow on food: pathogenic bacteria, which are especially dangerous as they cause foodborne illnesses, and spoilage bacteria, which develop and grow as food spoils.

Most pathogens do not multiply at freezer temperature and many of them perish because their enzymes do not work properly to maintain normal cell activity. Also, pathogens need water to grow and freezing turns the available water into solid ice crystals. Freezing is a common practice in the meat, fish and other animal protein-based industry, because it preserves the quality for an extended time and offers several advantages, such as insignificant alteration in the product dimensions, and minimum deterioration in products' colour, flavour and texture (Bibek and Marvin, 2008; USDA Food Safety Information, 2013). Almost any kind of food can be frozen; some foods require special treatment before they can be frozen safely. If defrost correctly, frozen foods are generally as safe as their original condition; however, freezing unwholesome food will not make it wholesome. When defrosting food, it is safest to do this in the fridge when food is above 8ºC and below (danger zone) (Leticia *et al*., 2009).

There are bacteria everywhere (Omaye, 2004; Kotsonis and Burdock, 2008; Dolan *et al*., 2010); therefore, allowing cooked food to stay out of cold environment (4.5°C) or in the warm-hot environment (4.5-60.5°C) for a long time may lead to bacterial contamination.

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Hutt *et al*. (2007) and Ladyatiey (2011) posit that a good environment for bacteria to grow is one typical of 4.5°C-60.5°C temperature and that it takes about 20 minutes for bacteria to double in number in such environment. Based on this, United States Department of Agriculture –USDA – (2011) advises that cooked food must not be left unconsumed after 2 hours of cooking or removal from freezers, and, if temperature of that environment is up to 32°C, cooked food must be consumed within an hour from cooking or reheating period.

The standard food safety rule provided by the Food and Drug Administration (FDA) for mitigating food-borne illnesses caused by bacteria states that food should not be held between the temperature of 4°C and 60°C for more than 2 hours. Below 4°C, the bacteria remain viable but will not have a chance to multiply to a sufficient quantity to bother people. Above 60°C, the bacteria will not be able to survive long (bacterial spores, however, can) (Danger zone rule). Danger zone is the temperature range in which harmful microorganisms can flourish. And it is recommended that food should not remain in this danger zone for longer than 2 hours (Hocking, 2003; FDA, 2014).

There are some disadvantages associated with frozen storage (Taha *et al*., 2004). These include freezer burn, product dehydration, rancidity, drip loss and product bleaching, which can have an overall effect on the quality of the frozen foods. There have been reports of illnesses involving frozen foods, ice cream and strawberries, and contaminated ice (Stephen & Phil, 2004). Freezing to 0^{O}F (-17.8^{O}C) or below inactivates microbes (bacteria, yeast, and moulds) present in food; once thawed, however, these microbes can again become active, multiplying under the right conditions to levels that can lead to food-borne illnesses. Since they will then grow at about the same rate as microorganisms on fresh food, thawed items must be handled as any perishable food (USDA Food Safety Information, 2011).

The manner and condition in which frozen food is stored and the way in which it is thawed can have a major impact on the quality of the final consumer product. Poor defrosting (thawing without refrigeration) encourages proliferation of microbial contamination (USDA, 2011), just like unhygienic handling and processing (inadequate cooking). These have led to outbreak of food-borne illnesses (James, 2000; Nester *et al*., 2007; Oscar, 2013). Storage at low temperatures prolongs the shelf life of many foods.

In general, low temperatures reduce the growth rates of microorganisms and slow many of the physical and chemical reactions that occur in foods. Low temperatures are used to preserve food by lowering microbial activity through the reduction of microbial enzyme activity. However, psychrophilic bacteria are known to grow even at commercial refrigeration temperatures (7ºC). These bacteria include members of the genera *Pseudomonas*, *Alcaligenes*, *Micrococcus* and *Flavobacterium* (Mossel and Pflug, 2009). Some of the fungi also grow at refrigeration temperatures. Freezing reduces the number of microorganisms in food but does not kill all of them and damage is caused by ice crystals. Refrigeration slows down the biological, chemical, and physical reactions that shorten the shelf life of food. Exposure of microorganisms to low temperatures reduces their rates of growth and reproduction. Microbes are not killed at low temperature for a considerable period of time. In refrigerators at 5ºC, foods remain unspoiled but, in a freezer at -5ºC, the crystals formed tear and shred microorganisms and may kill many of the microbes. However, some are able to survive, like *Salmonella* spp. and *Streptococcus* spp. For these types of microorganisms, rapid thawing and cooking is necessary (John and Nino, 2009).

According to Kai *et al*. (2015), freezing is one of most commonly used processes commercially and domestically for preserving a very wild range of foodstuffs, including prepared foodstuffs, which would not have required freezing in their unprepared state. Freezing makes water unavailable to microorganisms; the chemical and physical reactions leading to deterioration are slowed by freezing. Many home freezers are held at -10ºC, while commercial freezers are under -18ºC, a temperature at which the growth of microorganisms is almost stopped. As noted by Angela *et al*. (2017), frozen foods can have long shelf life, but continuous exposure to warmer temperature, such as the opening and closing of the freezer doors, can introduce microbes into the freezer. Microorganisms live in every part of the biosphere and some of them are even capable of growing at low temperature, including those below the freezing point. These microorganisms live in the sea or in high mountains, but also in the refrigerators, where they may spoil or, as pathogens, contaminate foods (Foster and Mead, 2008; Bibek and Marvin 2008).

Therefore, although storing foods in the refrigerator is the best way to keep them safe from bacterial contamination, there are also bacteria that can grow in cold temperature as well as inside refrigerators.

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Examples of pathogenic bacteria capable of surviving in refrigerated/frozen foods are *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, and *Pseudomonas* spp. Most of these microorganisms are well able to grow down to 0-2ºC, can enter the refrigerator through raw and improperly packaged foods (for example meats, eggs, and milk) or even through an open refrigerator door during opening and closing, and from warm temperature. If ingested, they cause dangerous food-borne illnesses, including sepsis, diarrhoea, meningitis, dysentery, food poisoning, urinary tract infections, and gastrointestinal infections (Geoffrey *et al*., 2007).

In view of the foregoing, this work investigated why bacteria are able to survive in cool temperature in foodstuffs stored in the freezer and how they contaminate food during thawing.

Materials and Method

Sample Collection

Freshly cooked vegetable soup, jollof rice, and steamed beef were left for three (3) hours to cool down before being put in the freezer, and were stored for three (3) days in the freezer before letting them out for thawing. The thawing process took three (3) hours before subjecting the samples to analysis.

Isolation of the Bacteria in the Samples

Ten grams (10 g) of each food sample was blended in sterile warring blender and homogenized and inoculated into triplicate tubes of 90 ml Brain Heart Infusion (BHF) broth (Oxoid). The tubes were incubated aerobically and anaerobically for 24-48 hours pre-enrichment at 15ºC, later serially diluted. The spread-plate method was used to isolate the bacteria present. Ten grams (10 g) portion of each sample was also blended in 90 ml peptone water (Germany). One millilitre (1 ml) of each of the homogenates was diluted 10^{-3}, 10^{-4}, 10^{-5} and 0.1ml pipetted into nutrient agar plates and BHF agar plates, Mac-Conkey agar and Eosine Methylene Blue agar (Oxoid). The plates were incubated in triplicates aerobically and anaerobically at 37^0C for 24 hours. Later, subculturing was done and stored on agar slants and kept in the fridge at 4ºC for further tests. Mannitol salt agar (Biolab. Hungary) was inoculated for isolation of *Staphylococcus aureus*, while *Salmonella/Shigella* agar (Oxoid) was inoculated after pre-enrichment in Selenite-F broth (Oxoid) for the isolation of *Salmonellae*. A gram of each was inoculated into lactose broth in a capped test tube with inverted Durban tubes for coliform test. Identification of the isolates obtained was carried out using colonial morphology and Gram-staining, as recommended by Nester *et al*. (2007), and biochemical tests were carried out as described by Ochei and Kolhather (2001) and Cheesbrough (2005).

Total Aerobic Plate Count

This was carried out according to the method of Adullahi *et al*. (2004). Total bacterial counts were enumerated on nutrient agar plates by the spread plate method using 0.1 ml of 10-fold dilution (10^{-2} to 10^{-5} of bacterial suspensions. Colony counts were made with colony counter (Oxoid). The bacterial colonies on plates were counted and randomly picked and purified by sub-culturing on fresh nutrient agar plates using the streak plate technique. Counts were expressed as colony forming units per gram ($cfug^{-1}$) sample; characteristic discrete colonies on the different media were isolated.

Characterization and Identification of Isolates

The following tests were carried out for the characterization and identification of the bacterial isolates: Gram Staining, Oxidase Test, Coagulase Test, Indole Test; Urease Test, Catalase Test, Methyl Red Test, Citrate Utilization Test, Voges Proskeur Test, Motility Test, Sugar Fermentation Test, as recommended by Nester *et al*. (2007) and Cheesbrough (2005).

Results

The microbial load/count of the various samples is presented in Table 1. The total heterotrophic bacterial mean counts of the samples were as follows: soup samples – 5.90 x 10^4 CFU/g; jollof rice –6.61 x 10^4 CFU/g; and steamed beef samples –6.43 x 10^4 CFU/g.

Table 1: Mean values of Total Bacteria Count in Colony Forming Unit (CFU/g) of the Food Samples

S/N	Food Sample	Total Bacteria Count (CFU/g)
1	Vegetable Soup	5.90 x 10^4
2	Jollof Rice	6.61 x 10^4
3	Steamed Beef	6.43 x 10^4

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The results of the morphological and biochemical characteristics of the bacterial isolates from the food samples are presented in Table 2. Morphologically, the colonies were placed according to their colour, shape, size, elevation, transparency, and many others. The gram stain showed the shape of the organisms as either gram positive or gram negative. The biochemical features of the isolated bacteria were used to fully ascertain the identity of the isolates. A total of ten (10) bacterial isolates were identified from the samples. Four were identified from the soup: *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes* and *Streptococcus* sp.; three from the jollof rice; *Bacillus cereus, Micrococcus* sp., and *Salmonella*; and three from the steamed beef: *Pseudomonas* sp., *Escherichia coli* and *Listeria monocytogenes*, as shown in Table 3. Although *Bacillus cereus* appeared in both soup and jollof rice samples and *Listeria monocytogenes* also appeared in soup and steamed beef samples. The percentage occurrence of the bacteria isolated is presented in Table 4. It shows that the occurrence of bacteria in the soup sample was 40%, jollof rice and steamed beef 30% each. The contamination level of the bacteria in the various food samples is also shown: *Bacillus cereus* and *Listeria monocytogenes* had 20% each; while *Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus aureus, Salmonella* sp., *E. coli*, and *Streptococcus* sp. had 10% each. Also a chart is used to present the percentage occurrence of the bacteria in the food samples.

Table 3: Cultural, morphological, biochemical characteristics and probable identity of bacteria isolated from Foodstuff Samples

Isolates	Gram Reaction and Cell Morphology	Shape	Colour	Size (mm)	Texture	Elevation	Spore Stain	Catalase	Coagulase	Oxidase	Citrate	Motility	Met Red	Voges Proskaeur	Indole	Glucose	Sucrose	Manitol	Lactose	Probable Organisms
Iso 1	Gram+ rods	Circular	Cream	3	Translucent	Flat	+	-	-	-	+	+	-	+	-	+	+	-	-	*Bacillus cereus*
Iso 2	Gram- rods	Circular	Yellow	1.3	Opaque	Convex	-	-	-	+	+	+	+	-	-	+	-	-	-	*Pseudomonas* sp.
Iso 3	Gram+ cocci	Circular	Yellow	2.8	Opaque	Flat	-	-	-	-	+	-	-	+	-	+	+	+	+	*Micrococcus* sp.
Iso 4	Gram+ rods	Circular	Cream	2	Opaque	Raised	-	+	+	-	+	-	-	+	-	+	+	+	+	*Staphylococcus aureus*
Iso 5	Gram- rods	Circular	Cream	2	Translucent	Raised	-	+	+	-	+	+	-	+	-	+	+	-	+	*Salmonella* sp.
Iso 6	Gram+ rods	Circular	Colorless	2	Translucent	Raised	-	+	+	+	-	+	+	+	+	+	+	+	+	*Listeria* sp.
Iso 7	Gram- cocci	Circular	Red	2	Opaque	Elongate	-	+	+	+	-	+	+	.	+	+	+	+	-	*Escherichia coli*
Iso 8	Gram+ cocci	Round	Red	0.9	Opaque	Raised	-	-	+	-	+	-	+	+	-	+	+	-	+	*Streptococcus sp.*

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Table 3: Bacteria Isolated from Various Food Stuff Samples

Food Sample	Organisms Isolated
Vegetable soup	*Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Streptococcus* sp.
Jollof rice	*Bacillus cereus, Micrococcus* sp., *Salmonella* sp.
Steamed beef	*Pseudomonas* sp., *Escherichia coli, Listeria monocytogenes*

Table 4: Distribution and Percentage Occurrence of Bacterial Isolates from the Food Samples

Organism	Vegetable soup	Jollof rice	Steamed beef	Contamination level	%
Bacillus cereus	+	+	-	2	20
Staphylococcus aureus	+	-	-	1	10
L. monocytogenes	+	-	+	2	20
Streptococcus sp.	+	-	-	1	10
Micrococcus sp.	-	+	-	1	10
Salmonella sp.	-	+	-	1	10
Pseudomonas sp.	-	-	+	1	10
Escherichia coli	-	-	+	1	10
Total occurrence of bacteria in sample	40	30	30	10	100

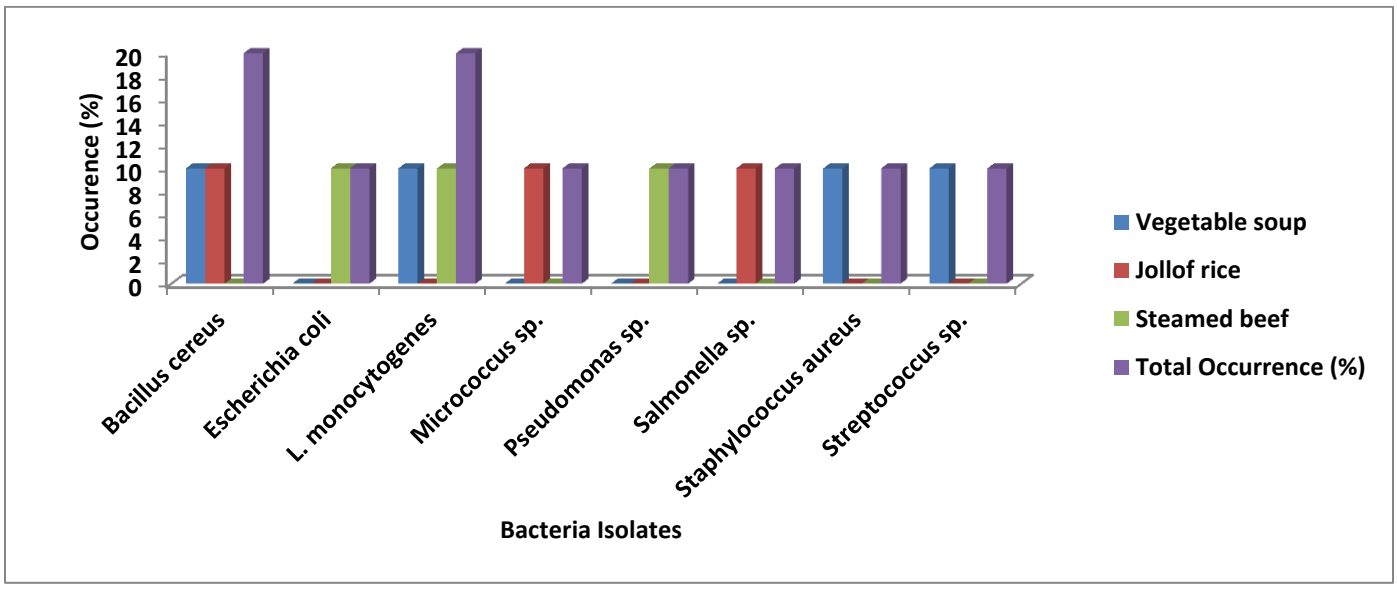

Fig. 1: Percentage Occurrence of Bacterial Isolates from the Food Samples

Discussion

Foodstuffs stored in the freezer can be contaminated by microorganisms during thawing which is known to allow the growth of microbes that have been inactive during refrigeration. Some of these organisms are known to cause food-borne infections and some cause food poisoning.

For example, *Staphylococcus aureus* and *Bacillus cereus* are capable of producing enterotoxins and are noted to survive for extended periods in hostile environment, such as heating (thermal stable), and they are resistant to heat and ultraviolent radiation (Sanni *et al*., 2000).

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The spread plate technique was used to isolate the bacteria associated with the foodstuffs. A total of eight (8) bacterial species were identified, namely: *Bacillus cereus, Pseudomonas* sp., *Micrococcus* sp., *Staphylococcus aureus, Salmonella* sp., *Listeria monocytogenes, Streptococcus* sp., and *Escherichia coli* (Table 2). The isolation of these organisms in the foodstuffs showed the level of contamination.

The bacterial load/counts of the various food samples, presented in Table 1, showed that the mean bacterial load/counts of the soup sample was $5.90x10^4$ (log cfu/g), that of jollof rice was $6.61x10^4$ (log cfu/g), and that of steamed beef was $6.43x10^4$ (log cfu/g). Thus, the soup sample had the lowest bacterial counts and the jollof rice sample had the highest. Stephen and Phil (2004) present a similar result; bacteria counts in the order of 10^4 bacteria per gram were reported in freeze-thawed tomatoes sauce. The quantity of bacteria on food is expressed as the number of colony units per gram (CFU/g). Levels of 100 CFU/g in food at point of consumption are regarded as safe, meaning people consuming foods with low levels of these microbes are at low risk; only those that consume more than one million CFU per serving are at risk of food infections.

The microbial loads of the samples were not within the minimum acceptable microbiological level required by the United States Department of Agriculture (USDA) and National Agency for Food and Drug Administration and Control (NAFDAC), which stipulated that the maximum allowable number of microorganisms in a sample unit of food should not be more than 10^4/ml or 10^4/g.

These organisms have previously been identified from foods stored in the freezer (Taha *et al*., 2004). The presence of these organisms in the foodstuffs may be due to contamination during thawing or inactive spores in the foods that sprang up during thawing. Another study indicated that the spores of Pseudomonas sp., a major spoilage organism of meat and meat product, can survive freezing storage. Nester *et al*. (2007) assert that spores can remain viable more than 100 days at 2ºC. Also, James (2000) posit that *Bacillus* and *Clostridium* spore-formers species would survive daily cycles in dry ice followed by 4.5 hours of thawing at 25ºC. The pathogens that survive the freezing procedure constitute a threat to consumers. In addition, the toxins produced by these pathogens are less affected by freezing.

Listeria monocytogenes can be spread with an infected products or surface, such as hands or kitchen counters, during food preparation. Thoroughly cooking products to 165ºF/74ºC will kill the bacteria *L. monocytogenes*. Consumers at high risk of contracting Listeriosis should reheat food immediately before consumption. Contamination may be through surfaces, utensils, plates, and hands during thawing.

Food-poisoning is not just something one gets outside the home; the meals one prepares can be a source of food-poisoning too. The United States Department of Agriculture says that all food should be frozen or refrigerated within 2 hours of being out. Otherwise, perishable food kept at room temperature goes into the "danger zone" of 40 and 140ºF or 4.44 to 60ºC.

Previous research has shown that *Salmonella* and other Gram negative species are able to survive freezing and frozen storage, with varying degrees of injury depending mainly on the freezing rate and temperature. This injury, however, is rapidly repaired, hence their capability to cause disease once favourable environment conditions return. *Salmonella* has been proven able to survive freezing, particularly when in non-fluid substrates (Oscar, 2013). Another study indicates that the spores of *Clostridium welchii*, a major spoilage organism of meat and meat product, can survive freezing storage (Colin and Da-Wen, 2011).

There are bacteria everywhere, as noted by Omaye (2014). Therefore, allowing cooked food to stay out of cold environment (4.5^0C) or in the warm-hot environment ($4.5\text{-}60.5^0$C) for a long time may lead to bacterial contamination. Kotsonis and Burdock (2008) and Dolan *et al*. (2010) note that bacterial food contamination occurs in foods that are kept for long periods in the danger zone ($5.0\text{-}60^0$C). Similarly, Nester *et al*. (2007), Oscar (2013) and Taha *et al*. (2004) reported the prevalence of same microorganisms in traditional foods. Also in agreement with this study is James (2000), who isolated the same bacterial species from different freeze-thawed foodstuffs. He claims that these foods are potential vehicles for transmitting food-borne illnesses if not heat treated thoroughly. Temperature is one factor that determines whether a bacterial cell can grow and reproduce or not. At optimum temperature, bacterial cells grow and increase in number and some produce toxins at certain temperatures.

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This study is in agreement with Hocking (2003) and Ladyatiey (2011), who aver that a good environment for bacteria to grow is one typical of 4.5-60.5^{0}C and that food left too long at unsafe temperature could be dangerous to eat. Based on these findings, United States Department of Agriculture –USDA – (2011) advises that cooked food must not be left unconsumed after 2 hours of cooking or removed from freezers

Also the standard food safety rule provided by Food and Drug Administration (FDA) states that food should not be held between the temperatures of 4^{0}C and 60^{0}C for more than 2 hours (FDA, 2014). This is supported by the findings of Hutt *et al.* (2009), which noted that most bacteria grow well at freezer temperatures of 0 -18^{0}C (psychrophilic) and can contaminate foods when the food is left at temperature range of 35-40^{0}C. Therefore, freeze-thawing food must be maintained at temperatures below 0-27^{0}C and rapid thawing should be done to prevent bacterial contamination.Works done by Omaye (2004), Kotsonis and Burdock (2008) and Dolan *et al*. (2010) are in agreement with this study. It is, therefore, possible that contamination of the foodstuffs took place during thawing of the food, as a result of the long period of time taken for the frozen foods to thaw. This finding also agrees with that of Sanni *et al*. (2000), who isolated *S. aureus* and other Psychrophiles from food products after four (4) hours of thawing. Spoilage microorganisms generally grow and cause spoilage when the cooked food product is held for a long time before freezing, frozen at a very slow rate (slow freezing conditions), thawed too slowly or held under thawed condition for a long time (Colin and Da-Wen, 2011).

Freezing does not kill germs and bacteria; instead, it essentially puts them into hibernation. They are inactive while the food is frozen and will “wake up” as soon as the food thaws, which mean the bacteria will have the moisture needed to survive. Frozen food has higher risk of causing food poisoning (Cheng-Chou and Shu-Jen, 2000; James, 2000). There have been reports of illnesses involving frozen foods, ice cream and strawberries, and contaminated ice (Stephen & Phil, 2004). Freezing to 0^{O}C (-17.8^{O}C) or below inactivates microbes (bacteria, yeast, and moulds) present in food.

Once thawed, they can again become active, multiplying under the right conditions to levels that can lead to food-borne illnesses.

Since they will then grow at about the same rate as microorganisms on fresh food, thawed items must be handled as any perishable food (USDA Food Safety Information, 2011). The manner and condition in which frozen food is stored and the way in which it is thawed can have a major impact on the quality of the final consumer product. Poor defrosting (thawing without refrigeration) encourages proliferation of microbial contamination (USDA, 2011), just as unhygienic handling and processing (inadequate cooking). These have led to outbreak of food-borne illnesses (James, 2000; Nester *et al*., 2007; Oscar, 2013). Examples of pathogenic bacteria capable of surviving in refrigerated/frozen foods are *Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica, Aeromonas hydrophila,* and *Pseudomonas* spp. Most of these microorganisms are well able to grow down to 0-2^{O}C, can enter the refrigerator through raw and improper packaged foods (such as meats, eggs, and milk) or even through an open refrigerator door during opening and closing, and from warm temperature, and, if ingested, can cause dangerous food-borne illnesses, including sepsis, diarrhoea, meningitis, dysentery, food poisoning, urinary tract infections, and gastrointestinal infections (Geoffrey *et al*., 2007).

This study reveals that, freezing does not kill germs and bacteria; instead, it puts them into hibernation. They are inactive while the food is frozen and will “wake up” as soon as the food thaws, which implies that the bacteria will have the moisture needed to survive and proliferate. Frozen food has higher risk of causing food poisoning. Freezing to 0^{O}C (-17.8^{O}C) or below inactivates microbes (bacteria, yeast, and moulds) present in food. Once thawed, they can again become active, multiplying under the right conditions to levels that can lead to foodborne illness. Since they will then grow at about the same rate as microorganisms on fresh food, thawed items must be handled as any perishable food. Frozen foods can have long shelf life, but continuous exposure to warmer temperature, such as opening and closing of the freezer doors, can introduce microbes into the freezer.

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Below 4^{O}C bacteria remain viable but will not have a chance to multiply to sufficient quantity to harm anyone. Also above 60^{O}C, bacteria will not be able to survive long. Therefore, food should not be held between the temperature of 4^{O}C and 60^{O}C for more than 2 hours.

Spoilage microorganisms generally grow and cause spoilage when the cooked food product is held for a long time before freezing, frozen at a very slow rate (slow freezing conditions), thawed too slowly or held under thawed condition for a long time. The manner and condition in which frozen food is stored and the way in which it is thawed can have a major impact on the quality of the final consumer product.

Poor defrosting (thawing without refrigeration) encourages proliferation of microbial contamination, just as unhygienic handling and processing (inadequate cooking). These have led to outbreak of food- borne illnesses.

Frozen food has higher risk of causing food poisoning and food infections. From the findings of this study, it is recommended that, freezing, thawing, and processing of food should be conducted according to hygiene standards. Since microorganisms can grow in cold temperatures at about the same rate as microorganisms on fresh food, thawed items must be handled as any perishable food, rapid thawing (defrosting) and thorough cooking are necessary for frozen foods. Cooked foods must not be left unconsumed after 2 hours of cooking or removal from freezer and food should not remain in the danger zone (4^{O}C and 60^{O}C) for more than 2-4 hours.

In conclusion, this study has shown that there are many types of bacteria that can grow in cold temperatures as well as inside the freezers. Examples of pathogenic bacteria capable of surviving in refrigerated/frozen foods are *Campylobacter jejuni*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Pseudomonas* spp., *Bacillus cereus*, *Micrococcus* spp., *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli*, and *Streptococcus* spp. If ingested, they cause dangerous food-borne illnesses, including sepsis, diarrhoea, meningitis, dysentery, food poisoning, urinary tract infections, and gastrointestinal infections.

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