EFFECTS OF FREEZING AND THAWING ON THE MICROBIOLOGICAL AND PHYSICOCHEMICAL QUALITIES OF FROZEN PORK

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ABSTRACT

This study was designed to establish the roles played by freezing and subsequent thawing of meat on the microbiological and physicochemical qualities of frozen pork. Initial microbiological and physicochemical assay of fresh pork bought from Marian market Calabar – Nigeria was carried out. A 10g sample of the meat was frozen for two weeks and later thawed and analyzed for pathogens and nutrient composition. The total microbial load of fresh pork ranged between 1.20x10⁴ cfu/g to 4.9x10⁵ cfu/g. Total coliforms was between 2.0x10⁴ cfu/g to 6.1x10⁵ cfu/g and total fungal count was between 1.96x10⁴ cfu/g to 5.7x10⁵ cfu/g. Total microbial load for freeze-thawed pork ranged between 5.0x10⁴ cfu/g to 2.6x10⁵ cfu/g. Total coliform was 3.2x10⁴ cfu/g to 7.8x10⁵ cfu/g. A total of 18 isolates including the following genera Pseudomonas, Salmonella, Staphylococcus, Escherichia, Enterobacter, Serretia, Penicillium and Aspergillus were prominent in fresh sample. There was a drastic disappearance of some of these organisms in the freeze-thawed sample as only Salmonella and Pseudomonas sp. survived. Decrease in nutrients especially the water soluble vitamins. It is worthy of note that freezing does not bring about sterility of frozen meat. Microbial load and types were reduced while some physicochemical qualities like tenderness, colour and vitamin content were negatively affected. However, freezing, safe thawing, proper cooking and hygiene can greatly reduce the microbial load to as low as 1x10² cfu/g which is the acceptable level of microorganisms in food by the World Health Organization.

KEYWORDS: Pork, freeze-thawing, Salmonella, Pseudomonas, nutrients.

INTRODUCTION

Meat has been a regular food for man as far as there has been any evidence of civilization on the face of the earth. Meat is animal flesh that is eaten as food. Generally, this means the skeletal muscle and associated fat and other tissues such as offals (Lawrie and Ledward, 2006). Often meat is used in a more restricted sense, the flesh of mammalian species (pigs, cattle, lambs etc) raised and prepared for human consumption to the exclusion of fish and other seafood, poultry and other animals (Collins English Dictionary, 2017). Meat can either be fresh or processed. Meat is a nutrition dense food which provides high quality protein and essential nutrients like iron, zinc, vitamin B₁₂ and omega-3s. Contrary to popular belief, lean red meat is not a major contributor to the total saturated fat in the diet as reported by Lawrie and Ledward (2006). Pig is one of the oldest form of livestock, having been domesticated as early as 500BC as reported by Nelson (1998). It is believed to have been domesticated either in the near East or in China from the wild bear (Nelson, 1998). The adaptable nature and omnivorous diet of this creature allowed humans to domesticate it much earlier than many other forms of livestock such as cattle. Pigs were mostly used for food, but people also used their hides for shields and shoes, their bones for tools and weapons and their bristles for brushes (Nelson, 1998). Pig can be slaughtered and used for meat called pork. Presently, meat and other food products can be preserved by freezing. The application of freezing for the preservation of foods has been practiced for several years to maintain their quality during storage, distribution and marketing (Person and Londahl, 1993). The overall process include; first, the actual freezing operation, where most water in the food is converted into ice, resulting in a hard solid material; second, frozen storage and finally, thawing where the frozen storage is more or less transformed back into its original state (Osman and Faruk, 2016). According to Martino and Zaritzky (1998), most physical and chemical changes occurring in foods during freezing are caused either directly or indirectly from water to ice transformation. Damage pertaining to the size and location of ice crystals within the food structure, mechanical damage cause by volume changes in the food structure and mechanical damage resulting from concentration of non-aqueous constituents are factors considered to be involved in food damage during freezing operation (Sun, 2006). Thawing is also considered to be a more significant cause of
quality damage than freezing. Generally speaking, the quality of frozen food is closely related to freezing and thawing processes as reported by Jacek and Paulius (2002). They also discovered that the rate of freezing is critical to minimize tissue damage and drip loss in thawing. Thawing generally occurs slowly than freezing. During thawing, foods are subjected to damage by physical and chemical changes and microorganism (Jacek and Paulius, 2002). Therefore, optimum thawing procedures should be of concern to food technologists. Quick thawing at low temperature and expensive dehydration of food is desirable to ensure food quality (Jacek and Paulius, 2002 and Kalichesky et al., 1995).

MATERIALS AND METHODS

Sample collection
Forty grams (40g) of fresh pork was purchased from Marian market abattoir, Calabar, Nigeria, packed in a plastic container and placed in a cooler containing ice block. This was immediately transported to microbiology laboratory, University of Calabar for microbiological analysis.

Sample Preparation/Microbiological Analysis
Ten grams (10g) of the sample was aseptically weighed using a digital weighing balance. This was homogenized with 90ml of peptone water (PW) using a sterile electric blender. A 10-fold serial dilution was carried out using 1ml of the homogenized pork sample pipetted aseptically into 9ml of PW contained in sterile, well labeled test tubes arranged in a test tube rack. 10⁻⁴, 10⁻⁵ and 10⁻⁶ dilutions were plated in duplicates using the spread plate technique on Nutrient Agar for general isolation, Mac Conkey Agar, Mannitol salt Agar and Potatoes dextrose Agar for total coliform, isolation of Staphylococcus aureus and isolation of yeast and mold respectively. The plates were incubated at 37°C for 24-48 hours (except for plates containing PDA which was left on the bench to grow at room temperature for 72hours) to obtain total viable aerobic bacteria, coliform and fungal count. Another ten grams (10g) of the sample was left in the freezer at -8°C for two weeks after which it was thawed in the refrigerator and analyzed using standard microbiological technique as described above for fresh pork sample.

Media Preparation
All media used were prepared according to manufacturer’s specification and sterilized by autoclaving at 121°C for 15minutes.

Physicochemical Analysis
Fresh and freeze-thawed pork samples were analyzed for physicochemical properties of colour, tenderness etc by 10 trained and untrained panelists familiar with meat evaluation after thawing. Panelists were selected among staff and students of the department and trained according to the American Meat Science Association guidelines (AMSA, 1995). Prior to sample evaluation, all panelists participated in orientation session to familiarize with the scale attributes (tenderness, colour, overall and so on) of raw meat using intensity scale. Sensory qualities were evaluated after thawing using the 5-point scoring method. Sensory scores were 5 for excellent, 4 for very good, 3 for good, 2 for fair and 1 for poor (Rahman et al., 2012). Microbiological detection according to Ford (1952) was used to determine the water soluble vitamin (vitamin B₁₂) loss in freeze-thawed pork sample. 10g of fresh pork was homogenized in 10ml of sterile water and strained to get the juice which was used as control for this analysis while the pork exudates after freeze-thawing was the test sample. A previously cultured Lactobacillus leichmannii was grown for 24 hours inside a liquid skim-milk-based medium with a carefully regulated pH before being added to the fresh pork juice and freeze-thawed pork exudates assay and autoclaved for 15mins at 120°C and incubated for 24hours at 37°C (Skeggs et al., 1950).

Enumeration and Isolation of Microflora of Pork
At the end of the incubation period, colonies were counted manually. The counts for each plate were expressed as colony forming unit of the suspension or dilution (CFU/g). Discreet colonies were subcultured into fresh agar plates aseptically to obtain pure cultures of isolates. Pure isolates of resulting growth were stocked in agar slants and stored in the refrigerator for subsequent characterization and identification test.

Characterization and Identification of Isolates
Colonies identified as discreet on the different media used were carefully examined microscopically for cultural size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests according to Whitman et al. (2012) were carried out. The fungal isolates were characterized by their cultural properties, stained with cotton-blue lactophenol solution and observed under low power (x40) objective lens.

Gram Staining
The Gram staining technique was carried out to characterize isolates into two main groups; Gram positive and Gram negative based on their cell wall composition (Fawole and Oso, 2001).

RESULT
Fresh pork and freeze-thawed pork samples were analyzed. The fresh sample was found to contain bacterial isolates identified as Escherichia coli, Staphylococcus aureus, Salmonella sp., Pseudomonas aeroginosa, Enterobacter sp and Pseudomonas sp, and fungal isolates identified as Aspergillus sp., and Penicillium sp. The freeze-thawed pork sample contained only Pseudomonas sp., and Salmonella sp by comparing their morphological and biochemical characteristics with standard reference organisms as described by Bergey’s Manual for Determinative Bacteriology (John et al., 2000).
Table 1 shows the total aerobic bacteria, coliform and fungi count grown on different culture media with fresh pork sample showing a higher number of colonies compared with freeze-thawed pork sample.

Table 1: Total aerobic bacteria, coliform and fungi count.

<table>
<thead>
<tr>
<th>Medium of isolation</th>
<th>Dilution factor</th>
<th>Number of colony Fresh pork</th>
<th>Freeze-thawed pork</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>10^{-5}</td>
<td>4.7x10^5</td>
<td>3.5x10^5</td>
</tr>
<tr>
<td>MSA</td>
<td>10^{-5}</td>
<td>5.8x10^7</td>
<td>-</td>
</tr>
<tr>
<td>MCA</td>
<td>10^{-5}</td>
<td>5.9x10^7</td>
<td>-</td>
</tr>
<tr>
<td>PDA</td>
<td>10^{-5}</td>
<td>5.4x10^7</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: NA= Nutrient agar, MSA=Mannitol salt agar, MCA=Mac Conkey agar, PDA=potatoes dextrose agar

Table 2 shows the morphological and colony characteristics of the isolates on different culture media ranging from creamy to milky white to golden yellow to greenish in colour and curved rods, rods and cocci in cellular morphology.

Table 2: Colony characteristics on different culture media.

<table>
<thead>
<tr>
<th>Medium of isolation</th>
<th>Colony colours</th>
<th>Edges</th>
<th>Colony elevation</th>
<th>Cellular morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>Greenish</td>
<td>Entire</td>
<td>Raised</td>
<td>Curved rod</td>
</tr>
<tr>
<td></td>
<td>Creamy</td>
<td>Entire</td>
<td>Flat</td>
<td>Rod</td>
</tr>
<tr>
<td></td>
<td>Milky white</td>
<td>Entire</td>
<td>Raised</td>
<td>Rod</td>
</tr>
<tr>
<td></td>
<td>Golden yellow</td>
<td>Entire</td>
<td>Flat</td>
<td>Coci</td>
</tr>
<tr>
<td></td>
<td>Creamy</td>
<td>Undulated</td>
<td>Raised</td>
<td>Rod</td>
</tr>
<tr>
<td></td>
<td>Red</td>
<td>Entire</td>
<td>Raised</td>
<td>Rod</td>
</tr>
<tr>
<td>MSA</td>
<td>Entire</td>
<td>Entire</td>
<td>Raised</td>
<td>Coci</td>
</tr>
<tr>
<td></td>
<td>Entire</td>
<td>Raised</td>
<td>Rod</td>
<td></td>
</tr>
<tr>
<td>MCA</td>
<td>Entire</td>
<td>Undulated</td>
<td>Raised</td>
<td>Rod</td>
</tr>
<tr>
<td></td>
<td>Entire</td>
<td>Flat</td>
<td>Rod</td>
<td></td>
</tr>
</tbody>
</table>

Some fungal isolates appeared blue-green, gray green, and pale in colour, flat, filamentous, and velvety in texture while some appeared pale brown, brown in colour, smooth walled and larger in size.

Table 3 shows the percentage occurrence of isolates with Pseudomonas sp. and Salmonella sp. showing a higher percentage of occurrence (22.2) compared with Serretia marcescens and Aspergillus sp. with the lowest percentage of occurrence (5.6).

Table 3: percentage (%) occurrence of isolates.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Frequency of occurrence</th>
<th>% of occurrence</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>2</td>
<td>11.1</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2</td>
<td>11.1</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Salmonella sp.</td>
<td>4</td>
<td>22.2</td>
<td>Fresh and freeze-thawed pork</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>4</td>
<td>22.2</td>
<td>Fresh and freeze-thawed pork</td>
</tr>
<tr>
<td>Enterobacter sp.</td>
<td>2</td>
<td>11.1</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Serretia marcescens</td>
<td>1</td>
<td>5.6</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>2</td>
<td>11.1</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>1</td>
<td>5.6</td>
<td>Fresh pork</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Physicochemical Properties

Colour
The colour of the sample was observed after thawing against fresh pork sample as control. The colour was almost similar to control but slightly varied by the thawing process. Assessment of pork after refrigerator thawing showed a moderate score 4, very good.

Tenderness
Significant change was observed in pork sample after thawing in the refrigerator. Tenderness of the frozen pork sample increased as the ice crystal thaws and showed a moderate score 4, very good.

Table 4 shows the sensorial changes of freeze-thawed pork compared to fresh pork sample.
The plates were read after the period of incubation. The control plate showed a significant proliferation of the microorganism *Lactobacillus leichmannii* while the test plate showed significant reduction in the growth of this organism.

**DISCUSSION**

A total of eighteen (18) isolates comprising of five different genera of Gram negative bacteria, one genera of Gram positive bacteria and two genera of fungi were isolated in this study from both fresh and freeze-thawed pork sample with an average incidence of 100%. The bacteria isolated were identified as *Staphylococcus aureus*, *Salmonella sp.*, *Pseudomonas sp.*, *Enterobacter sp.*, *Serretia marcescens*, *Pseudomonas aeruginosa*, and *Escherichia coli* with *Salmonella sp.* and *Pseudomonas sp.* occurring more frequently (22.2%) especially in the freeze-thawed pork sample while the fungi isolates were identified to be *Penicillium sp.* and *Aspergillus sp.* Microorganisms isolated from fresh and freeze-thawed pork sample in this study have been earlier found in other meats and meat products and is agreement with previous reports by Clarence et al., (2009). The presence of these organisms in fresh pork depicts a deplorable state of poor hygiene and sanitary practices employed in the slaughtering processing and packaging of fresh pork. Faecal coliforms such as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals (Huis et al., 1992). Fresh pork purchased from Marian market abattoir in Calabar, Nigeria was analyzed and results displayed in table 1 shows a total aerobic bacteria count which ranged from 4.9x10^6 cfu/g to 1.2x10^7 cfu/g, coliform count which ranged from 6.1x10^6 cfu/g to 2.0x10^7 cfu/g and a fungal count which ranged from 5.7x10^6 cfu/g to 1.9x10^7 cfu/g. This indicates a high rate of contamination especially faecal contamination by coliform bacteria and from fungi which can survive in extreme conditions (Nesbakken et al., 1994). There was as shown in this table great reduction in microbial load in the fresh pork sample subjected to freezing and thawing conditions. Very few organisms survived and their population spread possibly during the thawing process with a total bacteria count which ranged between 2.6x10^5 cfu/g to 5.0x10^6 cfu/g, total coliform count which was remarkably high and ranged from 3.2x10^5 cfu/g to 7.8x10^5 cfu/g. This result shows that some bacteria (grown on NA and MCA) which survived the freezing condition are either psychropiles as in the case of *Pseudomonas aeruginosa* and *Pseudomonas sp.* or resistant to freezing temperature or are possibly shielded by fat contained in pork as in the case of *Salmonella sp.* isolated and identified in the freeze-thawed pork sample. In contrast, the freeze-thawed sample plated on MSA gave a microbiologically unaccepted number which explains that more than 90% of the organism (*Staphylococcus aureus*) could not survive the freezing and thawing condition. The presence of *Escherichia coli* (11.1%) in fresh pork sample indicates faecal contamination of the meat which might be due to the unhygienic handling of the meat during slaughtering and processing or due to possible contamination from the skin, mouth, hand or nose of the handlers which might be introduced directly in to the meat (Schroeder et al., 2005). *Escherichia coli* is a normal flora of the human and animal intestine and has been identified as a leading cause of food borne illnesses all over the world (Hussein, 2007). However, diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) is highly prevalent in young children in developing countries as well as travelers (Duffy et al., 2005). The isolation of *Enterobacter sp.* (11.1%) may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering (Talaro and Talaro, 2006). *Salmonella sp.* (22.2%) found in both fresh and freeze-thawed pork sample is a pathogenic organism of public health significance and concern (Okonkwo et al., 2009).

*Staphylococcus aureus* (11/1%) isolated from fresh pork is also pathogenic. Before antimicrobials were discovered, the mortality or *Staphylococcus aureus* bacteremia was over 80% and more than 70% of patients developed metastatic infections. *Staphylococcus aureus* is however resistant to many antibiotics developed today (Lowry, 2003). *Serretia marcescens* (5.6%) isolated from fresh pork sample is an opportunistic pathogen and is responsible for a variety of infections which include; bacteremia, pneumonia, endocarditis (Engel et al., 2009). *Pseudomonas sp.* (22.2%) isolated from both fresh and freeze-thawed pork has constantly been a threat to the freezing industries and cause human infections such as septicemia, meningitides, pneumonia (McGraw, 2002). Freezing and thawing alter both the content and the distribution of moisture in pork tissue. Moisture as a quality characteristic in pork can be evaluated in several ways including tenderness, thaw loss, drip loss, colour leach etc. Tenderness may be a measure of damage to muscular tissue a structure in the freezing process, reflecting the effectiveness of the thawing process and this is in agreement with Kondratowicz et al. (2008). The significant growth of *Lactobacillus leichmannii* on plate containing fresh pork juice indicates the abundance of vitamin B12 in fresh pork sample because *Lactobacillus leichmannii* requires corrinoids as a growth factor and their growth depends solely on externally supplied vitamin B12 but the reverse was the case with the exudates from freeze-thawed pork sample as there was poor growth of this organism on the plate indicating that some of this water soluble vitamin was leached out of the meat during the thawing process, this corroborates with the work of Skeggs et al. (1950).

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<table>
<thead>
<tr>
<th>Thawing process</th>
<th>Colour</th>
<th>Tenderness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.00 ± 0.00</td>
<td>5.00 ± 0.00</td>
</tr>
<tr>
<td>Refrigerator temperature (-8°C)</td>
<td>3.73 ± 0.24</td>
<td>4.00 ± 0.26</td>
</tr>
<tr>
<td>Level of significance</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS* – not significantly different

Vitamin Loss (Vitamin B<sub>12</sub>)

The table shows a significant reduction in the growth of *Lactobacillus leichmannii* while the test plate showed significant reduction in the growth of this organism.
CONCLUSION
Fresh pork sold to the public in open abattoirs are grossly contaminated with coliform bacteria as well as other bacterial forms and fungi. The findings from this study revealed that fresh pork sold at Marian market abattoir in Calabar, Nigeria is contaminated with pathogenic Gram positive and Gram negative bacteria. The possible sources of these contaminants are due to the unhygienic manner of handling meats in the abattoir. This implies that pork is a source of various diseases and can pose serious health hazards. Freezing does not sterilize food but only reduces the microbial load and type to a very large extent and also preserves the quality of meat and meat products. Irrespective of the presence of these bacteria in pork analyzed. However, freezing, safe thawing, proper cooking and hygiene can greatly reduce the microbial load to as low as 1x10^2 cfu/g which is the acceptable level of microorganisms in food by the World Health Organization.

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