

EFFECTS OF FREEZING AND THAWING ON THE MICROBIOLOGICAL AND PHYSICO-CHEMICAL QUALITIES OF FROZEN PORKUmoafia G. E.*¹ and Okoro C. U.²

Department of Microbiology, University of Calabar, Calabar – Nigeria.

*Corresponding Author: Umoafia G. E.

Department of Microbiology, University of Calabar, Calabar – Nigeria.

Article Received on 19/12/2017

Article Revised on 09/01/2018

Article Accepted on 30/01/2018

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 1.20×10^6 cfu/g to 4.9×10^4 cfu/g. Total coliforms was between 2.0×10^5 cfu/g to 6.1×10^6 cfu/g and total fungal count was between 1.96×10^5 cfu/g to 5.7×10^6 cfu/g. Total microbial load for freeze-thawed pork ranged between 5.0×10^4 cfu/g to 2.6×10^6 cfu/g. Total coliform was 3.2×10^6 cfu/g to 7.8×10^4 cfu/g. A total of 18 isolates including the following genera *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Escherichia*, *Enterobacter*, *Serratia*, *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 1×10^2 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 Zartzyk (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, MacConkey 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 aeruginosa*, *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.

Medium of isolation	Dilution factor	Number of colony Fresh pork	Freeze-thawed pork
NA	10 ⁻⁵	4.7x10 ⁵	3.5x10 ⁵
MSA	10 ⁻⁵	5.8x10 ⁵	-
MCA	10 ⁻⁵	5.9x10 ⁵	-
PDA	10 ⁻⁵	5.4x10 ⁵	-

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.

Medium of isolation	Colony colours	Edges	Colony elevation	Cellular morphology
NA	Greenish	Entire	Raised	Curved rod
	Creamy	Entire	Flat	Rod
	Milky white	Entire	Raised	Rod
	Golden yellow	Entire	Flat	Cocci
	Creamy	Undulated	Raised	Rod
	Red	Entire	Raised	Rod
MSA		Entire	Convex	Cocci
		Entire	Raised	Cocci
		Entire	Convex	Rod
MCA		Undulated	Raised	Rod
		Entire	Flat	Rod
		Entire	Flat	Rod

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 *Serratia marcescens* and *Aspergillus sp.* with the lowest percentage of occurrence (5.6).

Table 3: percentage (%) occurrence of isolates.

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

$$\text{Percentage (\%)} \text{ of occurrence} = \frac{\text{Frequency of occurrence}}{\text{Total number of isolates}} \times 100$$

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.

Table 4: Sensorial changes of freeze-thawed pork compared to fresh pork sample (mean \pm SE).

Thawing process	Colour	Tenderness
Control	5.00 \pm 0.00	5.00 \pm 0.00
Refrigerator temperature (-8°C)	3.73 \pm 0.24	4.00 \pm 0.26
Level of significance	NS	NS

*NS – not significantly different
Vitamin Loss (Vitamin B₁₂)

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.*, *Serratia 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 4.9x10⁶ cfu/g to 1.2x10⁴ cfu/g, coliform count which ranged from 6.1x10⁶ cfu/g to 2.0x10⁴cfu/g and a fungal count which ranged from 5.7x10⁶ cfu/g to 1.9x10⁵ 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 2.6x10⁶ cfu/g to 5.0x10⁴ cfu/g, total coliform count which was remarkably high and ranged from 3.2x10⁶cfu/g to 7.8x10⁴ cfu/g. This result shows that some bacteria (grown on NA and MCA) which survived the freezing condition are either psychrophiles 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). *Serratia 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).

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 that is why some frozen foods still spoil. This study shows that freezing 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 1×10^2 cfu/g which is the acceptable level of microorganisms in food by the World Health Organization.

REFERENCES

1. AMSA Research guidelines for cookery, sensory evaluation, and instrumental tenderness measurements of fresh meat. Chicago III. America Meat Science Association and Nutritional Live Stock and Meat Board, 1995.
2. Clarence, S., Obinna, C. and Shalom, N. *African Journal of Microbial Research*, 2009; 3: 276-279.
3. Collins English Dictionary, Meat definition and Meaning. www.collinsdictionary.com. Retrieved, 2017-06-16.
4. Duffy, G., Butler, F., Cummins, E., O'Brien, S., Nally, P., Carney, E., Henchion, M., Mahon, D. and Cowan, C. *E. coli* O157: H7 in minced beef produced in Ireland: A quantitative risk assessment, draft report. The National Food Centre, Teagasc, Ashtown, Dublin, 2005.
5. Engel, H. J., Collignon, P. J., Whiting, P. T. and Kennedy, K. J. *Serretia sp.* bacteremia in Canberra, Australia. *European Journal of Clinical Microbiology and Infectious Diseases*, 2009; 28: 821-824.
6. Fawole, M. O. and Oso, B. A. Laboratory Manual of Microbiology. Revised Edition, Spectrum Books Ltd Ibadan, 2001; 127.
7. Ford, J. E. The microbiological assay of vitamin B₁₂. *Brit. J. Nutri*, 1952; 6: 324-330.
8. Huis in't Veld, J. H., Mulder, R. W. A. W. and Snijders, J. M. A. Impact of Animal Husbandary and Slaughter Technologies on Microbial Contamination of Meat: Monitoring and Control; Clement-Ferrand France, 1992; 79-100.
9. Hussein, H. *Journal of Animal Science*, 2007; 85: 63-72.
10. Jacek, K. and Paulius, M. Use of Low Temperatures for Food preservation, ISSN 1392-2130, 2002.
11. John, G. H., Peter, H. S. and Noel, R. K. *Bergey's Manual of Determinative Bacteriology*. 9th ed., The Williams & Wilkins, Philadelphia, 2000.
12. Kalichevsky, M. T., Knorr, D. and Lillford, P. J. Potential Food Applications of High-Pressure Effects on Ice-Water Transitions. *Trends in Food Science and Technology*, 1995; 6: 253-258.
13. Kondratowicz, J., Chwastowska-Siwiecka, I. and Burczyk, E. Techological properties of pork thawed in the atmospheric air or in the microwave oven as determined during a six-month deep-freeze storage. *Animal Sci. Papers Reports*, 2008; 26: 175-181.
14. Lawrie, R. A. and Ledward, D. A. *Lawrie's Meat Science (7th ed.)*. Cambridge: Woodhead Publishing Limited. ISBN 978-1-84569-159-2, 2006.
15. Lowry, F. Antimicrobial Resistance: *The Example of Staphylococcus aureus*, 2003; 111: 1265-1273.
16. Martino, N. and Zaritzky, E. Ice Crystal Size Modification during Frozen Beef Storage. *Journal of Food Science*, 1998; 53: 1631-1637.
17. McGraw, H. Concise Encyclopedia of Bioscience, 2002.
18. Nelson, S. M. *Ancestors for the pigs Pigs prehistory*. University of Pennsylvania Museum of Archaeology and Anthropology, 1998.
19. Nesbakken, T., Nerbrink, E., Rotterud, O. J. and Borch, E. Reduction of *Yersinia enterocolitica* and *Listeria spp.* On Pig Carcasses by Enclosure of the Rectum During Slaughter. *International Journal of Food Microbiology*, 1994; 23: 197-208.
20. Okonkwo, o., Ogun, A., Adebayo, A., Ogunjobi, A., Nkang, A. and Adebayo, B. *African Journal of Food Science*, 2009; 3: 35-50.
21. Osman, E. and Faruk, B. T. Food Preservation by Low Temperature. *Food Microbiology*, 2016; 29: 1002.
22. Person, J. O. and Lohndal, K. F. Freezing Technology. Principles and Operations. *Journal of Food Microbiology*, 1993; 36: 53-77.
23. Rahman, S. M., Park, J., Song, K. B., Al-Harbi, N. A., and Oh, D. H. Effect of slightly acidic low concentration electrolyzed water on microbiological, physicochemical, and sensory quality of fresh chicken breast meat. *Journal of Food Science*, 2012; 77: M35-41.
24. Schroeder, C., Naugle, A., Schlosser, W., Hogue, A., Angulo, F. and Rose, J. *Emerging Infectious Diseases*, 2005; 8: 23886-23888.
25. Sun, D. Handbook of Frozen Food Processing and Packaging. Boca Raton, FL: Taylor & Francis Group, LLC, 2006.
26. Skeggs, H. R., Nepple, H. M., Valentik, K. A., Huff, J. W., and Wright, L. D. Observations on the use of *Lactobacillus leichmannii* 4797 in the microbiological assay of vitamin B₁₂. *Journal of Biochemistry*, 1950; 184: 211-221.
27. Talaro, k. and Talaro, A. *Foundation in Microbiology*, 2006; 1: 781-783.
28. Whitman, W. B., Goodfellow, M., Kampfer, P., Busse, H. J., Trujillo, M. E., Ludwig, W. and Suzuki, K. I. (eds). *Bergey's Manual of Systemic Bacteriology*, 2nd ed., vol. 5, parts A and B, Springer-Verlag, New York, NY, 2012.