PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 6 (6): 576-581, 2007
ISSN 1680-5194

Effect of Refrigeration and Frozen Storage on the Shelf-life of Beef Purchased from Local Markets and Abattoir in Calabar Metropolis-Nigeria

C.A. Eneji[1], C.E. Ikpeme[2] and J. Ubua[1]
[1]Department of Animal Science, [2]Department of Biotechnology,
University of Calabar, Calabar, Nigeria

Abstract: Samples of the beef (biceps femoris muscle) obtained from Watt and Marian Markets and Bacocco slaughter-house in Calabar, Cross River State, Nigeria were stored in a refrigerator (8-10°C) and in a freezer (-10 to -20°C). Shelf life assessment based on p^H monitoring, sensory evaluation of colour, odour and sliminess and microbial proliferation indicate that beef stored under refrigeration had a keeping quality or shelf-life of two-six days. While that stored under freezing condition had a shelf-life of 16 days. The refrigerated beef samples maintained high p^H (8-12) hence poor shelf-life. Frozen samples had more or less constant p^H range (3-7). The bacteria load per gram was higher in fresh samples followed by those refrigerated and lastly, the frozen samples. Predominant organisms isolated in this study were species of *Monococus, Staphylococcus*, *E. coli*, *Bacillus, Candida and Streptococcus*. This showed that beef samples from the two markets and the abottoir were actually contaminated. The need to improve on the sanitary conditions is advocated.

Key words: Beef samples, refrigeration, freezing, slime growth, microbial load, shelf-life, market sources

Introduction

Meat is simply defined as animal tissues which are suitable as food. Forrest *et al.* (1975), stated that all processed or manufactured products prepared from animal tissues are included in this definition.

Beef, like other meat types is highly nutritious but extremely perishable food and its preservation has been a subject of concern to individual households and food industries. Preservation is important to maintain the beef quality during distribution and marketing. In developing nations, Nigeria inclusive, refrigeration facilities are not readily available to many people and the situation is aggravated by unreliable power supply. This implies that beef could easily get spoilt due to the physical, chemical and bacteriological degradation which will make it unpalatable and unwholesome (Agiang, 1976).

According to Ihekoronye and Ngoddy (1985) beef quality refers to the degree of suitability or fitness as food and acceptability to consumers. However, Price and Schweigert (1971) stated that for quality of beef and beef products most consumers have in mind a set of attributes rather than a single characteristic which makes quality a relative concept. Generally, the quality of beef can be based on colour, odour, appearance, taste, culinary properties as well as bacteriological status (Eneji, 1985).

Meat preservation therefore involves the application of measures to delay or prevent certain changes which makes beef unwholesome as food. These changes could arise from microbial decomposition, enzymatic/ nonenzymatic reactions and damage from physical abrassions (Frazier, 1976). Such conditions are likely to be more prevalent during slaughtering, transportation and distribution to market settings. The present study therefore is to determine the shelf-life of beef procured from local markets and abattoir in South Eastern Nigeria using refrigeration and freezing preservation techniques.

Materials and Methods

Collection of samples: Samples were collected from Watt and Marian markets as well as abattoir, all in Calabar municipality in Cross River State of Nigeria.

2kg of biceps femoris muscle was bought from both markets on six different occasions. These samples were picked with sterilized forceps and wrapped with sterilized aluminum foil before being taken into the laboratory for analysis. Samples were collected from the abattoir at about the same time but on different days. The sample was stored frozen until laboratory analysis.

Data collection: The initial temperature of beef samples was taken in the market and at the abattoir. The atmospheric temperature of the markets and the abattoir were also taken. Fifteen grams of the beef samples (markets/abattoir) were cut-off aseptically and blended into homogenous quantity in a heat sterilized blender. Distilled water was added to the beef paste for initial p^H determination.

Five grams of the homogenized samples were used for microbiological analysis. The remaining samples from the collection sources were sliced aseptically into 15 parts each. The parts were packed into sterilized polythene bags for refrigeration and freezing storage methods. Initial colour and odour of all samples from

Table 1: Refrigeration P^H profile at 48 hours interval (8-10°C)

Rso	Atm.temp at markets/ abattoir	Sample temp. at markets/abattoir	Initial p^H of sample	Days of Storage									
				2^{ND}	4^{TH}	6^{TH}	8^{TH}	10^{TH}	12^{TH}	14^{TH}	16^{TH}	18^{TH}	20^{TH}
RSM_1	29.5°C	32.0°C	6	7	8	9	10	10	10	12	12	12	12
RSM_1	31.0°C	32°C	7	7	7	8	9	10	10	10	10	10	10
RSA_1	28.4°C	29.0°C	6	6	6	7	7	8	9	9	10	11	12
RSM_2	33.5°C	29.0°C	7	7	7	8	8	9	10	10	10	12	12
RSW_2	28.0°C	28.5°C	6	6	7	7	7	8	10	10	12	12	12
RSA_2	28.2°C	30.5°C	6	6	6	7	7	8	9	9	10	11	12
RSM_3	30. 0°C	29.5°C	7	7	7	8	9	9	10	10	10	12	12
RSW_3	30. 0°C	3`1. 0°C	7	7	8	8	8	9	9	10	10	10	12
RSA_3	28.4°C	30.5°C	6	6	6	7	7	8	9	9	10	11	11
RSM_4	29.4°C	31.5°C	7	7	8	8	8	8	9	9	10	10	12
RSW_4	29°C	29.5°C	7	7	8	8	9	9	9	10	10	10	10
RSA_4	28.7°C	31.5°C	6	6	7	7	8	9	9	10	10	11	12
RSM_5	32.5°C	29.5°C	6	6	7	7	8	8	9	9	9	12	12
RSW_5	31°C	32.0°C	6	6	6	7	7	8	8	9	9	10	10
RSA_5	28.7°C	31.5°C	6	6	7	7	8	9	9	10	10	11	12
RSM_6	33°C	29.5°C	7	7	7	8	8	9	9	10	10	12	12
RSW_6	29.5°C	30.0°C	7	7	7	8	8	9	9	10	10	11	12
RSA_6	28.7°C	31.5°C	6	6	7	7	8	9	9	10	10	11	12

RSO = Notation for Refrigeration sample number, RSW = Notation for Refrigeration Sample Watt market, RSM = Notation for Refrigeration Marian Market RSA = Notation for Refrigeration Abattoir

Table 2: Frozen beef p^H profile at 48 hours interval (-10°C TO-20)

Rso	Atm.temp at markets/ abattoir	Sample temp. at markets/ abattoir	Initial p^H	Days of storage									
				2^{ND}	4^{TH}	6^{TH}	8^{TH}	10^{TH}	12^{TH}	14^{TH}	16^{TH}	18^{TH}	20^{TH}
FSM_1	29.5°C	32.0°C	7	6	6	6	6	6	6	5	5	3	3
FSM_1	31.0°C	32.0°C	7	7	7	7	7	6	6	6	6	6	6
FSA_1	20.4°C	30.4°C	6	6	6	6	6	6	6	6	6	7	7
FSM_2	33.5°C	29.0°C	7	7	7	7	7	6	5	5	3	3	3
FSW_2	28.0°C	28.5°C	6	6	6	6	6	6	5	5	5	5	5
FSA_2	28.4	30.2	6	6	6	6	6	6	6	6	6	6	7
FSM_3	30. 0	29.5	7	7	7	7	7	7	7	6	6	6	5
FSW_3	30. 0	31. 0	7	7	7	6	6	6	6	5	5	5	5
FSA_3	28.4	30.6	6	6	6	6	6	6	6	6	6	7	7
FSM_4	29.5	3.5	7	7	7	7	7	7	5	5	5	5	4
FSW_4	29.0	29.5	7	7	7	7	6	6	6	6	5	5	5
FSA_4	28.7	30.7	6	6	6	6	6	6	6	6	6	7	7
FSM_5	32.5	29.5	6	6	6	6	6	4	6	6	5	5	3
FSW_5	310	32.0	6	6	6	6	6	6	5	5	5	5	5
FSA_5	28.7	30.4	6	6	6	6	6	6	6	6	6	6	7
FSM_6	33.0	19.5	7	7	7	7	7	7	7	7	7	7	6
FSW_6	29.5	30.0	7	7	7	7	6	6	6	5	5	5	5
FSA_6	28.7	30.5	6	6	6	6	6	6	6	6	6	7	7

FSO = Freezer sample numbers, FSW = Notation for freezer sample number from Watt Market, FSM = Notation for freezer sample number from Marian Market

markets and abattoir were recorded using sensory evaluation. Zero (o) was awarded to normal acceptable colour and odour, while the positive sign (+) was awarded to any change in colour or odour detected. The intensity of deteriorative changes was progressively recorded by + signs. The point considered unacceptable and unwholesome was also recorded with a negative sign (-).

Microbiological analysis: Ten grams of beef, aseptically cut-off from the beef bulk samples of the 2 markets and the abattoir sources were separately blended into a homogenous paste in a heat sterilized blender using 10ml of distilled water (p^H 7.0). Five grams each of the homogenized beef samples were used for analysis. A series of 21 sterile plugged test-tubes (seven for each sample) were laid out and 9ml of distilled water aseptically pipetted into the test-tubes. One ml of the already inoculated fresh sample was pipetted into the first test tube labelled 10^{-1} dilution. The pipette was discarded and another sterile pipette used in pipetting 1ml from the 10^{-1} dilution into the second test tube (10^{-2}) and the pipette also discarded. Dilutions up to 10^{-7} for each sample were made after mixing for homogeneity, each time with another sterile pipette.

Table 3: Colour scores for refrigerated samples taken every 48 hours AT 8-10°C storage

Sample Codes	Initial Colour of sample	Days of Storage									
		2ND	4TH	6TH	8TH	10th	12th	14th	16TH	18TH	20TH
RSM_1	Pinkish red	0	0	+	++	+++	++	++	-	-	-
RSW_1	"	0	0	0	+	++	++	++	-	-	-
RSA_1	"	0	0	+	++	+++	-	-	-	-	-
RSM_2	"	0	0	0	+	++	-	-	++++	-	-
RSW_2	"	0	0	0	0	+	+++	++++	-	-	-
RSA_2	"	0	0	+	++	+++	++	-	-	-	-
RSM_3	"	0	0	0	+	++	-	-	++++	-	-
RSW_3	"	0	0	0	+	++	++	+++	-	-	-
RSA_3	"	0	0	++	+++	+++	-	-	-	-	-
RSM_4	"	0	0	0	+	++	-	-	++++	-	-
RSW_4	"	0	0	0	0	+	+++	+++	-	-	-
RSA_4	"	0	0	++	+++	-	++	-	-	-	-
RSM_5	"	0	0	0	0	+	-	-	++++	-	-
RSW_5	"	0	+	0	+	++	+	++++	-	-	-
RSA_5	"	0	0	++	+++	-	-	-	-	-	-
RSM_6	"	0	0	0	+	+	-	-	-	-	-
RSW_6	"	0	0	0	++	++	++	+++	-	-	-
RSA_6	"	0	+	++	+++	-	-	-	-	-	-

Zero (0) = Normal Colour, Positive + = Any change in colour and intensity of such change is noted by the number of (+) sign, Negative (-) = Unacceptable and unwholesome state of sample, RSM = Refrigerated sample from Marian market, RSW = Refrigerated sample from Watt Market, RSA = Refrigerated sample from Abattoir

Table 4: Colour profiles of beef samples stored AT-10°C TO-20°C

Sample Codes	Initial Colour of sample	Days of storage									
		2ND	4TH	6TH	8TH	10th	12th	14th	16TH	18TH	20TH
FSM_1	Pinkish red	0	0	0	0	0	0	0	0	+	+
FSM_1	"	0	0	0	0	0	0	0	0	+	++
FSA_1	"	0	0	0	0	0	0	0	0	+	++
FSM_2	"	0	0	0	0	0	0	0	0	0	+
FSW_2	"	0	0	0	0	0	0	0	0	+	+
FSA_2	"	0	0	0	0	0	0	0	0	0	+
FSM_3	"	0	0	0	0	0	0	0	0	0	+
FSW_3	"	0	0	0	0	0	0	0	0	+	++
FSA_3	"	0	0	0	0	0	0	0	0		+
FSM_4	"	0	0	0	0	0	0	0	0	+	+
FSW_4	"	0	0	0	0	0	0	0	0	+	++
FSA_4	"	0	0	0	0	0	0	0	0	0	+
FSM_5	"	0	0	0	0	0	0	0	0	0	0
FSW_5	"	0	0	0	0	0	0	0	0	+	++
FSA_5	"	0	0	0	0	0	0	0	0	+	++
FSM_6	"	0	0	0	0	0	0	0	0	+	+
FSW_6	"	0	0	0	0	0	0	0	0	+	++
FSA_6	"	0	0	0	0	0	0	0	0	+	++

Subscript 1,2,3,4,5 and 6 are replicate samples, 0= Normal colour, + = Time when colour changes, intensity of change depicted by number of (+) sign, FSM = Freezer sample from Marian market, FSW = Freezer sample from Watt Market, FSA = Freezer sample from Abattoir

Different growth media (Malt extract, MacConkey agar and Nutrient agar) were poured into 15 clean and sterile petri-dishes (five petri-dishes for each sample). One ml of the inoculum from the two markets and abattoir samples were pipetted into the petri-dishes.

All the petri-dishes containing the same growth media were tied together with a cellotape and placed in the incubator maintained at a temperature of 37°C for 24 hours. Characterization of isolates were based on culture, microscopic and biochemical tests (Bunchanan and Gibbons, 1974; Harrigan and McCane, 1976; Baird-Parker, 1976).

Results and Discussion

p^H: The p^H values of samples are summarized in Table 1 and 2.

p^H range varied between 6 and 12 for refrigerated samples and between 3 and 7 for freezer samples.

Table 5: Odour profile of refrigerated beef samples at 8-10°C

Sample Codes	Initial odour description	Days of storage 2ND	4TH	6TH	8TH	10th	12th	14th	16TH	18TH	20TH
FSM_1	Normal	0	0	0	+	++	+++	-	-	-	-
FSM_1	"	0	0	0	+	++	-	-	-	-	-
FSA_1	"	0	0	+	++	+++	-	-	-	-	-
FSM_2	"	0	0	0	+	++	+++	-	-	-	-
FSW_2	"	0	0	0	+	++	-	-	-	-	-
FSA_2	"	0	0	+	++	+++	-	-	-	-	-
FSM_3	"	0	0	0	+	++	+++	-	-	-	-
FSW_3	"	0	0	0	0	+	++	-	-	-	-
FSA_3	"	0	0	+	++	+++	-	-	-	-	-
FSM_4	"	0	0	0	+	+	++	-	-	-	-
FSW_4	"	0	0	0	+	++	-	-	-	-	-
FSA_4	"	0	+	++	+++	-	-	-	-	-	-
FSM_5	"	0	0	0	0	+	++	-	-	-	-
FSW_5	"	0	0	0	+	++	-	-	-	-	-
FSA_5	"	0	+	++	+++	-	-	-	-	-	-
FSM_6	"	0	0	0	0	++	+++	-	-	-	-
FSW_6	"	0	0	0	0	+	++	-	-	-	-
FSA_6	"	0	+	+++	+++	-	-	-	-	-	-

RSM = Refrigerated sample from marian market, Zero = Normal odour, Positive (+) = Any change in odour as soon as noticed. Intensity of such change depicted by number of (+) signs, RSW = Refrigerated sample from watt market, Negative (-) = Unwholesome samples from Abattoir, RSA = Refrigerated sample from Abattoir

Table 6: Odour profile of frozen beef samples stored at-10°C to-20°C

Sample Codes	Initial Colour of sample	Days of storage 2ND	4TH	6TH	8TH	10th	12th	14th	16TH	18TH	20TH
FSM_1	Normal	0	0	0	0	0	0	0	+	+	+
FSM_1	"	0	0	0	0	0	0	0	0	+	+
FSA_1	"	0	0	0	0	0	0	0	0	+	+
FSM_2	"	0	0	0	0	0	0	0	+	+	+
FSW_2	"	0	0	0	0	0	0	0	0	0	+
FSA_2	"	0	0	0	0	0	0	0	0	0	+
FSM_3	"	0	0	0	0	0	0	0	+	+	+
FSW_3	"	0	0	0	0	0	0	0	0	+	+
FSA_3	"	0	0	0	0	0	0	0	0	0	+
FSM_4	"	0	0	0	0	0	0	0	+	+	+
FSW_4	"	0	0	0	0	0	0	0	0	0	0
FSA_4	"	0	0	0	0	0	0	0	0	+	+
FSM_5	"	0	0	0	0	0	0	0	0	+	+
FSW_5	"	0	0	0	0	0	0	0	0	0	0
FSA_5	"	0	0	0	0	0	0	0	0	+	+
FSM_6	"	0	0	0	0	0	0	0	0	+	+
FSW_6	"	0	0	0	0	0	0	0	0	0	+
FSA_6	"	0	0	0	0	0	0	0	0	+	+

+ = Time when odour changes becomes noticeable

These tables revealed different degrees of acidity and alkalinity. Samples which tended more towards alkalinity suggests that microbial activity was already initiated which probably should lead to spoilage.

Colour: Variation in colour intensity of the samples are shown in Table 3 and 4; Beef colour is sorely dependent on the concentration of myoglobin. The normal colour of beef is pinkish-red. On storage, appreciable colour changes occurred. Pale green to deep green colours were observed on refrigerated samples from Marian and Watts markets sources at the 8th and 4th day of storage, respectively while much dark brown colour were observed on all freezer samples at the 18th day of storage. The pale green may have been caused by the reduction in myoglobin and brown colour due to the oxidation of myoglobin to met-myoglobin. From the consumer's point of view, at about the 8th day of refrigerated storage and 18th day of freezer storage, colour changes are appreciable and such beef could be classified as spoilt. Frazier (1976) revealed that beef could be conveniently stored for 10-12 days under refrigeration and 20-25 days under freezer conditions without any appreciable change in colour.

Odour: Odour observations shown in Table 5 and 6 reveal that refrigerated samples from Marian and watt markets gave off-odour from the 8th day, but off-odour of samples from abattoir was indicated on the 6th day.

Table 7: Odour profile of frozen beef samples stored at-10°C to-20°C

Sample	Dilution	TVC	C.C	Eijkiman	Indole	Inferences
MS (Fresh)	10^{-1}	112	13	-ve	-ve	Bacillus
WS (Fresh)	10^{-1}	150	12	-ve	-ve	Streptococcus spp
AS (Fresh)	10^{-1}	Too many to count	NIL	-ve	-ve	Mucor and Candida
RSM	10^{-1}	103	10	-ve	-ve	Staphyloccus
RSW	10^{-1}	70	7	-ve	-ve	Streptococcus spp.
RSA	10^{-1}	Too many to count	29	-ve	-ve	Bacillus spp.
FSM	10^{-1}	49	NIL	-ve	-ve	*E. coli*
F	10^{-1}	49	NIL	-ve	-ve	Staphylococcus aureus
FSA	10^{-1}	Too many to count	NIL	-ve	-ve	Staphylococcus aureus

TVC = Total Viable Count, C.C. = Coliform Count

Samples from the abattoir gave off-odour earlier probably because of poor sanitary conditions around the abattoir, because some beef purveyors wash beef before placing on the beef tables for sales in markets. However, the keeping quality from odour evaluation could be said to last for only two - six days on refrigeration storage. Frozen samples from the markets and the abattoir did not give any appreciable off-odour.

Slim development: The development of slime on beef surface is an early indicator of beef spoilage due to the growth of micro-organisms. (Agiang, 1976). Slime growth is associated with green colour and is characteristic of refrigerated samples at the 8th day of storage for samples from Marian and Watt markets while samples from the abattoir developed slime on the 4th day of storage. All frozen samples did not show any sliminess up to the 20th day of storage. This is in agreement with the findings of Price and Schweighrt (1971) who stated that slime growth is an important parameter in determining the effectiveness of meat storage techniques.

Bacterial count: Results of bacteria counts and isolated organisms are summarized in Table 7.

The results showed that frozen beef had less bacteria load than refrigerated samples, while refrigerated samples had less bacteria load than fresh samples.

This pattern is expected and confirms the report of Oguntimehin (1985) that microbial growth on beef during storage is limited to only psychrophilic organisms that can thrive under freezer conditions. The mesophiles and thermophiles were inhibited since they grow at a much higher temperature. A uniform dilution was used in this research to standardize results. Results showed that there exist an association between the growth of certain micro-organisms and the production of off-odour, colour changes and slime growth. Bacterial counts of 60 x 10^6 was observed in this work. However, these results fall within the range of other workers (Frazier, 1976; Bunchanan and Gibbons, 1974; Ayres *et al.*, 1962; Harrigan and McCane, 1976; Oguntimehin, 1985). Empey and Scott (1939) reported a population of 50×10^6 micro organisms per cm^3 of beef. Ayres *et al.* (1962) observed slime on sliced beef when microbial load exceeded 100×10^6 per cm^2, but slime growth was observed in this study when microbial population exceeded 60×10^6 per cm^2. The predominant organisms isolated in this study were species of *Monococcus, Staplylococcus, E. coli, Bacillus, Candida* and *Streptococcus*. These isolated microorganisms testify to the fact that samples from the two markets and the abattoir were actually contaminated and the various microbial strains isolated might be associated with poor handling/marketing methods.

Conclusion: Stability of beef samples from Marian and Watt markets as well as an abattoir, in Calabar Municipality were found to be different under refrigeration and freezing storage conditions. Beef stored under refrigeration had a keeping quality or shelf-life of only two-six days based on colour, odour, slime growth and microbial load evaluation whereas beef stored under freezing conditions had a better shelf-life of 16 days. It is necessary therefore to improve the sanitary conditions in the markets, abattoirs and their environs.

Acknowledgements

The author is grateful to the management of the University of Calabar for the funding of this research and Mr. Emmanuel H. Idio, Mr. Imoh J. Akpan and Mr. Eseme Umo for their various contributions.

References

Agiang, E., 1976. A Study of the Keeping Qualities of beef from Nsukka Maket University of Nigeria, Nigeria Unpublished B.Sc. Project.

Ayres, J.C., A.A. Kraft, H.E. Snyder and H.W. Walker, 1962. Chemical and Biological hazards in Foods. The Iowa State University Press, Ames, Iowa.

Baird-Parker, A.C., 1976. Methods for classifying *Staphylococci In:* Identification methods for Microbiologists. Part A Gibbs, B.M. and Skinner, F.A. (Editors). Academic Press, London.

Bunchanan, R.E. and W.E. Gibbons, 1974. Bergey's Manual of Determinative Bacteriology (8th edition.) Williams and Wikins Co., Baltimore.

Empey, W.A. and W.J. Scott, 1939. Investigations on Chilled beef: I, Microbial Contamination acquired in the Meat works. Council for Science of Industrial Research (Australia) Bulletin, pp: 126.

Eneji, C.A., 1985. Determination of the heat treatment equivalent and calculated biological value of canned meat. Unpublished Ph.D. Project. Institute of Food Technology, University of Horticulture, Budapest Hungary.

Forrest, J.C., E.D. Alerle, H.P. Hedrick and R.A. Merkely, 1975. Principles of Meat Science. Freeman and Co. U.S.A.

Frazier, W.C., 1976. Food Microbiology. Second edition. AVI, Publication. Co. Westport. Connecticut.

Harrigan, W.F. and M. McCane, 1976. Laboratory Methods in Food and dairy Microbiology, Academic Press, London.

Ihekoronye, A.I. and P.O. Ngoddy, 1985. Integrated Food Science and Technology. Longman Publication Ibadan-Nigeria.

Oguntimehin, G.B., 1985. Preservation through application of energy (heat processing, freeing, drying and Radiation). Nigeria Food J., 2: 221-245.

Price, J.F. and B.S. Schweigert, 1971. The Science of Meat and meat Products American Meat Institute Publication, W.H. Freeman publisher San Francisco.