Some Biochemical and Organoleptic changes due to Microbial growth in Minced Beef packaged in Alluminium polyethylene trays and Stored under Chilled condition

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Abstract -Changes in the quality of minced beef were studied under two refrigerated conditions (1-2^{0}C and 5-7^{0}C) using some biochemical organoleptic and bacterial assays during a six-day period. Meat's crude protein concentration remained constant (19.7-19.8%) in 4 days and 3 days at 1-2^{0}C and 5-7^{0}C respectively. There was an increase of only 0.4 to 0.6% and 0.9 to 1.4% during the 6 day period at 1-2^{0}C and 5-7^{0}C respectively. Between the 4th-6th day under both chill temperatures the meat has spoilt turning dark brown, slimy and putrid. This period coincided with meat's bacteria log number of 8.6/g and above, increased alkalinity from 0.5 in fresh meat to between 4.3 and 5.7 ml of 0.02N HCl/g sample and increased total volatile nitrogen (TVN) from 12.6mgN/100g protein in the fresh state to between 23.8 and 39.8 mgN/100g protein under the two chill temperatures. The gradual increase of meat's pH from 5.4 in fresh state to 5.8 at 1-2^{0}C in 5 days and 5.7 at 5-7^{0}C in 3 days, and its subsequent pronounced increase to between 6.1 and 6.9 may be ascribed to proteolysis on one hand. Increased TVN may also be responsible for the elevated meat's pH and alkalinity in the spoiling meat. Minced meat for use in hamburger, corn beef, hotdog and other meat products may enjoy a shelf stability of only 3 day holding period under chill temperatures otherwise it is deemed unsafe for human consumption. [Life Science Journal. 2010; 7(2): 47– 51] (ISSN: 1097 – 8135).

Keywords: Beef, organoleptic, bacterial, chill temperatures,biochemical, volatile nitrogen, alkalinity,protein, pH

1. Introduction

Meat (beef) owes its perishability to its high water content and preponderance of nutrients such as high molecular proteins, low molecular substances such as glucose, free amino acids, peptides and very minute amount of glycogen (Koutsoumanis *et al*., 2006, Jay, 2000, Ingram and Dainty, 1971). The quality of beef depends, among other factors, upon the pre-slaughter handling, care and state of animal, age, sex, species, hygiene precautions in the slaughter house and meat pH (Anon, 2001).

In a situation when a cow is well rested before slaughter, its post-rigor pH of 5.4 is achieved (Ingram and Dainty, 1971). Further changes (chemical, microbial and organoleptic), depending upon prevailing or storage temperature, relative humidity, bulkiness of meat, degradation of low molecular substances by the activity of the dominant indigenous microorganisms, are also overt (Ellis and Goodacre, 2001; Koutsoumanis *et al*., 2006; Labadie, 1999). The main bacteria implicated in the spoilage of refrigerated beef include *Brochothrix hermosphacta, Lactobacilli spp., Leuconostoc spp., Carnobacterium spp., Pseudomonas spp.* and Enterobactriaceae (Borch *et al*., 1996, Nuchas *et al.,* 2008).

Lactic acid bacteria (LAB) have been reported to play a role in the spoilage of refrigerated raw meat (Labadie, 1999). Spoilage signs are often attributed to the undesirable growth of microorganisms to unacceptable levels. In meat, microbial spoilage leads to the development of off-odours and often slime formation (Hilario *et al*. 2004, Huis in't Veld, 1996, Jackson *et al.,* 1997). Meat's organoleptic changes may vary according to the microbial association contaminating the meat and to the conditions under which the meat is stored (Danilo *et al.*, 2006).

It has also been reported that development of organoleptic spoilage is related to metabolization of meat's low molecular substances, such as sugar and free amino acids and the release of undesirable volatile metabolites. As soon as the glucose present in aqueous phase has been exhausted other substrates are consequently utilized by metabolizing microorganisms to produce odoriferous nitrogenous compounds, the most predominant of which is ammonia (Pearson and Muslemuddin, 1968, Stanbridge and Davis, 1998). Microbial loads from 10^7 cfu/cm^{-2} have been associated with occurrence of off-odours when the loads increased to as high as 10^9 cfu/cm^{-2} when the meat becomes putrid (Dainty *et al*., 1985, Jay, 2000).

Several devices have been shown to influence increased shelf life of meat at refrigeration conditions without the use of chemical additives (Brody, 1996, Fiber, 1991, Ilario, 2009, Danilo *et al*., 2006). Vacuum packaging of raw meat under chill conditions has proved to be effective in extending the shelf life and preventing growth of pathogens (Labodie, 1999). The main objective of this study was to assess the quality and to determine the optimum storability of minced beef packaged in polyethylene trays within one week period at chill storage temperatures.

2. Materials and Methods

Freshly cut stewing steaks, from the same meat muscle (*Longissimus dorsi*) were bought at a local abbatoir. The steaks were aseptically trimmed of visible fat, minced in the laboratory, packaged in portions of 60g in polyethylene trays with interior covered with multilayer barrier film.

To study the effect of storage temperature and storage period (days) on biochemical and organoleptic changes and bacteriological growth (counts), a factorial design was used. There were two levels of storage temperatures (1-2^0 and 5-7^{0}C) and six (6) levels of storage periods (0, 1, 2, 3, 4, 5 and 6 days). Three replicates were performed for each experiment for a total of 42 samples/packages) and the standard error was calculated. All samples were stored in cold room at lower (1-2^{0}C) and upper (5-7^{0}C) chill temperatures. By mincing, the natural microflora of the meat were thoroughly mixed with sterile spatula.

2.1. Analytical Procedures

2.1.1. Crude Protein

Kjeldahl method of analysis (AOAC, 1990) was used to determine crude nitrogen, multiplied by 6.25 to obtain crude protein.

2.1.2. Total Volatile Nitrogen (TVN)

Total Volatile Nitrogen was determined by modified Lucke and Geidel macro distillation at atmospheric pressure (Pearson, 1975).

The TVN was released by boiling the flesh directly with magnesium oxide, which prevents volatile acids from distilling from the protein.

2.1.3. Amino acid

Amino acid was determined by formol potentiometric titration to pH 9.0 end point as specified by Pearson, (1975).

2.1.4. Degree of alkalinity and pH

Titrimetric method was used to determine the degree of alkalinity and pH of meat by the technique of Shelef and Jay (1970).

2.1.5. Total bacterial count (TBC)

Samples (10g) arising from each tray were aseptically weighed after mixing thoroughly with sterile spatula and blended with 40ml sterile ringer's solution for 2 minutes in a stomacher at room temperature. Decimal dilutions were spread in triplicate in nutrient agar, incubated at 35 ± 2^{0}C for 48h. Results were calculated as the means for 3 determinations.

3. Results

Table 1 reports the changes in crude protein, amino acid and total volatile nitrogen (TVN) during chill storage. Crude protein in beef stored at 1-2^{0}C for the first 4 days and at 5-7^{0}C for the first 3 days remained identical and constant being 19.7-19.8%. Crude protein increased from 19.7 at zero day to between 20.1 to 20.3% in 5 days at 1-2^{0}C and to between 20.1 to 21.1% in 4-5 days at 5-7^{0}C. Fresh beef with formol value (amino acids) 17.5 decreased gradually from 17.2 to 15.6 and from 17.1 to 16.2 at 1-2^{0}C and 5-7^{0}C respectively from day 1-day 6.

After consistent decrease in amino acid from day 1 to day 5, an increase in amino acid value on the 6th day in each storage environment was observed. Conversely TVN increased consistently in meat samples stored in both media. Rate of increase in TVN was apparently higher at 5-7^{0}C than at 1-2^{0}C. On the 4th day of storage at both storage temperatures their TVN values were about doubled the TVN value of fresh sample. During the last 2 days TVN rose to between 27.3 and 29.1 at 1-2^{0}C and between 34.3 and 39.8 mgN/100g protein at 5-7^{0}C. TVN values of 14.2-16.9 at 1-2^{0}C in 3 days and 15.1-17.5 at 5-7^{0}C in 2 days corresponded to periods in storage at which bacterial log number were 7.0 and 6.6 respectively.

Fig 1 shows the changes in pH levels, degrees of alkalinity and bacterial log number in meat stored under the two storage conditions. pH values from 5.4 in fresh state increased within narrow limits of 5.4-5.8 in meat stored for 5 days at 1-2^{0}C and 5.4-5.7 in meat stored for 3 days at 5-7^{0}C. Under the latter condition, pH has increased tremendously to between 6.1 – 6.9 as found between 4 and 6 days. Although the degrees of alkalinity were identical at both refrigeration temperatures they showed constant increase from day 1 to day 6. The bacteria log number of fresh beef was 5.5 per gram. Bacteria log number has increased to 5.6 and 6.0 per gram at 1-2^{0}C and 5.9 and 6.6 per gram at 5-7^{0}C within two days. Bacteria log number per gram and titration values showed a remarkable increase from the third day at both 1-2 and 5-7^{0}C.

Table 2 shows the changes in bacterial population and organoleptic signs in beef under storage condition with bacteria log of number of 5.6 and 5.9 per gram meat retained the bright red colour above these levels i.e. 6-6.6 Cfu/g beef colour has turned pale red but odourless. During 3rd and 4th day storage periods at 1-2^{0}C with bacteria log number 6.9 and 8.1 per gram and 7.7 per gram on 3rd day at 5-7^{0}C all samples have changed to dark red but remained odourless. On the 5th day at 1-2^{0}C and on the 4th day at 5-7^{0}C beef colour has turned brownish with about 8.6 per gram bacterial log number per gram and off odour. Bacteria log number of 9.2 and above at both storage conditions meat has turned dark brown (6th day at 1-2^{0}C and days 5th day at 5-7^{0}C) and became starky, slimy and putrid.

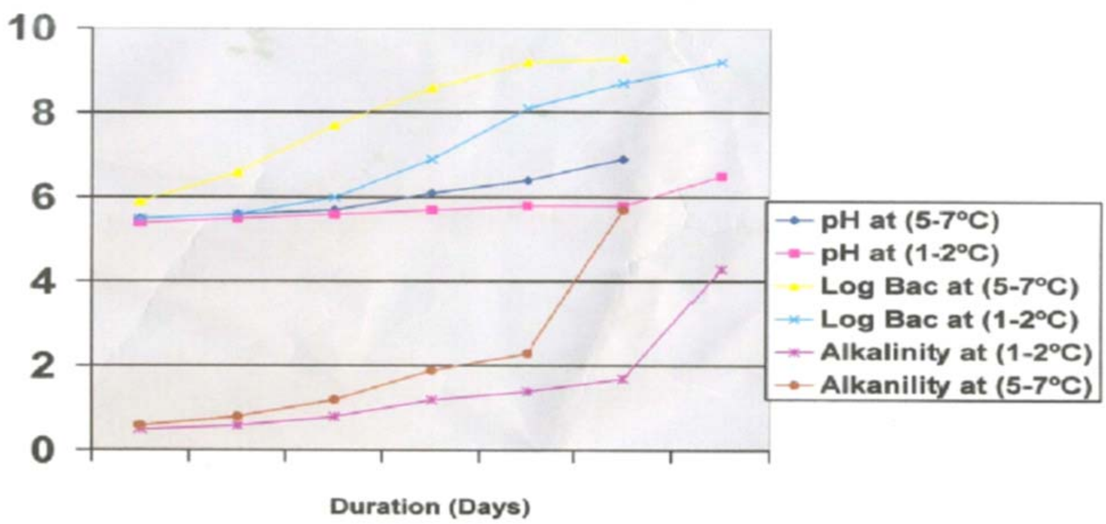

Figure 1: Changes in Bacteria log numbers, pH and Alkalinity (ml 0.1 Hcl /g Sample)

Table 1

Changes in crude protein amino acid and total volatile nitrogen during chill storage temperatures

Storage Temp.	Storage Time (days)	Crude Protein %	Amino acid ml o.IN NaoH	Total Volatile Nitrogen (TVN) mgN/100g Protein
Fresh	Zero	19.7 ± 0.8	17.5 ± 1.2	12.6 ± 0.1
1-2^{0}C	1	19.7 ± 0.8	17.2 ± 1.1	14.2 ± 0.2
"	2	19.7 ± 0.7	17.0 ± 0.2	16.4 ± 0.6
"	3	19.7 ± 0.7	16.2 ± 0.2	16.9 ± 0.5
"	4	19.8 ± 0.8	15.4 ± 0.1	23.8 ± 0.7
"	5	20.1 ± 0.9	15.0 ± 0.1	27.3 ± 0.7
"	6	20.3 ± 1.0	15.6 ± 0.3	29.1 ± 0.7
5-7^{0}C	1	19.7 ± 0.8	17.1 ± 1.2	15.1 ± 0.4
"	2	19.7 ± 0.7	16.4 ± 1.4	17.5 ± 0.6
"	3	19.8 ± 0.7	15.1 ± 1.5	20.9 ± 0.8
"	4	20.1 ± 0.9	14.4 ± 1.2	26.1 ± 1.0
"	5	20.6 ± 1.0	14.8 ± 0.8	34.3 ± 1.0
"	6	21.1 ± 1.1	16.2 ± 1.2	39.8 ± 1.1

Values are means ± of triplicate determinations

Table 2
Bacterial loads and subjective orgalonoleptic signs during chill storage

Storage temp. ^0C	Duration (days)	Bacterial log Nos	Colour	Slime growth	Smell
Fresh	Zero	5.5	Bright red	-	Odourless
1-2^{0}C	1	5.6	Bright red	-	"
	2	6.0	Pale-red -		"
	3	6.9	Dark red-		"
	4	8.1	Dark red-		Odourless
	5	8.7	Brown	-	Off odour
	6	9.2	Dark brown	Slimy	Putrid
5-7^{0}C	1	5.9	Bright red	-	Odourless
	2	6.6	Pale red -		Odourless
	3	7.7	Dark red	-	Odourless
	4	8.6	Brown	-	Off odour
	5	9.2	Dark brown	Slimy	Putrid
	6	9.3	Dark brown	Slimy	Putrid

Values are means of log numbers of bacterial

4. Discussion

The crude protein, 19.7% of meat as determined fresh from abbatoir and periodically verified frozen at -18^{0}C was constant. The crude protein value apparently remained constant for 4 and 3 days when stored at 1-2 and 5-7^{0}C respectively. The increase of 0.4-1.4% during these periods in storage as found in the two media is seemingly insignificant. This increase, however, may be due to synthesis of protein from non-protein nitrogenous substances by resident microorganisms in agreement with previous reports (Jay & Kontou, 1967; Ellis and Goodacre, 2001). The proliferating microorganisms in meat ecological niche at growth and death phase, being themselves proteinaceous, may also cause increase in crude protein in the spoiling meat. Further investigation is however needed to get this latter assertion established.

Amino acid concentration, decreasing gradually from fresh state of the meat to spoilt state at both storage temperatures is not accidental. This may be indicative of the metabolization of free amino acids by spoilage organisms resident in the spoiling meat (Dainty, 1985, Jay, 2000, Ellis and Goodcre, 2001, Labadie, 1999). The increased bacterial log no. as high as 9.0/g and the observed off-odour, slimeness and putrid stance resulting from free amino acid metabolization has been confirmed by previous reports (Ercolini *et al*., 2006, Jackson, *et al*., 1997, Huis in't Veld, Hilario *et al*., 2004).

The slight increase in amino acids on the 6th and 5th days at 1-2^{0}C and 5-7^{0}C respectively, may result from proteolysis of some kind by meat's microflora, particularly *Pseudomonas*, commonly implicated in the spoilage of beef at chill temperatures (Dainty *et al*., 1975, Ercolini *et al*., 2006).

The gradual but consistent rise in the degree of meat's alkalinity as storage and spoilage progressed may be due to production and accumulation of odoriferous nitrogenous compounds, most predominantly ammonia (Pearson and Muslemuddin, 1968, Standbridge, 1998, Dainty *et al*. 1985). Ammonia, determined as TVN, may be used as an index of meat quality under chill temperature according to the scheme of Pearson (1975) showing meat's fresh TVN to be 13 and its acceptable value to be ≤ 17. In this report it was evident that beef storage for 4 days at 1-2^{0}C and barely 3 days at 5-7^{0}C is acceptable for human consumption. However, in four days TVN values of 23.8 at 1-2^{0}C in 2 days and 20.9 with bacteria log nunmber 8.1/g and TVN 20.9 with bacteria log no 7.7/g are adjudged fit for human consumption being odourless, non-slimy and non-putrid.
All the parameters used to determine the wholesomeness of meat under chill conditions seem to have worked in consonance. The narrow limit within which pH changes occur makes pH measurement in spoiling meat restrictive (limiting). However, pH measure is useful for detecting proteolysis in spoiling meat when a sharp increase in post rigor pH is observed. Both TVN and degree of alkalinity accurately measure meat spoilage as they consistently increased as spoilage advanced and bacteria log number increased with increasing storage time.

Meat colour changes with passage of time in fresh state from bright red, through pale red to dark red during chill storage when the meat was odourless and still acceptable. With a change in colour to brown or dark brown, the meat has become slimy and putrid at high bacterial log numbers of between 8.6-9.3 cfu/g. from this study it is obvious that the maximum holding period for minced beef to keep under chill conditions is about four days pending use in meat products.

5. Conclusion

Meat preservation by freezing or refrigeration coupled with good packaging materials, has proved to be of a greater advantage than other alternative preservation methods such as dry salting or wet curing. In dry salting or salt curing, the amounts of salt used depend upon the end product desires. High levels of salt in meat or meat products may be fraught with danger. High dietary salt content is linked with hypertension. Application of nitrites on meat has been widely acknowledged for their functionalities as retainer of meat's reddish colour and their bacteriostatic effect on *Clostridium botulinum*. Reactions of meat's amines with nitrites have been implicated in information of nitroso- compounds, a carcinogen due to uncontrolled application of nitrites. This work has, however, predicted the maximum period of time it takes minced meat, without additive, to spoil under chilled storage. This holding period will give a meat processor a relief from having to be passing through labour intensive processing on daily basis.

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