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Original Article

Effect of Low Temperature on the Bacterial Load in Chicken, Mutton and Beef Meat in Relation to Meat Spoilage

¹*Anbalagan, M., ²Ganesh Prabu, P., ³Krishnaveni, R.E and ¹Manivannan, S.

¹Department of Zoology, Annamalai University, Annamalainagar, Tamilnadu, India.

²PG Department of Zoology, Govt. Arts College, C. Mutlur, Chidambaram, Tamilnadu, India.

³Department of Microbiology, Idhaya College for Women, Sakkottai, Kumbakonam, Tamilnadu, India.

¹*Corresponding Author: Dr. M. Anbalagan, Ph.D., Department of Zoology, Annamalai University, Annamalainagar, Tamilnadu, India. E-mail: manbuarivu@gmail.com

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Abstract

Meat spoilage during low temperature can be considered as an ecological phenomenon, which encompasses the changes of the available substrata, during the prevailing of a particular microbial association of specific spoilage organisms. In fact, spoilage of meat (Chicken, Mutton and Beef) depends on a temperature and duration of storage time. These specific spoilage organisms are the consequence of factors that dynamically persist or imposed by processing, transportation and temperature of the container and storage time in the market. In the present study the effect of low temperature (-18°C) on the bacterial load in Chicken, Mutton and Beef meat in relation to meat spoilage. In these three meats the high Total Viable Count (TVC) was recorded the first day of Beef meat (203×10^5 bacterial cell/gram). The low Total Viable Count (TVC) was recorded the fifth day of Chicken meat (35×10^2 bacterial cell/gram) and low pH range (pH: 5) also recorded in the fifth day of Chicken meat. Finally the low temperatures were arrest the pathogenic microbial growth and gradually reduce the pH range in the selected meat samples.

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Key Words: Bacterial load, Temperature, Chicken meat, Mutton meat, Beef meat, Meat spoilage

Introduction

The microbiological spoilage flora of aerobically stored fresh chicken meat consists of a wide range of bacterial species. Microbiological spoilage indicators of chicken, mutton and beef include a wide variety of off odors and flavors (Russell *et al.*, 1995). These defects have been attributed, among others, to the action of extracellular compounds, such as lipases and proteases, produced by dominant spoilage microorganisms (Nychas and Tassou, 1997). In recent years, foodborne infections and intoxications have assumed significance as a health hazard. Epidemiological reports suggest that poultry and other meat is still the primary cause of human food poisoning (Mulder, 1999). Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people (Yashoda *et al.*, 2001). However, the presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains a significant concern for suppliers, consumers and public health officials worldwide. Bacterial contamination of these foods depends on the bacterial level of the poultry carcasses used as the raw product, the hygienic practices during manipulation on the time and temperature of storage

(El-Leithy and Rashad, 1989).

This study was designed to evaluate the bacteriological quality of meats and to compare the level of contamination of three groups of meat such as chicken, mutton and beef meats. The scientific attention on meat microbiology, increased when large amounts of meat started being shipped long distances and long storage time (e.g., from Australia to the UK) and continued in the 1950s with the growth of supermarkets. Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odors, and the development of slime would constitute the main qualitative criteria for meat rejection. In general, spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual and the intensity of the change. Temperature control is completely lacking from the store to domestic storage and until the time of preparation and consumption. Some quantitative evidence is available from studies and surveys at distribution, retail and domestic level to illustrate the magnitude of the problem. In South European countries 30% of refrigerated foods were kept above 10°C in retail cabinets and household refrigerators

and even in North Europe 5% were above 13 °C in retail and 21% above 10 °C in households (Kennedy *et al.*, 2005).

Cold-tolerant *Enterobacteriaceae* (e.g., *Hafnia alvei*, *Serratia liquefaciens*, *Enterobacter agglomerans*) also occur on chilled meat stored aerobically (Nychas *et al.*, 1998) but in terms of numbers they do not contribute to the microbial associations. Although rarely, if ever, contributing significantly to the spoilage flora on meat and meat products, *Enterobacteriaceae* have been considered as indicators of food safety. With ground beef, *Pantoea agglomerans*, *Escherichia coli*, and *Serratia liquefaciens* were the major representatives of this family. *Brochothrix thermosphacta* and lactic acid bacteria have been detected in the aerobic spoilage flora of chilled meat but they are not considered to be important in spoilage except possibly for lamb (Holzapfel, 1998). These organisms have been isolated from beef carcasses during boning, dressing and chilling. Moreover, lairage slurry, cattle hair, rumen contents, walls of slaughter houses, the hands of workers, air in the chill room, neck and skin of the animal as well as the cut muscle surfaces have been shown to be contaminated with this organism. Both lactic acid bacteria and *B. thermosphacta* are of the main, if not the most important, cause of spoilage, which can be recognized as souring rather than putrefaction (Nychas *et al.*, 2008). Chemistry of spoilage was well established that glucose, lactic acid, and certain amino acids followed by nucleotides, urea and water-soluble proteins (Nychas *et al.*, 2008) are catabolized by almost all the bacteria of the meat microflora (Gill, 1986; Mc Meekin, 1982; Nychas *et al.*, 2007). The former compounds are the essential energy sources for the massive growth of microcosm on the meat despite their negligible quantity in comparison to proteins. It is shown that actual concentration of these compounds can affect the type (e.g., saccharolytic, proteolytic), the rate of spoilage and, moreover, seems to be the principal precursor(s) of those microbial metabolite(s) that we perceive as spoilage (Skandamis and Nychas, 2002; Tsigarida and Nychas, 2001).

Temperature seems to be the most important factor that influences the spoilage as well as the safety of meat (Koutsoumanis and Taoukis, 2005). Indeed modern lifestyle and the evolution of consumer requirements over the past decade have led to significant increase of demand for fresh (raw) meat. The mass consumption of fresh meat and meat products, as well as the new consumer patterns, i.e., reduced cooking times for minimal quality loss, microwave cooking, have accentuated the need for constant and systematic control of the temperature handling of raw meat products, throughout their distribution in the chill chain, from the point of production to their final consumption. Several studies have been recently carried out to assess the importance of low temperature handling of these meat products, focusing on the effect of temperature fluctuations or temperature abuses during handling on product quality (Koutsoumanis and Taoukis, 2005; Koutsoumanis *et al.*, 2006; Mc Meekin *et al.*, 2006). Thus an important aspect of meat fresh (raw) distribution and consumption is the effective monitoring of

time/temperature conditions that affect both safety and overall quality of meat. It is generally recognized by the European industry, retailers, food authorities and even consumers that several stages of the actual chill chain, such as transfer points or storage rooms, are found to be the weakest link in chilled perishable food management. Meat products, unless appropriately packaged, transported and stored, spoil in a relatively short time (Mc Meekin *et al.*, 2006).

Materials and Methods

Collection of the meat samples

Three different types of meat samples such as Chicken, Mutton and Beef samples were collected from the different areas (Vadavar Thalaippu, Anaikarai, Chinnavalayam, Periyavalayam, Silal) in and around Jayankondam, Arialoor District, Tamilnadu, India.

Microbiological analysis

The samples were examined for total microbial load. Microbiological media and media components were obtained from Hi-media Laboratories, Mumbai, India. Ten grams of sample was homogenised with 90 ml sterile peptone water. Multiple decimal dilutions were made with the sample diluents. The standard plate count (SPC) on plate count agar, yeast and molds on potato dextrose agar acidified to pH 3.5 using 10% tartaric acid. The *Pseudomonas sp.* on Baird Parker agar (Harrigan and Mc Cance, 1976) and *E. coli* using 4-methyl umbelliferyl b-D-glucuronide agar (Srihari and Vijaya Rao, 1998) were also enumerated. From the homogenates serial dilutions were made (up to 10⁻⁷) using buffered peptone water.

Enumeration and screening for total microbial load in meat samples

0.05 ml of inoculum was pipetted out into sterile petri plates from each of the selected dilution tubes. For the assessment of total viable count, pour plate technique was followed employing nutrient agar, duplicated and control plants were maintained. The inoculated plates were incubated for 24 hours at 37 °C. Selective media used for enumeration of *E. coli* and *Pseudomonas sp.* For the counts 2 × 0.05 ml of each of the serially diluted homogenate was inoculated on to the appropriate growth media in well-portioned out petri dishes using the drop plating technique (Reuter, 1970). All isolates obtained from days zero and five were cultured in nutrient broth and incubated at 37 °C in air for 24 hrs. Each culture was screened for using the drop collapse method as described by Youssef *et al.* (2004).

Results

Table 1 showed the effect of storage time at low temperature (-18 °C) on bacterial load in Chicken sample, First day of the Chicken sample have 156×10⁵ TVC of bacterial cells/gram, the pH range is about 7, second day of the chicken sample have 121×10⁴ TVC of bacterial cells/gram, the pH range is about 6.6 (slightly acidic condition), third day of the chicken sample have 98×10³ TVC of bacterial cells/gram, the pH range is about 6.3 (slightly acidic condition), fourth day of the chicken sample have 54×10² TVC of bacterial cells/gram, the pH range is about 5 (acidic condition) and fifth day of the chicken sample have 35×10² TVC of bacterial cells/gram, the pH range is about 5.6 (acidic condition). This data was well

Table 1. Effect of storage time at low temperature on bacterial load in Chicken sample

S.No	Number of days (Storage –18 °C)	Total Viable Count (TVC) cells/gram	pH Range
1	Day One	156×10 ⁵	7.0
2	Day Two	121×10 ⁴	6.6
3	Day Three	98×10 ³	6.3
4	Day Four	54×10 ²	5.6
5	Day Five	35×10 ²	5.0

Table 2. Effect of storage time at low temperature on bacterial load in Mutton sample

S.No	Number of days (Storage –18 °C)	Total Viable Count (TVC) cells/gram	pH Range
1	Day One	186×10 ⁵	7.0
2	Day Two	142×10 ⁵	6.5
3	Day Three	120×10 ³	6
4	Day Four	101×10 ³	5.5
5	Day Five	73×10 ²	5.1

Table 3. Effect of storage time at low temperature on bacterial load in Beef sample

S.No	Number of days (Storage –18 °C)	Total Viable Count (TVC) cells/gram	pH Range
1	Day One	203×10 ⁵	7.0
2	Day Two	179×10 ⁵	6.6
3	Day Three	146×10 ⁴	6.2
4	Day Four	111×10 ²	5.8
5	Day Five	85×10 ²	5.4

evidence that the fifth day storage have shown the least colony count when compared to first day of storage. Further the reduction in the pH was also well evidence throughout the storage days.

Table 2 showed the effect of storage time at low temperature (–18 °C) on bacterial load in Mutton sample, First day of the Mutton sample have 186×10⁵ TVC of bacterial cells/gram, the pH range is about 7, second day of the chicken sample have 142×10⁵ TVC of bacterial cells/gram, the pH range is about 6.5 (slightly acidic condition), third day of the chicken sample have 120×10³ TVC of bacterial cells/gram, the pH range is about 6 (slightly acidic condition), fourth day of the chicken sample have 101×10³ TVC of bacterial cells/gram, the pH range is about 5.5 (acidic condition) and fifth day of the chicken sample have 73×10² TVC of bacterial cells/gram, the pH range is about 5.1 (acidic condition). This data was well evidence that the fifth day storage have shown the least colony count when compared to first day of storage. Further the reduction in the pH was also well evidence throughout the storage days.

Table 3 showed the effect of storage time at low temperature (–18 °C) on bacterial load in Beef sample, First day of the Beef sample have 203×10⁵ TVC of bacterial cells/gram, the pH range is about 7, second day of the chicken sample have 179×10⁵ TVC of bacterial cells/gram, the pH range is about 6.6 (slightly acidic condition), third day of the chicken sample have 146×10⁴ TVC of bacterial cells/gram, the pH range is about 6.2 (slightly acidic condition), fourth day of the chicken sample have 111×10² TVC of bacterial cells/gram, the pH range is about 5.8 (acidic condition) and fifth day of the chicken sample have 85×10² TVC of bacterial cells/gram, the pH

range is about 5.4 (acidic condition). This data was well evidence that the fifth day storage have shown the least colony count when compared to first day of storage. Further the reduction in the pH was also well evidence throughout the storage days.

In the present study the effect of low temperature (–18 °C) on the bacterial load in Chicken, Mutton and Beef meat in relation to meat spoilage. In these three meats the high Total Viable Count (TVC) was recorded the first day of Beef meat (203×10⁵ bacterial cell/gram). The low Total Viable Count (TVC) was recorded the fifth day of Chicken meat (35×10² bacterial cell/gram) and low pH range (pH: 5) also recorded in the fifth day of Chicken meat.

Discussion

The recovery of 21% of the aerobic and 37% of the anaerobic bacteria present in the original chicken meat after freeze-dehydration and rehydration at room temperature is in agreement with previous studies of May and Kelly, (1965). In contrast, Saleh and Goldblith, (1966) reported no salmonellae or coliform organisms, when they examined freeze-dehydrated chicken. However, Gunderson *et al.* (1954) revealed that *E. coli*, *Aerobacter aerogenes*, and their variants are frequently found in cooked, boned chicken meat. May and Kelly, (1965) also identified *E. coli* in their tests with freeze-dehydrated chicken meat. Our study confirms the previous report from this laboratory (May and Kelly, 1965) that vegetative cells of many types of bacteria can survive freeze-dehydration and rehydration at low temperature (50 °C). According to Chipley and May, (1968) the rehydration of freeze-dried meat at high temperature (85 to 100 °C) effectively selects for spore forming species of bacteria. Inoculated *C. sporogenes* had a survival rate of 81% after dehydration and rehydration at

100 °C for 10 min; these results are in accordance with the 81 % survival rate of *C. botulinum* reported by Wells, (1966) in chicken meat during freeze-dehydration. The rapid growth of *C. sporogenes* (up to 7.4×10^7 cells/g within 40 hrs.) at 37 °C suggests that surviving cells can multiply to dangerous levels in a very short period of time. Thus, the accidental or intentional delay of use of rehydrated meat containing any spores of pathogenic bacteria could lead to tragic results. These studies give additional evidence of the need for adequate microbiological control during processing of freeze-dehydrated chicken. Furthermore they point out that such control alone is not sufficient for the protection of the ultimate consumer. If the product is maintained at high temperature after rehydration, pathogenic spore formers are capable of survival and rapid growth. Thus, the consumer should be urged to refrigerate any rehydrated product promptly if it is not used immediately (Chipley and May, 1968).

Gracey *et al.* (1999) considered that microbial count of 105 CFU/cm² was satisfactory for fresh meat, while count of 106 CFU/cm² was considered unsatisfactory. Bacterial count of 106 CFU/cm² for chilled meat was considered satisfactory, but count of 107 CFU/cm² was considered unsatisfactory. Furthermore, ICMSF, (1980) reported that if meat is prepared under unhygienic conditions, the initial count was higher (exceeding 106 CFU/cm²). The results of our study are similar to Frank and Mallion, (1980) who recognized that a recent slaughtered and dressed carcass will be contaminated with bacteria count of 102 - 106 CFU /cm². Aerobic bacteria such as *pseudomonas* associated with spoilage of meat stored aerobically at 25 °C, cause discoloration due to metmyoglobin formation by reducing oxygen tension (Stanbridge and Davies, 1998). Alasnier *et al.* (2000) studied lipolysis in muscle during refrigerated storage and reported that free fatty acids formation is due to the breakdown of triglycerides and phospholipids. Patil *et al.* (2007) observed decrease in the levels of phospholipids during frozen storage of chevon. Both phospholipids and free fatty acids levels have been reported as good indicators of bacterial quality of fresh mutton (Vasundhara *et al.* 1983; Vasundhara and Kumudavally, 1989). Biogenic amine levels are positively related with storage time (Min *et al.* 2007) and are monitored as a measure of proteolytic activity.

The mean values of pH of aerobically packaged mutton *Harrisa* (a popular indigenous meat based product of Jammu and Kashmir) during storage at 4±1°C is observed on day 0 (6.06), day 4 (6.15) and day 7 (6.23) increased significantly during refrigerated storage (Bhat and Pathak, 2010). This increase in product pH during storage might be due to accumulation of metabolites of bacterial action on meat and meat products and deamination of meat proteins (Bachhil, 1982; Jay, 1986). Similarly, the increased pH was also reported by Nag, *et al.* (1998) in chicken nuggets, Kumar and Sharma, (2004) in chicken patties, Chidanandaiah *et al.* (2009) in buffalo meat patties, Sureshkumar *et al.* (2010) in buffalo meat sausages, Kumar and Tanwar, (2010) in chicken nuggets

and Bhat *et al.* (2010) in chevon *Harrisa*.

Conclusion

In the low temperature (-18 °C) the bacterial load was significantly decreased from first day to fifth day in all the three meats (Chicken, Mutton and Beef), it was evidence of the meat spoilage process the low temperature were arrest the pathogenic microbial growth and reduce the meat spoilage. The low temperature was gradually reduce the pH range (pH 7 to 5) from first day to fifth day storage, pH 5 was indicates the acidic condition on these selected meat samples. Reduce pH (acidic condition) was evidence for the reduction of meat spoilage process.

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