ABSTRACT

The study evaluated the shelf life of buffalo meat in refrigerated storage. The shelf life was evaluated for 9 days where all the physicochemical parameters were analysed on alternate days i.e. on the 0th, 3rd, 5th, 7th and 9th days. Buffalo meat was kept in polystyrene foam trays which were covered with PVC film and stored at refrigeration temperature. A significant change was recorded in physicochemical parameters during the storage period. pH, TVBN (total volatile basic nitrogen), FAA (free amino acid), and tyrosine value increased significantly (P<0.05) during storage period while concentration of glucose decreased significantly (P<0.05) during storage period. Significant positive (P<0.01) correlation was recorded among physicochemical parameters viz. TVBN, FAA, and tyrosine value. Significant negative (P<0.01) correlation was recorded between physicochemical parameters related to protein and glucose.

Keywords: buffalo meat, physicochemical parameters, shelf life

INTRODUCTION

Refrigeration is the key method for meat storage under household conditions. It is easily available, easy to execute, and best for short-term storage of meat. It retards undesirable changes in meat, but still deteriorative changes are bound to happen. During refrigerated storage changes that take place can be monitored to detect levels of meat spoilage and assess shelf life of meat. Different physicochemical parameters were used for detecting spoilage of meat during refrigerated storage. pH was suggested as a chemical indicator of bacterial spoilage in meat and meat products (Yano et al., 1995). The pH of fresh beef was 5.9 while that of spoiled beef was 6.59 (Shelef and Jay 1970). Total volatile basic nitrogen (TVBN) was established as a quality indicating metabolite in modified atmosphere packaged chicken fillets and correlated significantly with microbiological and sensory attributes (Balamatsia et al., 2007). The degree of autolysis and bacterial proteolysis has been assessed in fish and beef by means of estimating tyrosine value (Strange et al., 1977). Tyrosine value was used as one of the methods for detecting the microbial spoilage in meats, poultry and sea food Jay (1987). The depletion of glucose in meat was related to the onset of spoilage (Nychas et al., 2008). The concentration of free amino acids increased during the storage period and this corresponded well with colony counts, particularly with meat having high glucose concentrations (Nychas and Tassou, 1997). The titrable acidity in beef under refrigerated storage correlated well
with total plate count, extract release volume and pH. As the percentage of fat increased, the level of titrable acidity decreased (Shelef and Jay, 1970). Most of these studies were performed individually considering a single parameter at a time. Moreover the studies were mostly confined to beef as buffalo meat is not common in western countries. Therefore, the present study was planned to evaluate the shelf life of buffalo meat in a comprehensive manner using a group of physicochemical parameters related to meat spoilage and having high correlation with microbial growth.

MATERIALS AND METHODS

Buffalo meat required for the experiments was procured from the municipal slaughter house, Bareilly. The meat samples belonged to round portion of carcasses (consisting mostly of semimembranous, semitendinosous, biceps femoris and quadriceps muscle) of almost similar conformation. Meat was purchased within 2 hours of slaughter and immediately after purchase it was brought to the laboratory. Samples were trimmed of all visible fat and connective tissue. The meat was kept in polystyrene foam trays which were covered with PVC film and stored at refrigeration temperature for further studies. All parameters were analysed on alternate days of refrigerated storage i.e. on the 0th, 3rd, 5th, 7th and 9th days of storage. The samples were drawn aseptically and all the parameters were determined as per their standard procedures.

pH was evaluated by the method of Trout et al. (1992). TVBN was performed by the micro diffusion technique described by Pearson (1968) using Conway diffusion disk. The procedure of Strange et al. (1977) was followed for the estimation of tyrosine concentration during refrigerated storage. Concentration of D-glucose during the storage period was estimated with a commercial kit (GAGO-1KT, Sigma Aldrich) as per the method described by Washko and Rice (1961). The kit utilised glucose peroxidase enzyme, which converts glucose to gluconic acid that reacts with horse raddish peroxidase to give an orange colour. The method of Rosen (1957) with suitable modification was followed for the determination of free amino acid during storage. The method measures the amount of α-amino acids produced during storage as a result of bacterial degradation of meat proteins. First, 10 g of meat was homogenised with 100 ml of distilled water for 2 minutes. in an Ultra Turrax Tissue Homogenizer (Model T-25, Janke and Kenkel, 1 KA Labor Technik, Germany) and kept in refrigerator overnight. The next day the homogenate was centrifuged at 3000 rpm for 15 minutes. in a refrigerated centrifuge machine. Then, 10 ml of supernatant obtained on centrifugation was mixed with 10 ml of 10% trichloroacetic acid (TCA), left undisturbed for 10 minutes. and again centrifuged at 3000 rpm for 15 minutes. for precipitation of true protein. Next, 1 ml of supernatant was mixed with 1ml of freshly prepared 10% Ninhydrin reagent made in acetone. Then 1 ml of 80% phenol in ethanol and 10 µl of pyridine was added to the mixture, which was kept in a boiling water bath for 10 minutes. Then, 5 ml of ethanol was added to the mixture, and the optical density was measured at 570 nm in a spectrophotometer. The amount of free amino acid (mg/100 g) was calculated by comparing test values with a standard graph prepared using L-leucine as a source of α-amino acids. The titrable acidity of buffalo meat during the storage period was evaluated by the method of Shelef and Jay (1970).
The ammonia concentration in meat during the storage period was determined by the method prescribed by Sastry, Kamra and Pathak (1999). A 5 g meat sample was mixed with 50 ml of distilled water and homogenized for 2 minutes at 10000 rpm. The homogenate obtained was filtered with Whatmann filter paper No. 1 and the filtrate obtained was poured in a Kjeldahl flask. To 40 ml of the filtrate taken in the Kjeldhal flask, freshly prepared 10 ml of 10% sodium hydroxide and magnesium oxide of 25 mg/ml of filtrate were added. The distillation was performed in a kjeldahl distillation apparatus and 25 ml of distillate was collected in a beaker having 25 ml of Tashiro indicator, the colour of which changed to green on collection of distillate having ammonia. The distillate thus obtained was titrated with 0.1N sulphuric acid till the end, manifested by reappearance of pink colour. A blank was run in the same manner but without filtrate and titrated. The amount of ammonia was calculated by the formula:

\[
\text{Ammonia (mg/100 g) in Meat} = \frac{100 \times Y \times (A-B) \times 0.0014}{X \times W}
\]

- \(Y\) = Volume (ml) of filtrate made up to
- \(X\) = Volume (ml) of aliquot taken for distillation
- \(A\) = Volume (ml) of 0.1 N sulphuric acid used for titration of test sample
- \(B\) = Volume (ml) of 0.1 N sulphuric acid used for titration of blank
- \(W\) = Weight (g) of sample taken for estimation

**Statistical analysis**

The experiments were repeated a minimum of three times and the data generated for different quality characteristics were compiled and analysed using SPSS (version 17.0 for Windows; SPSS, Chicago, III., U.S.A.) with randomized block design. The data were subjected to analysis of variance. Duncan’s multiple range tests was carried out for comparing means to find the differences between storage periods and their interactions for various parameters. The smallest difference (\(D_{5\%}\)) for two means to be significantly different (\(P<0.05\)) was reported. The Pearson coefficient of correlation was also determined and the significance (\(P<0.01\)) of correlation was reported.

**RESULT AND DISCUSSION**

The pH of buffalo meat increased significantly (\(P<0.05\)) during the storage period (Table 1). The increase in pH was probably due to bacterial activity that resulted in the production of ammonia, amines and other alkaline substances (Nychas et al., 1998). The increased pH during storage period may be due to growth of Gram-negative bacteria such as *Pseudomonas, Moraxella, Acinetobacter* etc. The TVBN content increased significantly (\(P<0.05\)) during storage period (Table 1). A significant positive correlation (\(P>0.01\)) was recorded between pH and TVBN (Table 2) as increase in TVBN concentration increased pH due to alkaline nature. TVBN concentration reached beyond the recommended limit of 20 mg/100g (Byun et al., 2003) on the 9\(^{th}\) day of storage. The increase in TVBN during the storage period was due to breakdown of protein and deamination of amino acids.

The ammonia concentration in buffalo meat increased significantly (\(P<0.05\)) during the storage period (Table 1). The increase in ammonia concentration during storage was primarily due to deamination of amino acids by microbes as a source of energy after the availability of glucose declined. A significant correlation was observed between ammonia and TVBN as both are products
of microbial deamination (Table 2). The tyrosine concentration increased significantly (P<0.05) during refrigerated storage (Table 1). The increase in tyrosine concentration could be attributed to hydrolytic changes in meat due to inherent tissue enzymes and bacterial proteolysis (Strange et al., 1977). The result of the present study indicated a highly significant (P<0.01) correlation between tyrosine value and storage period (Table 2).

The glucose concentration recorded a significant decrease (P<0.05) during storage period (Table 1). The decrease in glucose was due to utilisation by microbes invading the meat. After glucose was depleted there was utilisation of proteinacious substances as reflected by significant (P<0.01) negative correlation between glucose concentration and concentration of metabolites released due to protein degradation like TVBN, ammonia, tyrosine, free amino acids during the storage period (Table 2). Similar findings were observed in beef under refrigerated storage (Byun et al., 2003). It is supported by the findings that under aerobic storage, spoilage is most frequently associated with the post-glucose utilization of amino acids by microbes (Gill, 1983). The titratable acidity of buffalo meat decreased significantly (P<0.05) during refrigerated storage (Table 1). The amount of acid required to bring the pH to 5.0 increased during storage period (Shelef and Jay 1970).

Concentration of free amino acids (FAA) increased significantly (P<0.05) during the storage period (Table 1). A slight increase in ninhydrin positive substances was observed during spoilage (Saffle et al., 1961). A significant correlation (P<0.001) between storage period, concentration of free amino acids and ninhydrin reactive material was observed by Keeton and Melton (1978). In the present research a highly significant (P<0.01) correlation was observed between free amino acid and tyrosine value (Table 2). The result could be attributed to proteolysis as tyrosine (aromatic amino acids) and α-amino acids are the product of proteolysis.

The present study provides a comprehensive overview of changes in important physicochemical parameters in buffalo meat during refrigerated storage with accompanying correlation between them. The correlation between different parameters provides an idea of relative change between different parameters. It provides an idea of correlated change between production of proteinacious metabolites and utilisation of glucose which reflects the decrease in concentration of glucose and degradation of proteins. Therefore it can be concluded from the present study that metabolites of protein degradation viz. tyrosine value, ammonia, free amino acid, and TVBN tend to increase during refrigerated storage while glucose tends decrease during refrigerated storage.

REFERENCES


