PHYSICO-CHEMICAL QUALITY OF BUFFALO MEAT UNDER REFRIGERATION

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Physico-chemical quality of buffalo meat under refrigeration"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"Physico-chemical quality of buffalo meat under refrigeration"** is a record of research work done independently by **Sri. P.A. Abdulkader Kunhy**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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P.A. ABDULKADER KUNHY

To My Wife

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Introduction

INTRODUCTION

In India water buffaloes (*Bubalus bubalis*) are reared primarily for milk production and draft purpose. India ranks first and contributes to 55 per cent of the world buffalo population. Since there is no ban on buffalo slaughter, they are slaughtered for meat by the end of their productive life. The meat obtained from buffalo is known as carabeef or buffalo beef. The contribution of this species to the meat sector is substantially high. As our country faces an overwhelming demand for animal protein, effective exploitation of the meat production potential of our rich buffalo population could be the way out to meet the demand.

It has been reported that a growth rate of 700 to 1200 g per day could be obtained from birth to 14 months of age of a buffalo male calf and at the age of 16 to 18 months it would produce highly acceptable meat which is considered superior to that obtained from prime beef breeds (Nagarcenkar, 1988).

As age advances, the meat obtained from buffaloes will be invariably tough. Since they are generally slaughtered late in their life, there is a general opinion that buffalo meat is unacceptably tough (Robertson *et al.*, 1983).

Deterioration in quality on storage of meat is a major problem that confronts the meat industry. Reduction of heat

by rapid chilling or freezing of meat can result in serious loss of quality, particularly the toughening due to cold shortening phenomenon. As buffalo meat from older animals is known to be of a relatively darker colour than beef, chilling could be considered as a practical way for making buffalo meat more acceptable in appearance (Ragab *et al.*, 1966).

Post-mortem storage of carcasses at refrigeration temperature has been known to improve meat tenderness by the conditioning process and it still continues to be an important procedure for producing tender meat (Koohmaraie *et al.*, 1988).

India presently exports more than Rs.750 millions worth of meat annually. Scientific rearing of male buffalo calves could be adopted to increase the buffalo meat production and meat export potential of our country (Nagarcenkar, 1988).

The information regarding the effect of time and temperature of storage on buffalo beef quality are scanty. A study to bring out the influence of storage on quality changes of buffalo meat will help the meat producers to store meat under optimum conditions to improve its quality.

The present work is undertaken to study the various changes in quality parameters of chilled buffalo meat namely, pH, water holding capacity, glycogen content, sarcomere length, fibre diameter and organoleptic attributes.

Review of Literature

REVIEW OF LITERATURE

2.1 pH

The pH of meat is closely associated with the chemical and physical properties of meat and hence is an important physico chemical quality. Several factors like onset of rigor, tenderness and water holding capacity are influenced by the pH of meat.

Briskey (1964) stated that the normal muscle had a slow glycolytic descend rate to an ultimate pH of 5.7 to 5.3 but an extremely poor quality muscle had a rapid decrease.

Cassens and Newbold (1966) observed that the ultimate pH at 37°C muscle samples was attained or closely approached 8 h post mortem, whereas in the 15°C samples 24 h were required. In the 1°C sample, ultimate pH was not reached until 42-72 h post mortem.

Ragab *et al.* (1966) observed that the pH of fresh buffalo meat ranged from 6.67 to 6.3. Miles and Lawrie (1970) obtained a high pH value with excised *Longissimus dorsi* muscle cooked as soon as possible after slaughter and required a relatively low shear force value. Vaneerd (1972) noticed that the time before rigor development was influenced by the temperature of the

muscle. The water holding capacity and pH dropped steadily as a function of time post-mortem.

When the meat was cooled after slaughter the fall in pH value became slower down to 10-15°C and accelerated again at lower temperature (Bendall, 1973).

Pierson and Fox (1976) observed that the muscle on the third day of ageing at 3°C had a significantly lower pH than at 20°C. They also found that temperature of ageing affected the rate of glycolysis.

Honikel *et al.* (1981a) observed that rigor mortis occurred in bovine neck muscles as soon as pH 5.9 and an ATP level of about 1 μ mol/g were attained. Honikel *et al.* (1983) reported that rigor shortening starts at pH 6.3-6.0 and at an ATP content of 50 per cent of the concentration in living muscle. It was also observed that cold shortening can begin at pH 7.0 and at the full ATP content in living muscle.

Honikel *et al.* (1981b) observed that the rate of pH fall in muscle depended on the incubation temperature. They also found that the period necessary for attaining pH 5.9 (onset of rigor morfis) increased with falling temperature, being about 10 h at 30°C and more than 24 h at 0.5°C.

Valin *et al.* (1984) found that buffaloes which had a lower muscle pH than cattle, displayed a significantly smaller amount of collagen in the muscles studied. Prabhakar *et al.* (1986) reported that buffalo meat recorded lower ultimate pH than beef.

Yu and Lee (1986) stated that high pH muscle exhibited an extensive degradation of Z-lines whereas low pH muscles showed a preferential degradation of M lines and myosin heavy chains. Intermediate pH muscle did not show much degradation of muscle proteins and resulted in tougher meat.

Chakradhardas *et al.* (1988a) in a study recorded the pH of buffalo meat after 6 h of slaughter as 6.48 $\pm$ 0.071 and it fell to 6.12 $\pm$ 0.077 after 48 h at 5 $\pm$ 2°C.

2.2 Water holding capacity

Water Holding Capacity (WHC) is the capacity of muscles to retain fluids during handling and processing (Kauffman *et al.*, 1969). It is an important attribute that affects both qualitative and quantitative aspects of meat and meat products. Meat with lower WHC will lose more weight during processing, storage, transit and display and will result in alteration of its composition and yield of the products.

Parrish *et al.* (1969) studied the effect of post-mortem ageing temperature and time on certain muscle attributes and found that meat attributes like tenderness, WHC and cooking loss were not affected by various ageing temperature and time. Processing of beef immediately after slaughter provides products of excellent WHC and fat emulsification capacity by the combined effects of high levels of ATP and high pH values in the pre-rigor muscle (Hamm, 1978).

Honikel *et al.* (1980) observed higher drip losses in contracted than in unshortened muscle. Honckel *et al.* (1981b) reported that neither shortening nor development of rigor had an immediate effect on WHC of muscle and unsalted muscle homogenates. The small decrease in WHC post mortem was due to pH fall only and independent of temperature. The decrease in WHC at 12-24 h post-mortem was accompanied by the enzymatic break down of ATP by a drop of pH caused by the formation of lactic acid and by the onset of rigor mortis.

Hamm (1981) reported a shrinkage of myofibrillar system with a decrease of WHC occurring post-mortem before the onset of rigor mortis because of the effect of decreasing pH. Honikel *et al.* (1983) stated that shortening of a muscle in general and the development of rigor influenced the tenderness and the water retension of beef.

Gault (1985) stated that the availability of charged molecules to associate with water molecule was dependent on ultimate pH of the muscle. At pH levels considerably above (>6.0) or below (<4.0) the iso-electric point ($\sim$5.0) the number of available charges is enhanced thus increasing WHC. Prabhakar and Narayana Rao (1986) in a comparative study between buffalo meat and beef observed that buffalo muscles recorded lower ultimate pH and WHC.

Kondaiah *et al.* (1986) found that buffalo head meat and tripe had higher pH resulting in higher WHC. Buffalo heart muscles showed lowest WHC. Drip loss was apparently due to changes of the microstructure of the muscle cells and drip loss of bovine muscle was lowest when pre-rigor muscles were kept at about 12°C (Honikel *et al.*, 1986). They also reported a close and linear relationship between shortening of sarcomeres in pre-rigor state and during the onset of rigor mortis to the extend of drip loss during the storage of meat post rigor as a factor which influences the WHC of meat. According to them release of drip from the muscles could be dependent on the state of contraction after the onset of rigor and due to the shrinkage of filamental spacing (perhaps also to changes in cell membrane) which results in the release of water into the extracellular space.

2.3 Glycogen

Glycolysis is one of the important biochemical reactions taking place in a muscle even after death of the animal, resulting in conversion of muscle to meat. The quantity of muscle glycogen at the time of slaughter is an important factor as far as the quality of meat is concerned.

Complete inhibition of enzymatic anaerobic glycolysis at pH 5.3 has been reported by Bate-Smith and Bendall (1949). According to Howard and Lawrie (1957) and Lawrie *et al.* (1959) the presence of residual glycogen even after the ultimate pH is attained was due to inaccessibility or insusceptibility of glycogen for the enzymatic action.

Newbold and Lee (1965) found 0.6 to 6.4 mg of residual glycogen per gram in ox muscles which had ultimate pH values ranging from 5.78 to 5.51. Disney *et al.* (1967) reported that glycogen content of 700 mg per cent was sufficient to bring the pH to 5.4.

Although post-mortem glycolysis levels and decrease in glycogen did not differ among treatments, there was a significant positive correlation between pH and glycogen level in post rigor tissue (Cook, 1967).

Merkel (1970) observed that depletion of muscle glycogen prior to slaughter leads to low level of lactic acid, high pH, dark colour and sticky texture of meat.

Newbold and Harris (1972) reported that even when there was an adequate supply of glycogen in the muscle at the time of slaughter, the ultimate pH was rarely less than 5.t to 5.4. Behnke and Fenna (1973) observed that storage of pre-rigor beef muscle at -3°C resulted in rapid glycolysis and did not necessarily cause toughness of meat.

Romans and Ziegler (1974) reported that the muscle contained about 0.5 to 1.0 per cent glycogen which gets converted to lactic acid that lowered the pH of the muscle. Lawrie (1975) reported that the muscle glycogen was only 0.1 g per cent by wet weight after rigor mortis and neither breed, age nor sex appeared to affect the glycogen levels and was of the opinion that a concentration of 0.8 per cent or less glycogen provided an ultimate pH of above 5.5. He further observed that in case of inanition or exercise immediately pre slaughter there was an appreciable fall in the reserves of glycogen in muscle. The conversion of glycogen to lactic acid continued until a pH was attained when the enzymes became inactivated. He also noticed

that post-mortem glycolysis increased with increasing external temperature above ambient.

Seidman and Cross (1982) stated that as the pH of the meat reached its minimum, most of the ATP disappeared and the glycogen got converted to lactic acid. Dreiling *et al.* (1987) observed a faster decrease in muscle glycogen by 48 h post-mortem.

Kuttinarayanan (1988) observed that the level of residual glycogen in mutton was 15-22 per cent of the initial level.

2.4 Fibre diameter and sarcomere length

During muscular contraction the contractile units of the myofibrils, the sarcomeres shorten. Shortening is induced by the release of calcium ions from the sarcoplasmic reticulum into the myofibrillar space. The movement of calcium ions within the muscle cells post-mortem depends on temperature, pH and ATP concentration present (Honikel *et al.*, 1983).

Herring *et al.* (1965b) observed that when muscles shortened there were corresponding decrease in sarcomere length, increase in fibre diameter and decrease in tenderness. Herring *et al.* (1965 a,b) reported a highly significant relationship between the amount of fibre contraction, as evaluated by change in sarcomere length and tenderness in beef

muscle. Sink *et al.* (1965) reported a high correlation between the rate of onset of rigor mortis and sarcomere length in porcine muscle. They also observed significant relationship between change in pH and change in sarcomere length.

Herring *et al.* (1965b) observed that in general the differences in sarcomere lengths of muscles were associated with differences in fibre diameter and shear force value. When muscles shortened there were corresponding decrease in sarcomere length, increase in fibre diameter and decrease in tenderness. Ragah *et al.* (1966) found that as age advanced the fibre diameter increased in buffalo muscles which in turn increased the toughness of meat.

Cook and Langsworth (1966) demonstrated a linear relationship between fibre shortening, sarcomere length and tenderness when differential contraction was induced by incubating unfrozen and pre-rigor frozen ovine muscles at different temperatures for 24 h post-mortem.

Hegarty (1970) demonstrated that during rigor development, the muscle fibre diameter decreases. Hegarty (1972) reported a positive correlation between long sarcomeres and tenderness. Honikel *et al.* (1980) stated that muscle shortening was minimum at 20°C provided the temperature was attained as quickly as possible. Honikel *et al.* (1981a) observed an unchanged sarcomere length until the onset of rigor mortis and sarcomere

length decreased at the onset without any effect of pH on sarcomere length.

Yates *et al.* (1983) concluded that high temperature and low pH caused proteolysis and less shortening of the myofibrils than refrigeration temperatures.

The mean sarcomere length in meat incubated for 4 h at 10°C to 30°C was significantly ($P<0.05$) longer than in samples kept at 37°C or 42°C (Petaja *et al.*, 1985). Honikel *et al.* (1986) found that below 6°C, sarcomeres contracted upto 70 per cent and the drip loss of muscles increased linearly with increased pre-rigor shortening. They also observed 10 per cent shortening at 0°C and above 22°C there was an abrupt increase of sarcomere shortening. During cold or rigor shortening the muscle became thicker and this could be caused by an increase in diameter either of the fibres or of extracellular space or of both.

The higher pH meat of the cold side carcass had almost the same sarcomere length as the intermediate pH meat but the high pH meat was obviously more tender than the intermediate pH meat. (Yu and Lee, 1986). They also found a high correlation between shear force and sarcomere length and stated that sarcomere length could be used as an indicator of meat tenderness, but only for the meat with the same ultimate pH.

Koohmaraie *et al.* (1988) observed that shorter sarcomere length had a greater ageing response.

2.5 Organoleptic qualities

Quality of a product is the product of various individual quality parameters and not the sum. Among the qualities of meat, organoleptic quality is the most important one which can be estimated objectively by mechanical and chemical methods and subjectively by sensory panel evaluation with the help of a score card.

According to Ragab *et al.* (1966) buffalo meat is darker than that of cattle and chilling favours the colour and pH of buffalo meat. They also suggested that chilling could be considered as a practical way for making buffalo meat more acceptable in appearance. Marsh and Leet (1966) studied the effect of cold shortening and observed that fibres allowed to contract 20-40 per cent of their initial length, were less tender than those which shortened to a greater or lesser extend.

Among the post-mortem changes taking place before and during rigor mortis, the extend of muscle shortening had an important role in the tenderness of meat.

Parrish *et al.* (1969) observed that in comparison with the opposite sides of carcasses aged continuously at 2°C, there was no difference in tenderness in the corresponding sides aged at 7°C, 15°C or 21°C and then at 2°C finally.

Goll *et al.* (1971) reported that degradation of Z-discs and weakening of the actin-myosin interaction occurs in myofibrils in post-mortem storage. Arganosa *et al.* (1973) found that the darker colour of buffalo meat was not due to difference in ultimate pH but probably to the greater pigment concentration in buffalo meat than beef muscles.

Meat tenderness has been shown to be related to the state of muscle contraction (Smith *et al.*, 1971) the ultimate pH (Bouton *et al.*, 1973), post mortem temperature (Parrish *et al.*, 1973), and the enzymatic proteolysis of myofibrillar proteins (Suzuki *et al.*, 1982). According to Locker and Daines (1975) there was greater toughness in shortened muscle.

Cia and Marsh (1976) observed that the pre-rigor tissue shortened considerably than that in which rigor was established, with a lower cooking loss. It was also significantly more tender when cooked within 3 h of slaughter. Matsukawa *et al.* (1976) found that *Longissimus dorsi* muscles from young buffalo, 19 to 25 months of age had similar mean Warner Bratzler shear force values and taste panel scores to

those from Sinhala, Red Sindhi and Friesian cattle of similar age.

Temperature had the greatest effect on tenderising rate, thirty two times than that due to animals and ten times than that due to muscles (Dransfield *et al.*, 1981). According to Marsh *et al.* (1981) meat was much more tender when kept at 37°C for 3 h after slaughter than when kept at 0 to 2°C after slaughter.

Colour measurements indicated that muscles from buffalo were darker than beef and darkened more at chill temperatures, although ultimate pH values of the groups did not differ adhesion, compression and Warner Bratzler shear force values were significantly greater in buffalo than in beef muscles; indicating greater connective tissue contribution to toughness of buffalo meat. Taste panel assessments of muscles of relatively low connective tissue content in beef, indicated that generic differences in tenderness were slight although buffalo meat was less juicy than beef. Flavour and overall acceptability of buffalo meat were significantly less than for beef (Robertson *et al.*, 1983). They also reported that the connective tissue in buffalo meat had a bigger contribution to toughness than it did in beef.

Robertson *et al.* (1984) studied the effect of heat on meat from young and old buffaloes and Brahman cattle and found that

myofibrillar strength was not affected by animal age or species as indicated by Warner Bratzler shear force value. But there were species differences in the mechanical properties of undenatured and partially denatured connective tissue.

In a comparative study of buffalo meat and beef, Valin *et al.* (1984) found that buffaloes which had a lower muscle pH than cattle, displayed a significantly smaller amount of collagen in the muscles studied, but the species did not differ significantly in the degree of intramuscular collagen cross linking.

The distribution and area of longissimus muscle fibre types from two intact male water buffaloes, one Angus bull and on Charolais bull indicated that only fibres of aerobic oxidative capacity were present in the muscle of the water buffalo examined. But, all three fibre types were present in muscles from the Augus and Charolais bulls. The findings identified distinct and unique differences in meat characteristics from water buffalo in case of organoleptic and or processing traits (West and Carpenter, 1985).

High temperature conditioning at 37°C or higher for 6 h just after slaughter made meat more tender than conventional systems (Petaja *et al.*, 1985). According to Robertson *et al.* (1986) there were no species differences among cattle and buffalo in case of tenderness and juiciness after tender

stretching. Prabhakar and Narayana Rao (1986) stated that beef and buffalo meat were almost similar in shear value, colour of lean, total myoglobin, fibre diameter, texture, juiciness and flavour.

Yu and Lee (1986) observed that the effect of elevated temperature above 4°C on muscle protein degradation and meat tenderness was manifested only when the incubation time extended beyond 24 h.

In an experiment to study factors associated with tenderness, Seidman and Koohmaraie (1987) found that shear force value variation in tenderness was associated with myofibrillar protein degradation. Koohmaraie *et al.* (1988) observed that calcium dependent protease activity which was highest in longissimus muscle, was the best determinant of tenderization resulting from post-mortem storage at refrigeration temperature and demonstrated that about 50 per cent of ageing response was completed by 24 h of post-mortem storage.

Chakradhardas *et al.* (1988b) reported the mean score for flavour of buffalo meat as 6.09 $\pm$ 0.134 for fresh meat and 6.14 $\pm$ 0.09 for 6 h samples.

Feedlot diets promoted the formation of marbling in buffalo muscles and produced good carcass confirmation and

higher percentages of hindquarter than grazing buffaloes (Dahlan *et al.*, 1988). According to Ziauddin *et al.* (1993) alterations in collagen characteristics rather than significant changes in collagen concentration are responsible for toughening of meat as animal age advances.

Koohmaraie (1994) stated that proteolysis of key myofibrillar proteins was the principal reason for improvement in meat tenderness during post mortem storage.

Materials and Methods

MATERIALS AND METHODS

Fifteen samples of buffalo meat were collected from the buffaloes slaughtered in the municipal slaughter house, Kuriachira, Thrissur. *Longissimus dorsi* muscle in the region of first to fifth lumbar vertebra was removed from the animals of six to eight years of age, approximately 45 minutes after exsanguination and transported in vacuum flasks. The samples collected were processed within one hour of collection which is designated as 1 h sample.

The samples were kept separately under refrigeration temperature (4°C) and were analysed at 8 h, 24 h and 72 h periods for pH, water holding capacity (WHC), glycogen content, fibre diameter and sarcomere length. Organoleptic evaluation of the cooked meat samples at 1 h, 24 h and 72 h was conducted. Statistical analysis of the data was carried out as per Snedecor and Cochran (1967).

3.1 pH

pH of the samples was estimated using the method described by Moeller *et al.* (1977). One gram of meat was homogenized in a domestic blender with 10 ml of 0.005 M sodium iodoacetate and the pH of the homogenate was taken using Beckman's zerometric pH meter.

3.2 Water holding capacity

Water Holding Capacity (WHC) was estimated using the method described by Wardlaw *et al.* (1973) with modifications given below. Five gram of minced meat was taken in 15 ml centrifugation tube and added 7.5 ml of 0.6 M sodium chloride solution. It was mixed properly and kept at room temperature for 15 minutes and centrifuged at 3500 rpm for 15 minutes. The volume of the supernatent (V) was measured and water holding capacity was calculated as follows.

$$\text{WHC (ml/100 g)} = \frac{(7.5 - V)\ 100}{5}$$

3.3 Glycogen

Glycogen content of the meat samples was estimated using the method described by Seifter *et al.* (1950).

3.3.1 Reagents

1. Potassium Hydroxide (KOH) solution 30 per cent

Three hundred grams of reagent grade KOH pellets was dissolved in distilled water in a beaker, cooled, transferred to one litre volumetric flask and made up the volume using distilled water.

2. Sulphuric acid

To 50 ml of distilled water, one litre of concentrated sulphuric acid was added carefully.

3. Anthrone reagent 0.2 per cent

Two hundred milligrams of anthrone was dissolved in 100 ml of already prepared sulphuric acid. This reagent was always prepared afresh.

4. Standard glucose solution

A stock standard of glucose solution was prepared by dissolving one gram of Analar grade glucose in saturated benzoic acid solution and diluting to 100 ml with the same. A standard glucose solution was prepared by diluting one ml of the stock solution to 500 ml with distilled water. Five millilitres of this standard solution contained 100 µg of glucose.

3.3.2 Procedure

One gram of meat was weighed and transferred to a test tube. Three millilitres of KOH was added into the test tube. The tissue was then digested by heating the tube for 20 minutes in a boiling water bath. After cooling, the contents were

transferred to a 500 ml volumetric flask and the volume was made up using distilled water and mixed thoroughly.

Five millilitres each of the digesta, standard glucose solution and distilled water was pipetted out in separate test tubes and marked as unknown, standard and blank respectively. The unknown, standard and blank were then kept in an ice cold water bath. By careful mixing, added 10 ml of anthrone reagent each to the test tubes from a burette. After cooling, the tubes were placed in a boiling water bath for 10 minutes. The tubes were immediately cooled in running water. The readings of standard and unknown were taken in a spectronic-20 (Bausch and Lomb, USA) at wavelength of 595 nm, after setting the instrument to 100 per cent transmittance with the blank.

3.3.3 Calculation

$$\text{Glycogen content in g/100 g wet tissue} = \frac{UxC.Sx500x100}{Sx1.11x5xWx1000x1000}$$

Where,

U = reading of the unknown

S = reading of the standard

W = weight of the tissue in grams

C.S = concentration of the standard in micrograms

1.11 = the factor used to convert glucose into glycogen

500/5 = dilution factor

100/1000x1000 = factor expressing the value in gram per cent

3.4 Fibre diameter

Fibre diameter was measured as per the method described by Jeremiah and Martin (1977).

Five gram of meat was cut into small pieces and homogenised in a domestic blender for 15 sec at low speed, interspaced with five seconds interval in a solution containing 0.25 M sucrose and 1.0 mM ethylene diamine tetra acetic acid (EDTA) to produce a slurry. One drop of the slurry was transferred onto a microscopic slide and covered with a coverslip and examined directly under 10x objective and 10x eyepiece of a light microscope containing a calibrated micrometer. Muscle fibre diameter was measured as the mean cross-sectional distance in micrometers between the exterior surfaces of the sarcolemma of 20 randomly selected muscle fibres.

3.5 Sarcomere length

The sarcomere length of myofibres was measured as per the method described by Hostetler *et al.* (1972). Five grams of meat was blended with 35 ml of sucrose solution (0.25 M) for one minute in a domestic mixer-grinder at low speed. Immediately after blending, a few drops of the slurry containing the fibre fragments were transferred to a

microscopic slide and covered with a coverslip. The length of 10 sarcomeres each of 25 randomly selected fibre fragments were measured under a microscope using a 10x eyepiece with a calibrated micrometer under oil immersion objective and the average was recorded.

3.6 Sensory evaluation

Sensory evaluation was conducted for flavour, juiciness and tenderness. Samples of meat were cut into uniform size of approximately 8 x 1.5 x 1.5 cm and packed in labelled polypropylene bags. For cooking, the bags were immersed in a boiling water bath for 40 minutes. The cooked meat samples were cut into one centimeter cubes and were served to semitrained taste panelists provided with a 9 point hedonic scale score card.

3.7 Statistical analysis

Statistical analysis of the data was conducted as per Snedecor and Cochran (1967).

SCORE CARD FOR TASTE PANEL STUDIES

Name of the product: Cooked Sample No.: Date:

SAMPLE CODE:		FLAVOUR 1	FLAVOUR 2		JUICINESS 1	JUICINESS 2		TENDERNESS 1	TENDERNESS 2
Delicious	9			More			Very		
	8			Juicy			Tender		
	7								
Desirable	6			Juicy			Tender		
	5								
	4								
Not So Desirable	3			Less			Tough		
	2			Juicy					
	1								

Guideline for judgement: If you feel that flavour of the product given to you for taste panel evaluation is delicious, put a tick mark in any one of the three boxes against flavour. The mark being made on the top box if it is delicious. Lower box signifies that it is not so desirable and a tick in the centre box signifies that it is desirable. Similarly mark for other characters viz. juiciness tenderness and overall acceptability.

Specific comments if any:

Name and Designation : Signature

Results

RESULTS

The influence of storage of buffalo meat under refrigeration temperature (4°C) on the physico-chemical and organoleptic qualities were recorded and analysed. The parameters such as pH, Water Holding Capacity (WHC), glycogen, fibre diameter, sarcomere length were studied at intervals of 1, 8, 24 and 72 h and organoleptic qualities at 1, 24 and 72 h of storage.

Fifteen meat samples were collected from buffaloes slaughtered at the municipal slaughter house, Kuriachira, Thrissur and brought to the laboratory. The samples were designated as 1 h samples. Meat samples were stored separately at refrigeration temperature (4°C) and subjected to analysis.

4.1 pH

The mean values of pH obtained from buffalo meat samples at different durations of storage are given in Table 1a. At 1 h, a mean pH of 6.83 ± 0.04 and at 8 h a mean pH of 6.44 ± 0.06 were observed. It came down to 5.76 ± 0.10 and 5.73 ± 0.016 by 24 and 72 h respectively. The pH recorded at 1 h was significantly different from that at 8, 24 and 72 h of storage ($P<0.05$). Student 't' values obtained in statistical analysis are given in Table 1b. pH fell steadily from 6.83 ± 0.04 at 1 h

Table 1a. Mean and standard error of pH

Hours of storage (h)	Mean ± S.E.
1	6.83 ± 0.04
8	6.44 ± 0.06
24	5.76 ± 0.10
72	5.73 ± 0.11

Table 1b. 't' values of pH

Sl.No.	Comparison	't' value
1	1 h Vs 8 h	10.1343*
2	1 h Vs 24 h	12.4586*
3	1 h Vs 72 h	14.0891*

* Significant ($P<0.05$)

to 5.76±0.10 at 24 h by 1.07 units while, the fall during 24 h to 72 h was only by 0.03 units. The pH declined by 0.68 units during the 8-24 h period. The trend of pH decline is depicted in Fig.1.

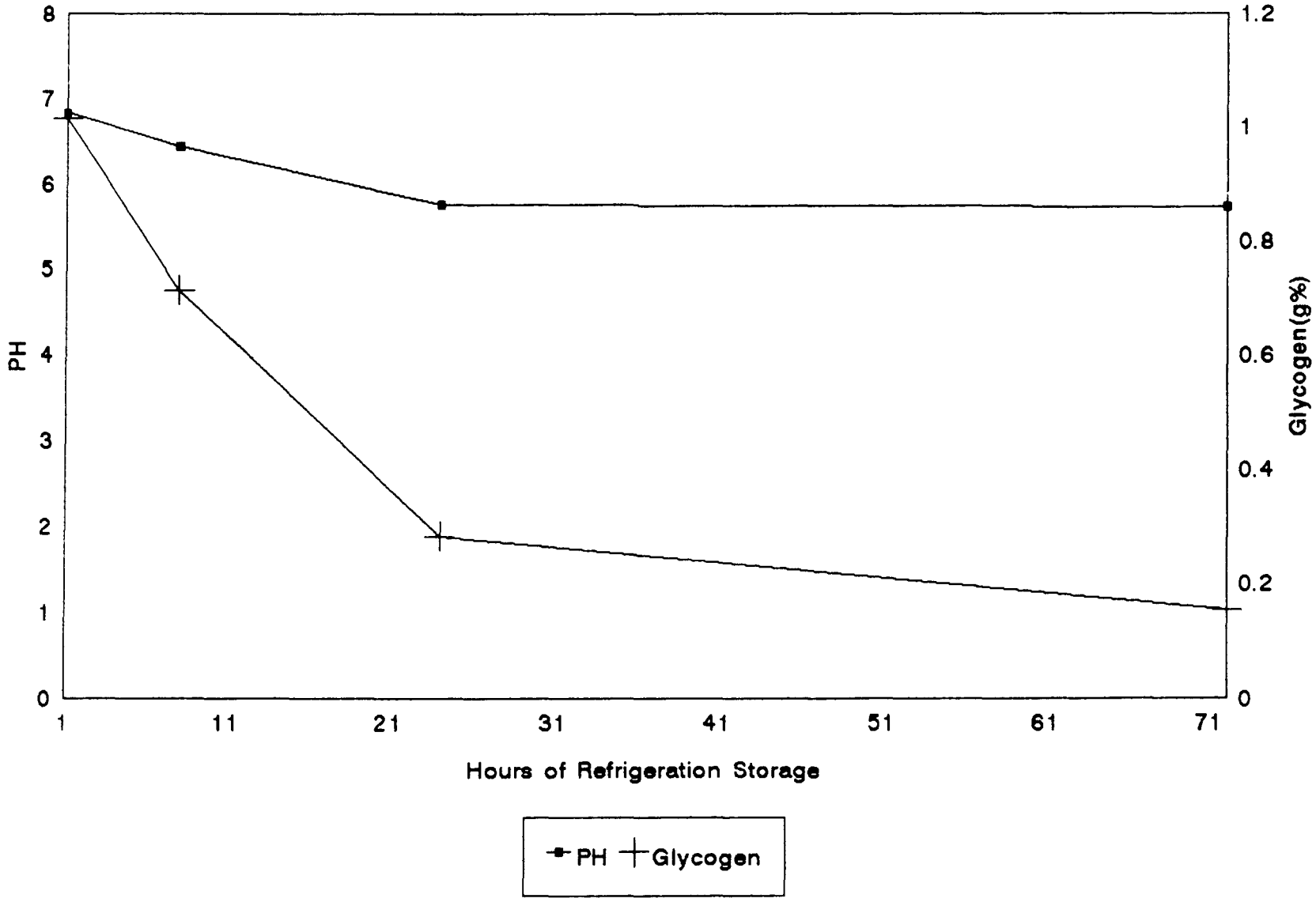
4.2 Water holding capacity

The mean values of water holding capacity are given in Table 2. Water holding capacity observed at 1 h storage, decreased from 18.4±0.88 to 17.33±0.95 ml per 100 g of meat at 72 hours. The maximum reduction in WHC was noticed during 8 and 24 h periods as evidenced by mean values, 18.8±0.91 and 17.60±1.04 respectively. The 8 h sample recorded the highest WHC and the lowest was with 72 h sample. Even though there was numerical difference in quantity of fluid centrifuged out, there was no statistically significant difference among the mean values at different storage periods. The trend of variation in WHC is depicted in Fig.2.

4.3 Glycogen

The mean and standard error of values obtained for glycogen content of buffalo meat are shown in Table 3a. At 1 h the mean glycogen content was 1.014±0.151 and it was reduced to 0.154±0.72 g per cent by 72 h. The change in pH was brought about by the conversion of about 84.83 per cent of glycogen to lactic acid. The mean values were 0.714±0.126 and

Fig.1

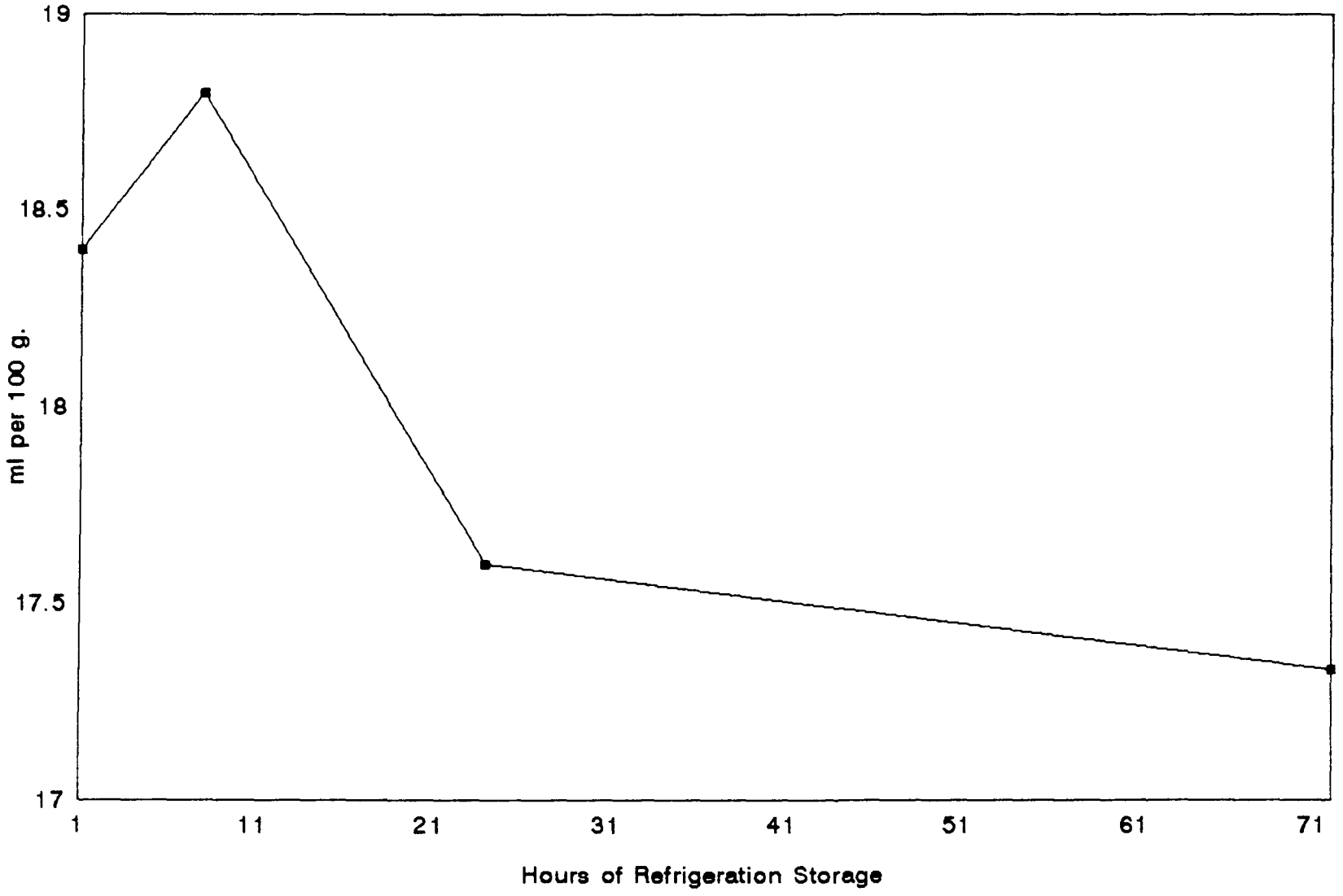


Time course of PH and glycogen decline

Table 2. Mean and standard error of water holding capacity

Hours of storage (h)	Mean ± S.E. (ml/100 g)
1	18.4 ± 0.88
8	18.8 ± 0.91
24	17.6 ± 1.04
72	17.33± 0.95

Fig.2



Trend of water holding capacity

Table 3a. Mean, standard error, percentage of glycogen

Hours of storage (h)	Mean ± S.E.	Percentage of utilisation	Percentage of glycogen unutilised
1	1.0143 ± 0.15	0.00	100.00
8	0.7140 ± 0.13	29.60	70.69
24	0.2834 ± 0.07	72.05	27.94
72	0.1538 ± 0.72	84.84	15.16

Table 3b. 't' values of glycogen

Sl.No.	Comparison	't' value
1	1 h Vs 8 h	4.9368*
2	1 h Vs 24 h	6.6622*
3	1 h Vs 72 h	7.3907*

* Significant ($P<0.05$)

0.283±0.066 g per cent at 8 and 24 h respectively. On comparison with 1 h sample, glycogen content of buffalo meat was 70.39 per cent at 8 h, 27.94 per cent at 24 h and 15.16 per cent at 72 h and were significantly ($P<0.05$) different (Table 3a and 3b).

A rapid fall in glycogen content was noticed at 8 h and 24 h. But there was only a gradual reduction at 72 h. During 1 and 8 h period utilization was 29.61 per cent of total glycogen. Between 8 and 24 h, 42.45 per cent of glycogen was utilised, whereas only 12.78 per cent of total glycogen was utilized between 24 and 72 h. The pattern of reduction in glycogen content is shown in Fig.1.

4.4 Fibre diameter

The mean and standard error of data pertaining to fibre diameter are shown in Table 4 and Fig.3. Mean value of fibre diameter at 1 h was 68.47±1.272 μ and increased to 71.13 ± 1.598 μ by 8 h of storage. The fibre diameter reduced from 69.860±1.041 at 24 h to 68.980±1.105 at 72 h. The variation in fibre diameter was not statistically significant during the course of study. It was noticed that the fibre diameter increased by 2.66 μ at 8 h as evidenced by difference in mean values, amounting to 3.88 per cent increase in fibre diameter from 1 h to 8 h duration.

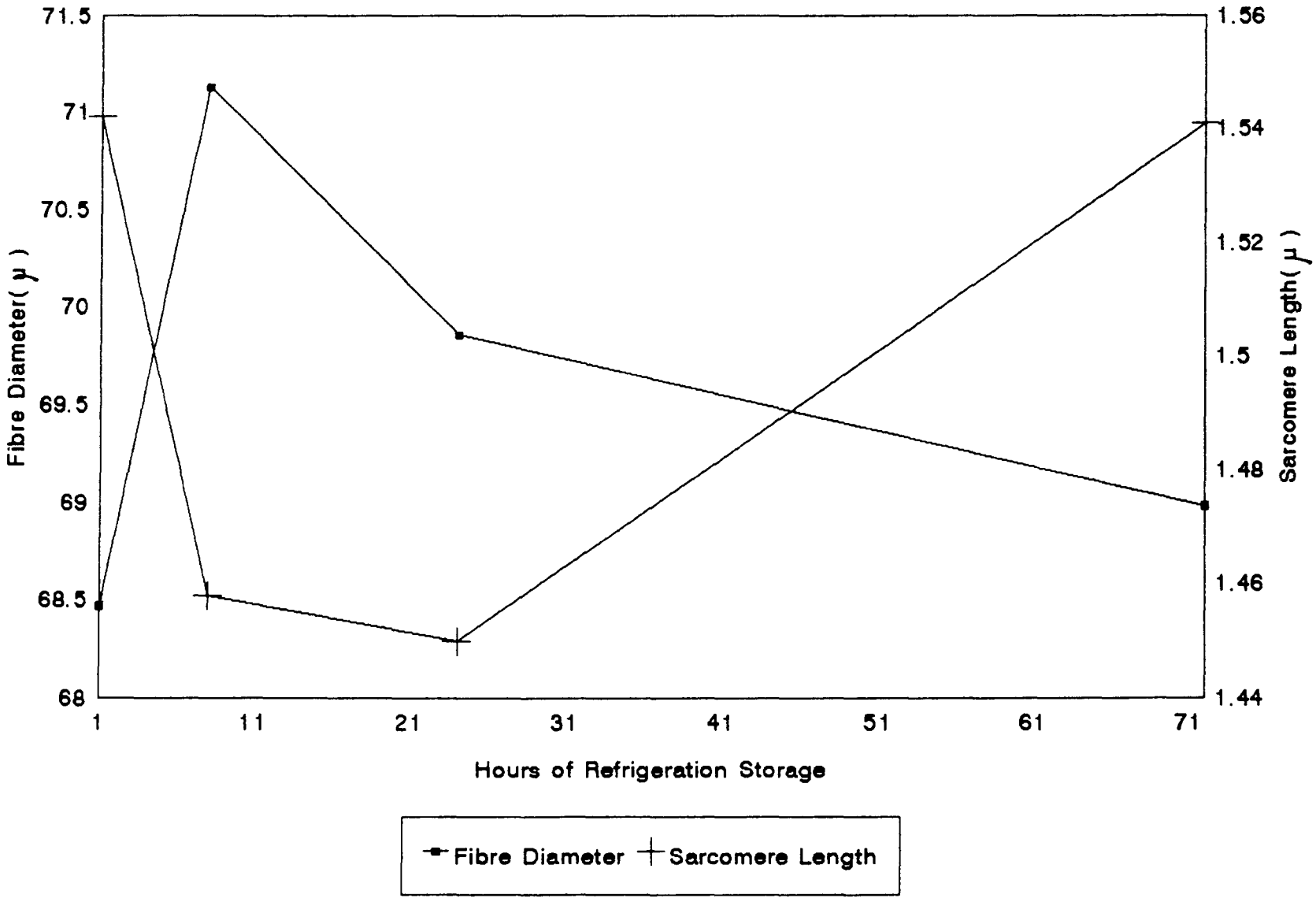
Table 4. Mean and standard error of fibre diameter

Hours of storage (h)	Mean ± S.E. (μ)
1	68.47 ± 1.27
8	71.13 ± 1.60
24	69.86 ± 1.04
72	68.98 ± 1.11

Table 5. Mean and standard error of sarcomere length

Hours of storage (h)	Mean ± S.E. (μ)
1	1.542 ± 0.03
8	1.458 ± 0.02
24	1.450 ± 0.03
72	1.541 ± 0.02

Fig.3



Dimensional changes of fibre diameter and sarcomere length

4.5 Sarcomere length

The mean value of sarcomere length at different storage periods is shown in Table 5. At 1 h the mean value was 1.542±0.031 μ which reduced to 1.458±0.021 μ at 8 h. It was observed that the reduction in sarcomere length was very negligible between 8 and 24 h and attained the initial value at 72 h (Fig.3). The means were 1.450±0.026 μ and 1.541±0.023 μ at 24 and 72 h of storage respectively. The sarcomere length reduced by 0.084 μ at 8 h and increased by 0.083 u at 72 h of storage which is almost equal to the value obtained during the initial hour of study. The sarcomere length was not significantly different among various hours of observation.

4.6 Taste panel studies

The results of the panel regarding flavour, juiciness and tenderness are shown in Table 6. The mean score for flavour was 6.05±0.19, 5.91±0.21, 5.96±0.21 at 1, 24 and 72 h of storage respectively. The mean panel score for juiciness was 5.85±0.19 during the 1 h of evaluation, 5.21±0.21 and 5.55±0.22 at 24 and 72 h respectively. The mean value of tenderness score was 6.10±0.22, 5.71±0.20 and 5.70±0.26 at 1, 24 and 72 h respectively.

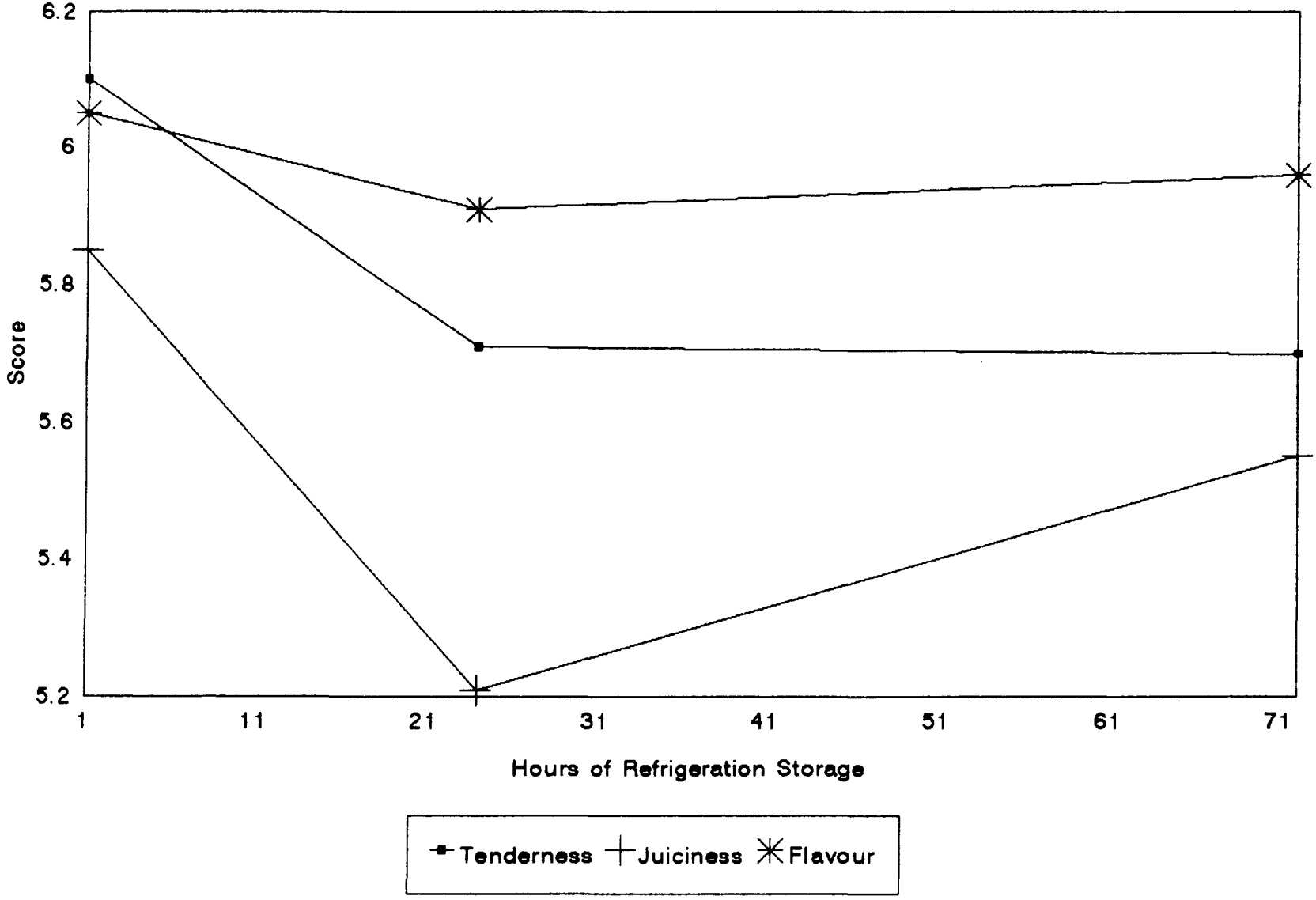
Except the juiciness ($P<0.05$) the scores were not significantly different at various hours of storage. The

Table 6. Mean and standard error of organoleptic qualities

Attributes	Hours of storage (h)		
	1	24	72
Flavour	6.05 ± 0.19	5.91 ± 0.21	5.96 ± 0.21
Juiciness	5.85 ± 0.19	5.21 ± 0.21	5.55 ± 0.22
Tenderness	6.10 ± 0.22	5.71 ± 0.20	5.70 ± 0.26

maximum mean value was noticed at 1 h sample. The score for 24 and 72 h were almost equal as far as flavour and tenderness were concerned. The pattern of organoleptic scores is shown in Fig.4.

Fig.4



Profile of organoleptic attributes

Discussion

DISCUSSION

The conversion of muscle to meat comprises of complex biochemical reactions which decide the ultimate quality of meat. This conversion is dependent on various pre-slaughter, peri-slaughter and post-slaughter handling of animal and or meat. Among the factors which influence the quality of meat, the physico-chemical and biochemical factors are of paramount importance.

5.1 pH

In the present study a mean pH value of 6.83 ± 0.04 was obtained at 1 h in the samples examined. However Ragab *et al.* (1966) reported a lower pH value of 6.67 to 6.3 for fresh buffalo meat. A higher initial pH observed in this study is beneficial in the case of meats used for processing.

A pH of 6.44 ± 0.06 was observed in samples at 8 h in this study. Cassens and Newbold (1966) reported attainment of ultimate pH in meat at 8 h and 24 h under storage temperature of 37°C and 15°C respectively. Chakradhardas *et al.* (1988) observed a pH of 6.48 in buffalo meat by 6 h of slaughter at 5±2°C and this is in agreement with the observation of pH at 8 h in this study.

Miles and Lawrie (1960) were of the opinion that high pH value in meat will give a relatively low shear force value of cooked samples. In the present study it was further observed that a pH of 5.76 was attained by 24 h at 4°C, whereas Honikel *et al.* (1981b) recorded attainment of a pH of 5.9 at 0.5°C taking more than 24 h. During 24 h period, under the present study, it was observed that pH declined from 6.83 to 5.76 steadily, but the fall of pH during 24 to 72 h period was insignificant (5.76 to 5.73 respectively). It was observed that pH had a positive correlation with water holding capacity and glycogen content as evidenced by lowest values at 72 h of storage.

5.2 Water holding capacity (WHC)

Water holding capacity of meat is an important physico-chemical quality since it affects the organoleptic qualities as well as the processing yields of the meat. Mean values of WHC of buffalo meat samples under the present study are given in Table 2. At 8 h of storage, WHC recorded a maximum of 18.8 ml/100 g of tissue. Water holding capacity did not differ significantly among the samples tested at different storage periods in this study. This observation is in agreement with the findings of Parrish *et al.* (1969) that various meat attributes like tenderness, WHC and cooking loss were not affected by various ageing temperature and time.

Honikel *et al.* (1981a) reported that neither shortening nor rigor had an immediate effect on WHC. Similarly in the present study also it was observed that at different stages of post-mortem holding, WHC did not differ significantly. In this study, meat sample with highest initial mean pH recorded better WHC than those at ultimate pH and this finding is in accordance with the finding of Kondaiah *et al.* (1986) that a higher pH was correlated with a higher WHC. Water holding capacity values recorded in samples tested at different periods of storage showed a downward trend which is depicted in Fig.2. During rigor, the sarcomeres shorten causing reduction in available sites for water binding, which may be one of the reasons for the reduced WHC during 8 to 72 h period. As the meat approaches its iso-electric point, there is a drastic reduction in the number of available charges for binding the water molecules, which is yet another reason for low values of WHC recorded (Gault, 1985). With a higher WHC during 1 h to 8 h under refrigeration storage, the processing of buffalo meat can be done more efficiently since, excellent WHC and fat emulsification capacity have been reported during high pH values (Hamm, 1978).

5.3 Glycogen

It was found that the initial glycogen content obtained in the meat samples tested, declined from 1.014 to 0.1538 g per

cent in 72 h amounting to utilisation of 84.84 per cent glycogen and bringing down the pH by 1.1 units at 72 h of storage (Fig.1). It was noticed that as the glycogen content decreased, pH also decreased. Residual glycogen, in the opinion of Lawrie *et al.* (1959) was due to inaccessibility or insusceptibility of glycogen for the enzymatic action. Level of residual glycogen recorded in this study was higher than that reported by Newbold and Lee (1965). In the present study, it was noticed that 0.860 g of glycogen was utilised to bring down the pH from 6.83 to 5.73 by 72 h whereas, Disney *et al.* (1967) achieved an ultimate pH of 5.7 by utilising 0.7 g of glycogen in beef. The results of the present study are in agreement with that of Newbold and Harris (1972) who observed an ultimate pH in beef rarely less than 5.5 to 5.4 even when there was an adequate store of glycogen. The residual glycogen level recorded in this study is in agreement with the values reported by Lawrie (1976) who obtained the residual level of above 0.1 g per cent after rigor mortis in beef whereas, Kuttinarayanan (1988) reported 15 to 22 per cent residual glycogen in mutton.

The results obtained in the present study showed a positive correlation in the decreasing mean values of pH, glycogen and WHC. This is supported by the findings of Cook (1967) who reported a positive correlation between pH and

glycogen in beef and the findings of Kuttinarayanan (1988) between pH, glycogen and WHC in mutton.

5.4 Fibre diameter and sarcomere length

The fibre diameter in the present experiment ranged from the initial reading of 71.13 μ at 1 h to 68.47 μ at 8 h, whereas sarcomere length at the same period decreased from 1.542 μ to 1.458 μ. The fibre diameter increase was 3.88 per cent while the sarcomere length decreased by 5.45 per cent at 8 h. Sarcomere length and fibre diameter attained their initial measurements of 1.54 μ and 68.98 μ respectively at 72 h. When muscles shortened there was corresponding decrease in sarcomere length and increase in fibre diameter (Herring *et al.*, 1965) and the results of the present study are in agreement with the above findings. But the data obtained in this study are not in agreement with Honikel *et al.* (1986), who reported 70 per cent contraction of sarcomere at 6°C. Honikel *et al.* (1981a) observed unchanged sarcomere length until the onset of rigor mortis whereas in the present study fibre contracted by 3.88 per cent during 8 h.

Yu and Lee (1986) were of the opinion that sarcomere length could be used as an indicator of meat tenderness whereas Koohmaraie *et al.* (1988) stated that shorter sarcomeres had a greater ageing response. In the present study it was observed

that 1.542 u long sarcomere provided comparatively tender meat (Table 6). The measurements at different periods showed fibre diameter and sarcomere length had a negative correlation (Fig.3). Cook *et al.* (1966) also demonstrated a linear relationship between fibre shortening sarcomere length and tenderness.

5.5 Organoleptic qualities

The results of the present study indicated that storage of buffalo meat under refrigeration (4°C) did not affect the organoleptic qualities except juiciness significantly. Juiciness of different samples under varying storage periods was significantly ($P<0.05$) different from that of 1 h sample. The other attributes like tenderness and flavour remained unaltered. This indicates that the quality parameters of buffalo meat except juiciness are not altered within the course of study. Parrish *et al.* (1969) did not observe significant changes in tenderness at different temperatures of ageing.

Yu and Lee (1986) were of the opinion that temperature above 4°C, will bring about protein degradation and meat tenderness if only the ageing period is extended beyond 24 h. The results of the present study is in agreement with the above findings and ageing of meat at 4°C even upto 72 h was not sufficient to bring about improved ratings in flavour and

tenderness. However this finding is in contradiction to the finding of Koohmaraie (1987) who reported that the desired improvement in tenderness could be obtained by keeping beef at 4°C within 24 hours. The score obtained for flavour of fresh buffalo meat was in agreement with that reported by Chakradhardas *et al.* (1988). According to Honikel *et al.* (1983) shortening of muscle and development of rigor influence tenderness and water retention of beef. But in this study significant shortening of the muscle was not observed. Hence there was no significant change in tenderness.

It was noticed that fibre diameter, sarcomere length and tenderness were, though not significantly, numerically altered during the storage and the findings are in agreement with those of Herring *et al.* (1965) who observed that when muscles shortened there was a corresponding decrease in sarcomere length, increase in fibre diameter and decrease in tenderness.

According to Honikel *et al.* (1986) during cold or rigor shortening the muscle became thicker due to an increase in fibre diameter. However the results of the present study did not show any significant increase in the fibre diameter of the stored samples compared to the values obtained in the 1 h samples tested. West and Carpenter (1985) examined fibre types in water buffalo and obtained only fibres of aerobic oxidative capacity. The absence of white fibres showed a significant and

distinct difference in muscle metabolic and functional characteristics than that of Angus and Charolais bulls. This distinct fibre type may be the reason for the non significant changes in the fibre diameter and sarcomere length throughout the storage period. Further studies are required in buffalo meat samples to identify the distinctive fibre types if any which may influence the post-motem changes considerably.

Summary

SUMMARY

The consumer preference of a product is dependent on its quality. Meat by virtue of its unique nature, is having different quality attributes related to physico-chemical, biochemical, nutritional, microbiological and organoleptic qualities. Since the information regarding the quality of buffalo meat are scanty, the present study was undertaken to evaluate the physico-chemical qualities of buffalo meat during refrigeration storage. The study was conducted on various parameters namely pH, water holding capacity, glycogen content, sarcomere length, fibre diameter and organoleptic qualities namely flavour, juiciness and tenderness.

Fifteen buffalo meat samples were collected immediately after slaughter from the municipal slaughter house, Kuriachira, Thrissur, and brought to the laboratory. Except for organoleptic qualities, the samples were analysed at 1, 8, 24 and 72 hours of storage. Flavour, juiciness and tenderness were assessed at 1, 24 and 72 h only.

The initial pH of 6.83 $\pm$ 0.04 decreased to 6.64 $\pm$ 0.06 by 8 h. By 24 h of storage, it was further reduced to 5.76 $\pm$ 0.10 and by 72 h it was only 5.73. These values were significantly different ($P<0.05$) from initial pH.

The difference in water holding capacity observed at different storage periods were not significant. A higher water holding capacity was noticed at 1 and 8 h (18.4 ± 0.88 and 18.8 ± 0.91 ml/100 g). The values were found reduced to 17.6 ± 1.04 and 17.33 ± 0.95 ml/100 g at 24 and 72 h respectively.

The mean glycogen level was 1.0143 ± 0.15 g per cent at 1 h and found reduced to 0.1538 ± 0.72 g per cent at 72 h. By 8 h 70.39 per cent and by 24 h 27.97 per cent glycogen was utilised.

The fibre diameter and sarcomere length observed at different storage periods were not significantly different from observations at 1 h sample. With a corresponding increase in fibre diameter, there was a decrease in sarcomere length. The fibre diameter and sarcomere length attained almost its initial measurements by 72 h of storage.

The organoleptic studies indicated that flavour and tenderness were not enhanced during the hours of storage. The 1 h sample had a better juiciness (5.85 ± 0.19) than the samples observed at 24 and 72 h. The flavour and tenderness ratings for initial sample was 6.05 ± 0.19 and 6.10± 0.22 respectively which was not altered by storage at 24 and 72 h. From the above results it was clear that storage of buffalo meat upto 72 h reduced the pH and glycogen significantly from initial levels. The water holding capacity, fibre diameter,

sarcomere length and organoleptic qualities were not altered significantly during 72 h of refrigeration storage.

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PHYSICO-CHEMICAL QUALITY OF BUFFALO MEAT UNDER REFRIGERATION

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ABSTRACT OF A THESIS

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ABSTRACT

Meat is accepted all over the world as a rich source of high quality assimilable protein containing all the essential amino acids. Meat quality per se is contributed by its physico-chemical, biochemical, nutritional, microbiological and organoleptic qualities. Though some of these are predetermined depending on animal physiology, others can be changed by manipulating the meat handling practices. It is learned from the literature that studies on physico-chemical quality of buffalo meat are scanty. Hence the present study was undertaken to assess the various physico chemical qualities of buffalo meat under refrigeration (4°C).

Fifteen meat samples were collected from the municipal slaughter house, Kuriachira, Thrissur and analysed for pH, water holding capacity, glycogen content, fibre diameter, sarcomere length and organoleptic qualities at 1 h and stored at refrigeration temperature (4°C) for further analysis at 8, 24 and 72 h of refrigeration.

The pH and the glycogen content of the samples reduced steadily during the storage period from 1 h to 72 h. However significant differences in water holding capacity, fibre diameter and sarcomere length were not observed during the course of study. In the organoleptic evaluation, flavour and

tenderness did not differ significantly during different hours of storage, though the meat was significantly juicier ($P<0.05$) with a score 5.85 ± 0.19 at 1 h and reduced further during subsequent hours of storage.