TENDERISATION OF BUFFALO MEAT BY CALCIUM CHLORIDE MARINATION

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DECLARATION

I hereby declare that this thesis, entitled **TENDERISATION OF BUFFALO MEAT BY CALCIUM CHLORIDE MARINATION** 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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KAVITHA RAJAGOPAL

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Certified that this thesis, entitled **TENDERISATION OF BUFFALO MEAT BY CALCIUM CHLORIDE MARINATION** is a record of research work done independently by **Dr. Kavitha Rajagopal,** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of Dr. Kavitha Rajagopal a candidate for the degree of Master of Veterinary Science in Livestock Products Technology, agree that the thesis entitled **Tenderisation of Buffalo Meat by Calcium Chloride Marination** may be submitted by Dr. Kavitha Rajagopal, in partial fulfilment for the degree.

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KAVITHA RAJAGOPAL

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Introduction

INTRODUCTION

The conversion of muscle to meat involves a number of chemical reactions which occur in postmortem muscle. Some of these enzymatic reactions essentially cease within the first 24 hours while other changes continue during postmortem conditioning period. The rate and extend of some of these reactions have important implications on the ultimate quality of muscle as a food.

Muscle is converted to meat after a lot of biochemical and biophysical changes at chilling temperature, which is known as conditioning, or ageing, during which the tenderness and other organoleptic qualities are enhanced to make it more palatable. Among the eating qualities of meat, tenderness has been indicated by numerous consumer surveys (Morgan, 1991a) as the most important palatability attribute. Therefore, the importance of tenderness and the factors that affect tenderness should be given emphasis.

It is well established that the texture and tenderness of meat depend on two principal factors, viz., the amount and properties of collagen of the muscle tissue which is termed as 'background toughness' and the state of the contractile proteins in the muscle system, which is referred to as 'processing toughness' (Morrissey and Fox, 1981). The proteolysis of myofibrillar proteins is now being emphasised as the key event in meat tenderisation during postmortem storage. Of the two characteristic enzymes implicated in hydrolysing the myofibrillar proteins, the calcium dependant protease is more involved in tenderisation than do the catheptic enzymes.

The calpain proteolytic system consists of two neutral proteases, μ-calpain, m-calpain and the endogenous inhibitor of the calpains called calpastatin (Koohmaraie *et al.*, 1991). μ-calpain, which requires micromolar calcium for activation is primarily responsible for the proteolysis of the structural proteins by undergoing activation and autolysis. Conversely, the activity of m-calpain, which

requires millimolar calcium for activation does not change appreciably, over time postmortem, indicating that insufficient calcium is present in postmortem muscle to activate this enzyme. This would suggest that a significant tenderising potential remains in the muscle in the form of non-activated m-calpain. Attempts have been made to utilise the tenderising potential of m-calpain through the addition of calcium ions to postmortem muscle. Calcium chloride has been approved by Food and Drug Administration as Generally Recognized as Safe (GRAS) for use in meat products (FSIS, 1973). Calcium chloride injection or infusion has been successful in shortening the period of ageing, when applied either prerigor or after the completion of rigor (Koohmaraie *et al.*, 1989; Wheeler *et al.*, 1992) in bovine and ovine carcasses.

The development of such a method could be an integral part of reducing the variation in tenderness of meat consumed in India, where there exists an altogether different pattern of meat production and consumption. In our country, animals are being slaughtered at the end of their productive life, the meat of which is abundant in connective tissue contributing to the background toughness. Apart from this, various other factors like inhumane slaughter practices, faulty processing, the rate of cooling during initial phase of rigor, several other pre slaughter and post slaughter factors and the habit of consuming tough muscle in rigor make meat a lesser palatable food. A possible solution to this problem is natural conditioning for up to 10 - 14 days, which might prove to be less cost effective.

Therefore, the post mortem calcium chloride marination of meat cuts or whole carcass may have special promise to the consumers in India in that it would enhance tenderness and reduce the time required for ageing. Many studies in this regard had been conducted with positive outcomes in exotic breeds of cattle or their crosses and in sheep. There is a pressing need for the same in buffalo meat, which is preferred to other meats in India with increased export. The production of buffalo meat is on the increase reaching 1.487 million tonnes

in 2005 contributing to 26 per cent of total meat production in India (FAOSTAT, 2006).

Therefore, this study on tenderisation of buffalo meat by calcium chloride marination was designed to address the following objectives.

1. To assess the effect of calcium chloride marination on the tenderisation of buffalo meat.

2. To compare the tenderising effect of calcium chloride with that of ageing, and

3. To determine the effect of calcium chloride marination on the other organoleptic qualities of meat.

Review of Literature

REVIEW OF LITERATURE

A detailed study was conducted on the effect of calcium chloride marination on the organoleptic qualities of buffalo meat especially, tenderness. A thorough scanning of the available literature has revealed that much of the studies had been conducted in beef, mutton and lamb and there is dearth of literature on the effect of calcium chloride marination on buffalo meat. The available relevant literature on the eating qualities of meat, various factors affecting them, role of endogenous proteases, calcium chloride marination and its effect on tenderness and other eating quality attributes are briefly reviewed in this chapter.

2.1 ORGANOLEPTIC QUALITIES OF MEAT

The national beef tenderness survey conducted by Morgan *et al.* (1991a) pointed out factors such as meat color, flavor, aroma, tenderness and the method of cookery to play a collective role in meat taste and more importantly in consumer acceptance. Pearson (1999) opined that meat, poultry and fish products must meet the same requirement as other foods in providing not only nutritionally adequate, but also palatable and acceptable products to the diet of human.

The organoleptic sensation may enhance or impair the efficacy of digestion by their reflex action on the production of gastric and intestinal juiciness; and thus the nutritive value of food. Of the attributes of eating quality, colour, water holding capacity and some of the odour of the meat are detected both before and after cooking and provide the consumer with a more prolonged sensation than do juiciness, texture, tenderness, which are detected on mastication (Lawrie, 1998).

2.1.1 Attributes

2.1.1.1 Colour

The appearance of meat surface to the consumer depends not only on the quantity of myoglobin present but also on the type of myoglobin molecule, on its chemical state and on the chemical and physical condition of other components in the meat (Lawrie, 1998).

Consumers often interpret pink or red colour in cooked meats as an indication of undercooking, based on the well accepted relationship between colour and internal temperature of beef steaks, where rare is equivalent to 60° C internal temperature, medium well done is equivalent to 71° C and well done is equivalent to 82° C. Consumers prefer bright red fresh meats and brown or grey coloured cooked meats (Cornforth, 1999).

Temperature of cooking naturally affects the degree of conversion of the pigments. Thus, beef cooked to an internal temperature of 60° C has a bright red interior and that cooked to an internal temperature of 70-80° C or higher is grayish brown (Lawrie, 1998).

2.1.1.2 Flavour and Aroma

Flavour is a complex sensation. It involves odour, taste, texture, temperature and pH. Of these, odour is the most important (Lawrie, 1998).

Meat flavour is thermally derived, since uncooked meat has little or no aroma and only a blood like taste. Sulfur compounds, derived from ribose and cysteine; seem to be particularly important for the characteristic aroma of meat (Mottram, 1998).

2.1.1.3 Juiciness

Unlike other key aspects of texture, juiciness remains a uniquely subjective property. The relationship between 'subjective' juiciness of meat and any objective measurement remains elusive and poorly understood (Hamm, 1960).

Weir (1960) defined juiciness as the impression of wetness during the first few chews which is produced by the rapid release of meat fluid and the sustained juiciness, largely due to the stimulatory effect of fat on salivation.

Meat juiciness is an important contributor to eating quality and also plays a key role in meat texture contributing between 10 per cent and 40 per cent to its variability. (Szczesniak, 1963; Dransfield *et al.*, 1984; Hutchings and Lillford, 1988).

If meat is consistently and acceptably tender and there is no off flavor, then juiciness becomes the sensory characteristic that is the primary determinant of meat quality. The only reliable and consistent measure of juiciness is achieved using sensory methods (Winger and Hagyard, 1999).

2.1.1.4 Tenderness

The sensory characteristic of tenderness is considered the most important factor of consumer perception and is the most important eating quality attribute in the acceptance of meat. The overall impression of tenderness to palate includes texture and involves three aspects. Firstly, the initial ease of penetration of the meat by the teeth; secondly the ease with which the meat breaks into fragments; and thirdly the amount of residue remaining after chewing (Weir, 1960). Bernholdt (1975) also defined tenderness as that quality of cooked meat that is recognised by the characteristics of easy chewability without loss of desirable texture.

Morrissey and Fox (1981) opined that it is extremely difficult to quantify the contributions to tenderness by either connective tissue or myofibrillar proteins.

Of all the attributes of eating quality, texture and tenderness are presently rated most important by the average consumer and appear to be sought at the expense of flavour or colour (Lawrie, 1998).

Texture is a sensory property of food embodying all the mouth feel characteristics, i.e., kinaesthetics. Tenderness is one attribute of texture, being the resistance to shear or the hardness of meat. Mechanical means are commonly used to provide a measure of tenderness (Chrystall, 1999).

2.1.2 Factors Affecting Organoleptic Quality

Species is the most general factor affecting tenderness and is a reflection of texture. Therefore, the large size of cattle in relation to sheep and pigs is generally associated with a greater coarseness of their musculature (Hammond, 1932).

Juiciness of meat is attributable to its content of fat (Blumer, 1963) and moisture content (Offer and Trinick, 1983).

Flavour formation in meat and meat products are influenced by age and species (Minor *et al.* 1965), fat content (Wasserman, 1979), sex (Brooks and Pearson, 1989) and volatiles from Maillard reaction (Mottram and Whitfield, 1994).

Differences in the colour of meat are due to electrical stimulation (Dutson and Pearson, 1985), species, age, exercise, oxygenation and oxidation of muscle pigments (Hunt and Kropf, 1987), chilling (Fox, 1987), breed, sex and type of muscle (Lawrie, 1998).

The collagen contribution to toughness is due to the presence of intermolecular cross links which, with increasing animal age, become more thermally resistant and thus less readily broken during cooking. By contrast, toughness due to the contractile proteins is determined by the conditions during the first few postmortem hours (Marsh *et al.*, 1981). Hydroxyproline is equated to higher connective tissue and the increased tenderness of veal in relation to beef is attributed to the type and the quantity of connective tissue (Lawrie, 1998).

Tenderisation process including the rate and extend of proteolysis occurs unequally in different breeds (Wheeler *et al.*, 1990) and in different animals within a breed (Koohmaraie, 1996).

According to the result of a study conducted by Pike *et al.* (1993) effect of early postmortem glycolytic rate is of paramount importance to beef *longissimus thoracis et. lumborum* tenderness. The implication is that early postmortem temperature is only important through its influence on early postmortem glycolytic rate.

Pearson (1999) reviewed in detail the major quality traits of meat, the factors associate with them and their effects on acceptability of different products.

2.1.3 Measurement of Organoleptic Quality

2.1.3.1 Colour

According to the guidelines of American Meat Science Association (AMSA) (1991) the visual appraisal of colour could be carried out by evaluating the steaks daily for attributes like lean colour on a scale of 8 (1 = bleached red, 4 = cherry red, 8 = very dark red); percentage of discolouration on a scale of 7 (1 = 0 per cent, 4 = 20-59 per cent and 7 = 100 per cent); colour uniformity on a scale of 5 (1 = uniform, 5 = extreme two toning); and display colour stability(1 = very bright cherry red or pale red, 5 = dark red to tan or brown).

The two major types of instruments used in meat colour measurement are spectrometers and tristimulus colourimeters. The tristimulus values obtained were transformed into the Hunter L, a, b colour space to approximate visual spacing. The important factor affecting meat colour is the variation in opacity or translucence because in meat colour, pigmentation and light scatter interact to affect colour appearance (MacDougall, 1999).

2.1.3.2 Flavour and Aroma

Sensory methods adequately measure flavour and aroma but can be very expensive to perform. Instrumental methods are economical to perform on a routine basis, but do not always reveal the complete flavour story. Both types of flavour measurement methods are important and have their place in flavour investigation (Bett and Grimm, 1999).

The subjective measurement of flavour include descriptive flavour profile sensory panel evaluation of steaks in accordance with American Society for Testing and Materials (ASTM) (1999) protocols which measures beef flavour identification, brown-roasted, bloody/serumy, metallic, soapy/chemical, cardboard, oxidized/painty and fishy flavours on a scale ranging from 1 (least intense) to 15 (most intense).

2.1.3.3 Juiciness

Juiciness/Water Holding Capacity could be assessed by methods applying either external mechanical force, including filter paper press method (Grau and Hamm, 1957), centrifugation methods (Wierbicki and Deatherage, 1958), capillary volumeter method (Fischer *et al.*, 1976) and imbibing method (Monin *et al.*, 1981); applying no force, like measurement of evaporation and weight loss (Honikel *et al.*, 1986); or those applying thermal force like percentage of cooking loss (Bendall and Restall, 1983).

The hydration properties of meat, expressed by water holding capacity, press juice, and cooking loss, determine its ability to retain water during processing. These properties influence tenderness, juiciness, firmness, and appearance of meat (Offer and Knight, 1988).

Juiciness can be looked at as a way of measuring water holding capacity which is measured by chewing the meat and using the human senses. While the physical methods for water holding capacity determination can be described and standardised exactly. But in juiciness evaluation, standardisation or calibration is difficult (Honikel and Hamm, 1999).

2.1.3.4 Tenderness

The methods for objective assessment of tenderness include methods making use of grinding forces (Miyada and Tappel, 1956); penetration forces (Bouton and Harris, 1972); methods employing shear and biting systems like, Kramer shear (Kramer *et al.*, 1951), nip tenderometer (Purchas, 1973), Warner-Bratzler shear (Wheeler *et al.*, 1997a); tensile assessment (Locker and Wild, 1982) and compressive forces in processed products (Jones *et al.*, 1985).

The fragmentation methods for assessing tenderness make use of the characteristic of ease of fragmentation. It has been used by Davey and Gilbert (1969), as a measure of ageing. The procedure could be applied to both raw meat (Calkins *et al.*, 1980) and cooked meat (Davis *et al.*, 1980). The results depend on the physical operations and the equipment used.

The tenderness of meat assessed by changes in structural framework makes use of the description of fibres as a characteristic. Muscle fibre diameter and its degree of shortening and breakdown had been examined as possible predictors or indicators of tenderness (Voyle, 1971).

The chemical changes that could serve as a means of tenderness measurement include; the ageing changes in myofibrillar component, assessed by

changes in electrophoretic patterns (Mac Bride and Parrish, 1977), the connective tissue description in terms of solubility, cross linking and general extractability (Seideman, 1986).

Peleg and Normand (1982) while comparing the relationship between subjective and objective measures of texture assessment, stated that the deformation rates used in mechanical systems often do not correspond to the variable rates used in human mastication motions, which would influence the relationship between subjective and objective assessment results.

The subjective measurement of tenderness could be accomplished by the method outlined by AMSA (1995), where meat steaks are evaluated for myofibrillar tenderness, juiciness; beef flavor intensity, connective tissue amount and overall tenderness based on an 8- point Hedonic scale.

There is considerable interest in being able to determine the tenderness of a product soon after slaughter, which could be accompanied by measuring characteristics that are highly correlated with tenderness, viz., mineral content, iron/zinc ratios, connective tissue shrink tension, and 3 h pH values (Chrystall, 1999).

2.2 MECHANISM OF TENDERISATION

Goll *et al.* (1970) pointed out the presence of a protein endogenous to the muscle which require calcium, and which catalyses the degradation of myofibril on Z line.

Yamamoto (1975) stated that, of the several cathepsins that have been identified, some appear to be of importance in the ageing of meat because their optimum pH is close to that existing in postmortem beef muscle.

Penny (1980) opined that proteolysis of myofibrillar proteins is a key event in meat tenderisation during postmortem storage of carcasses at refrigerated temperatures.

Bendall and Restall (1983) reported that structure of muscle proteins were altered according to temperature of heat treatments. Expulsion of water from individual muscle fibres was slow at 40- 53° C, but rapid at 60° C as the collagen of the basement membrane was shrinking. At 64 - 90° C, shrinkage of the endomysial, perimysial and epimysial collagen were noticed decreasing the myofibril diameter. Prolonged heating converted collagen to gelatin and concomitant tenderisation occurs.

Troy *et al.* (1986), reported that myofibrillar protein break down commences within 4 to 6 hour postmortem, which is supported by Koohmarie *et al.* (1987), who stated that tenderisation begins soon after exsanguination.

Many researchers have documented that myofibrillar proteolysis could be attributed to endogenous proteinase activity and reports indicated that calcium dependent proteinase system (calpains) may be more involved in postmortem proteolysis than the catheptic enzymes (Koohmaraie *et al.*, 1988, 1991; Wheeler *et al.*, 1990; Whipple *et al.*, 1990; Morgan *et al.*, 1991b).

Whipple *et al.* (1990) while evaluating the attributes that affect *longissimus* muscle tenderness in *Bos taurus* and *Bos indicus* cattle, observed a significant relationship between calpastatin 24 hour activity and the amount of postmortem proteolysis associated with tenderness. Calpastatin is susceptible to inactivation by freezing (Koohmaraie, 1990).

Wheeler and Koohmarie (1994) from their study on prerigor and post rigor changes on ovine *longissimus* tenderness, interpreted that ultimate tenderness in any one muscle from young animals depends on a combination of rigor shortening induced toughness and the extend of proteolysis tenderisation by the calpain proteolytic system.

The decreased tenderness associated with cattle of *Bos indicus* seemed to be highly correlated with increased calpastatin 24 hour activity. Moreover, it

seems that calpastatin activity is more highly related to aged meat tenderness than is intramuscular fat (Shackelford *et al.*, 1994).

The fact that the ultimate tenderness is influenced more by proteolysis than by sarcomere length is emphasised by Wheeler and Koohmarie (1994) by their finding in ovine muscle that when shear force decreased to one half (from 8.66 to 4.36 kgf) from 24 to 74 hour postmortem. sarcomere length did not change significantly during this time.

The activities of the enzymes involved in postmortem tenderisaton are pH and temperature dependent. So the rate of glycolysis influences the rate and extend of tenderisation process especially the proteolysis of myofibrillar component of toughness (O'Halloran *et al.*, 1997).

Storage of lamb carcasses at 4^{0} C resulted in proteolytic break down of calpastatin within 6 hours, and little nondegraded calpastatin was detected on 3 day and 7day of postmortem storage (Doumit and Koohmaraie, 1999).

2.3 CALCIUM CHLORIDE MARINATION

The calpains are located in the cytosol and at the Z disks where most of the changes occur in the muscle during postmortem storage (Dayton and Schollmeyer, 1981). Compared to μ-calpain m-calpain requires 200-300 μM calcium for activation (Dayton *et al.*, 1981). Because the calcium ion requirement for m-calpain far exceeds the calcium ion levels in muscle (Goll *et al.*, 1983) it has been concluded that m-calpain is not activated *in vivo* or during normal postmortem storage of meat (Koohmaraie *et al.*, 1987).

Slinde and Kryvi (1986) from their morphological investigation concluded that in the presence of calcium ions and in the absence of inhibitors, the calcium activated proteinase act at the Z disc level of sarcomere, and they suggested the enzyme to be essential in the postmortem tenderisation process in beef and of importance for the *in vivo* muscle protein turn over process.

Inomata *et al.* (1986) suggested that initial autolysis due to calcium ions is required for m-calpain to become active, the activation of which is the major causative factor associated with accelerated tenderisation at day 1 in calcium chloride infused carcasses.

Calcium chloride injection or infusion improved or accelerated tenderness in pre-rigor meat (Koohmaraie *et al.*, 1988, 1989, 1990, Koohmaraie and Shackelford, 1991; Morgan *et al.*, 1991b). Wheeler *et al.* (1992) obtained similar results with post-rigor injection, if the meat was aged 7 days postmortem.

Other than calcium chloride infusion and injection, there are no documented conditions that can affect m-calpain so dramatically, which is remarkably stable under normal postmortem conditions (Koohmaraie *et al.*, 1990).

Metallic off flavors were reported by Morgan *et al.* (1991b), when they injected 300mM calcium chloride at 10 per cent by weight to tenderise beef from mature cows.

Dransfield (1992) while emphasizing the role of calpain I in postmortem tenderisation, stated that the rate of tenderisation is proportional to the concentration of the calpain I which is activated when the pH falls to 6.1 which is then autolysed slowly reducing its concentration and the rate of tenderisation.

Wheeler *et al.* (1992) found that post rigor injection of beef with calcium chloride and ageing for 7 days resulted in tenderisation similar to that resulting from pre-rigor injection.

Wheeler *et al.* (1993) used a lower amount of calcium chloride, i.e., 5 per cent of 200mM calcium chloride and found no flavor problems in beef.

The degradation of troponin T, an indicator of tenderisation is much less in *M. Psoas major* than in *M. Semitendinosis* or *M. Longissimus dorsi*, and is attributed to its lower content of calpains (Dransfield, 1993).

Kendall *et al.* (1993) concluded that although m-calpain and caipastatin activities decrease with increasing ionic strength, they might be actively influencing the loss of structural integrity of the myofibrils in postmortem muscle even at elevated ionic strengths and decreased pH.

Diles *et al.* (1994) reported that loin strip steaks from mature cows injected with a 200mM calcium chloride solution did not have any off flavor, but were improved in WB shear force values and tenderness as rated by a trained sensory panel.

While studying the alteration of postmortem ageing in beef by the addition of enzyme inhibitors and activators, Alarcon-Rojo and Dransfield (1995) used solutions of calcium, sodium, potassium and magnesium chlorides at different concentrations, and found out that calcium chloride accelerated ageing and produced more tender meat than the controls. Moreover, sodium and potassium chlorides were 43 per cent and magnesium chloride 73 per cent as effective as calcium salts in tenderising the meat.

The calpain proteolytic system plays a key role in the tenderisation process that occurs during postmortem storage of meat under refrigerated conditions (Koohmaraie, 1996).

Boehm *et al.* (1998) monitored the changes in the activity of calpains and calpastatin during postmortem storage in bovine muscle and observed that extractable m-calpain activity changed slightly during postmortem storage. m-calpain activity from its initial level at death has been reduced to its 63 per cent on 7^{th} day of storage while caipastatin activity has been reduced to 30 per cent.

Calcium injection appears to tenderise meat by activating residual μ- and m-calpain activity in the muscle, bringing about a greater degree of postmortem protein degradation (Pringle *et al.*, 1999). Research has demonstrated that pre-rigor infusion or injection of calcium chloride can accelerate the tenderisation process (Gracey *et al.*, 1999).

The infusion of calcium chloride or sodium chloride into carcasses has been used for acceleration of postmortem proteolysis and tenderisation process (Polidori *et al.*, 2000).

A study by Rowe *et al.* (2004) established that exposure of postmortem bovine muscle tissue to oxidation via irradiation at 24 hour postmortem would result in inactivation of μ- and m- calpain and decreased proteolysis of myofibrillar proteins and higher shear force values in aged beef steaks.

Observations made by Maddock *et al.* (2005) provided new evidence that the rates of pH decline and increases in ionic strength are important variables to be considered when examining the variations in calpain induced proteolysis of meat proteins in postmortem muscle.

2.3.1 Concentration of Calcium Chloride

Calcium chloride had been approved by Food and Drug Administration as Generally Recognized as Safe (GRAS) at maximum levels of 3 per cent (w/w) using 0.8 M solution (FSIS, 1973).

Koohmaraie *et al.* (1988) employed infusion of 0.3M calcium chloride immediately after death in ovine carcasses, to accelerate tenderisation and proteolysis of myofibrillar proteins, so that they were completed at 24 hour postmortem.

Koohmaraie *et al.* (1989) demonstrated that when m-calpain activity was enhanced (as evidenced by the loss of enzyme activity due to autolysis) by infusion of ovine carcasses with 0.3M calcium chloride, no inhibitor activity could be detected. They observed that tenderisation that occurred by infusion of carcasses with calcium chloride was not due to ionic strength.

Fifteen mM calcium in meat obtained by injection and 10mM calcium ions by rehydration tenderised beef. About 4mM calcium ions may produce just detectable tenderisation (Koohmaraie *et al.*, 1989).

Injection or infusion of a solution of calcium chloride (300mM, 10 per cent (w/w) into pre rigor meat enhanced and accelerated postmortem tenderisation and maximum tenderisation was accomplished at 1day postmortem with this process (Koohmaraie *et al.*, 1990).

In a study involving lamb carcasses and Brahman cross beef carcasses to determine the effects of calcium chloride infusion on acceleration of postmortem tenderisation, Koohmaraie *et al.* (1990), used 0.3 M calcium chloride at 10 per cent of live weight within 45 min. of slaughter. Morgan *et al.* (1991b) used 0.3 M calcium chloride solution at 10 per cent of the subprimal weight in beef from mature cows to improve tenderness. Polidori *et al.*, (2001) infused longissimus muscle from cross bred beef cattle with a 0.3 M calcium chloride solution.

Wheeler *et al.* (1993) reported that an injection at 24 h postmortem of 200mM calcium chloride solution at 5 per cent (w/w) reduced the variation in beef tenderness without affecting other beef quality or palatability traits.

Whipple and Koohmaraie (1993) marinated steaks from mature cows (8-11 years of age) at 5 days postmortem in 150mM calcium chloride solution for 24 and 48 hours and obtained shear force values less than 5 kgf which were uncommonly low for mature cows.

Diles *et al.* (1994) injected beef longissimus from cow carcasses (more than 8 years of age) with 0.2M calcium chloride at 10 per cent and compared 7 day vs. 14 day post injection ageing. They reported that 14 day ageing resulted in decreased shear force, increased tenderness and flavor intensity rating and decreased purge relative to 7day post-injection ageing.

Alarcon-Rojo and Dransfield (1995), tested different concentrations of calcium chloride for their tenderising effects and concluded that 30mM calcium chloride is the lowest concentration for maximum tenderising effect mediated by calcium ions and reducing the concentration of added calcium ions below 30mM gave an approximately proportional decrease in the amount of ageing. More over,

30mM calcium chloride produced the largest tenderising effect compared to similar concentrations of potassium chloride or magnesium chloride. The calcium chloride treated meat had a toughness value 40 per cent lower than the control at 1day, and this tenderisation was maintained up to 8 days of storage. The inhibition of ageing by cysteine inhibitors was overcome in the presence of 30mM calcium chloride

Kerth *et al.* (1995) injected beef longissimus from beef carcasses with 0.2M calcium chloride at 5 per cent level. They reported that 14 day compared to 7 day post-injection ageing resulted in decreased shear force, increased purge, no effect on sensory traits, and no difference relative to control for discoloration Hunter a value, or browning during retail display.

Nurahmudi and Sams (1997) demonstrated that injection immediately postmortem with a 0.3 M solution (10 per cent (w/w) combined with vacuum tumbling is necessary for tenderisation of spent fowl meat deboned immediately after picking and that delaying injection until 24 hour postmortem gave no additional tenderisation.

According to Wheeler *et al.* (1997b) the currently recommended procedures for tenderising meat with calcium chloride solution include injecting cuts of meat at 1day or 2 day postmortem at 5 per cent of cut weight with a 0.2 M calcium chloride solution and ageing for an additional 7 days.

Gonzalez *et al.* (2001) used 0.25 calcium chloride solution for marinating *cutaneous trunci* muscles for 2 hours and were aged for 0, 1, 2, 3, 4, 5 and 7 days, and found that calcium chloride marination is effective in tenderising *cutaneous trunci* muscle.

2.3.2 Methods of Application

In order to accomplish infusion of ovine carcasses Koohmaraie *et al.* (1988) exteriorised the carotid artery, after slaughter and electrical stimulation,

into which was pumped 0.3 M calcium chloride solution using a pumping device, after which the carcasses were transferred to a holding cooler at 1-2° C for subsequent ageing.

Morgan *et al.* (1991b) used calcium chloride injection to improve tenderness of beef from mature cows. They injected the subprimals with a 0.3M calcium chloride solution at 10 per cent level, the solution being injected into the subprimals with an inject star automatic pumping device. After injection, the subprimals were vacuum tumbled for 15 min., vacuum packaged and transferred to a holding cooler (0° C-1° C).

Whipple and Koohmaraie (1992b) while assessing the effects of freezing and calcium chloride marination on beef tenderness and calpastatin activity; marinated the beef steaks for a period of 48 h in a 600ml of cold calcium chloride solution at 4°C.

Hoover *et al.* (1995) in their study of restaurant consumer acceptance of beef loin strip steaks tenderised with calcium chloride, used 200mM calcium chloride at 5 per cent (w/w), which they injected using a commercial multi needle Gunther pickle injector. The solution was made with tap water, which was injected into meat at 2° C. After injection, strip loins were allowed to equilibrate for 5 min., which were vacuum- packaged and stored at 2° C until 7 days postmortem.

Polidori *et al.* (2001) infused beef carcasses with 0.3 M calcium chloride solution using a pumping device (five injection sites, the distance between each injection sites was 7.5 cm) in a section of the *longissimus thoracis et lumborum* muscle, 40 cm in length, from first to the sixth lumbar vertebra, which was then transferred to a cold room at 2° C, in order to study the effects of calcium chloride infusion on postmortem proteolysis and tenderisation of beef muscle.

2.3.3. Effects of Calcium Chloride Marination on Quality Parameters of Meat

2.3.3.1 pH

Koohmaraie *et al.* (1986) studied the effect of low calcium requiring calcium activated factors on myofibrils under varying pH and temperature conditions and concluded that calpains can cause proteolysis of myofibrils at pH 5.5, although their activity at this pH is only 25 percentage of that at pH 7.5.

Several researchers reported a decrease in pH of beef up to 1.5 days after slaughter and then a steady increase up to 13 days (Boakye and Mittal, 1993).

Yu and Lee (1986) stated that beef with high ultimate pH values was most tender as a result of neutral proteases degrading the Z-discs and those with low pH values were more tender than beef with intermediate pH values due to acidic proteases degrading the M-lines and heavy myosin chains.

Cena *et al.* (1992) while assessing the proteolytic activity of isolated lamb calpains on myofibrils observed that the two calpains showed a relevant activity in the pH range of 5.5 to 6.5 with over 40 per cent of maximum activity found at pH 7.5. Both isoenzymes at 4° C retained about 25 per cent of their activity at 25°C.

Dransfield (1992) outlined that when pH falls from 7.1 to 6.1, the sarcoplasmic calcium ion concentration increased to about 10^{-4} M due to failure of calcium pump in the sarcoplasmic reticulum or due to proteolytic attack on the sarcoplasmic reticulum, which would activate calpain I. Further more, during the fall in the pH, the level of calpastatin, a specific inhibitor of calpains, decreases to about 70% of its initial value.

Dransfield (1992) concluded that tenderisation starts when a muscle pH of approximately 6.1 is attained, and predicted that beef tenderness at this point was approximately 12.5 kg of shear force.

Boakye and Mittal (1993) observed an increase in pH with ageing in both vacuum packaged and non-vacuum packaged carcasses; which they attributed to changes in charges caused by proteolytic enzymes during ageing. They also obtained positive correlations between pH, packaging and ageing time. The pH on day 16 was the highest.

Dransfield (1993) while modeling postmortem tenderisation predicted that calpain II would become activated at pH 5.8, when the concentrations of calcium ions reach 83μM.

Maddock *et al.* (2005) studied the effect of pH and ionic strength on μ-and m-calpain inhibition by calpastatin observed that μ-calpain had the greatest activity at pH 6.5 while that of m-calpain was greater at pH 7.5 than at pH 6.5. Inhibition of μ-calpain was not affected by pH.

2.3.3.2 Water Holding Capacity (WHC)

Grau and Hamm (1957) assessed the WHC by calculating the area of water diffused from meat on to a filter paper under the influence of a standardised but manually applied pressure. The area of fluid obtained around the meat film was proportional to the amount of free water in the meat.

Kauffman *et al.* (1986) used filter paper to estimate drip loss of porcine musculature, and found a positive relationship between fluid accumulation on filter paper and the actual per cent of drip loss. The relationship was linear and nearly perfectly correlated. WHC expressed by press juice could serve as a potential predictor of drip loss and so a better indicator of cooked meat juiciness (Boakye and Mittal, 1993).

Improved WHC is likely to improve tenderness because cooking losses will be lower and consequently a given cross sectional area of meat sample will contain more water and less structural components. A decrease in cooking loss from 28 per cent to 14 per cent would be expected to give rise to an appreciable

decrease in the cross sectional area of structural components, and thus an increase in tenderness (Purchas, 1990).

The temperature fall in hot boned muscle is generally faster and more uniform than in muscle left on the carcass. Therefore, WHC of hot boned meat would be higher than that of cold boned counterparts (Laack *et al.* 1992).

In a study conducted by Boakye and Mittal (1993), for assessing the changes in pH and WHC of *longissimus dorsi* muscle during beef ageing, it was found that the WHC expressed as press juice increased up to ageing day 13.

The post storage purge losses were 61.5 per cent higher for cuts injected with 150 mM and 62.5 per cent higher for cuts injected with 200 mM calcium chloride than for controls. On ageing the purge losses tend to reduce (Diles *et al.*, 1994).

Landsell *et al.* (1995) reported a higher drip loss from the beef steaks injected with calcium chloride than that of the control.

Many proteins involved in tenderization and drip loss are substrates of the enzyme μ-calpain (Huff-Lonergan *et al.*, 1996).

Ilian *et al,* (2001) opined that differences in μ-calpain, m-calpain ar... calpastatin activity may ultimately influence tenderness and WHC by impacting the rate and extend of proteolysis.

Melody *et al.* (2004) reported that μ-calpain activity, its autolysis and protein degradation are associated with differences in pork tenderness and WHC.

2.3.3.3 Cooking loss

Results from an experiment employing calcium chloride infusion in ovine and bovine carcasses indicated that 0.3M calcium chloride infusion at 10 per cent live weight did not affect cooking loss or cooking rate (Koohmaraie *et al.*, 1990).

Morgan *et al.* (1991b) observed no differences in cooking time and losses between treatment groups, when the steaks from mature cows were injected at 10 per cent level with 0.3 M calcium chloride.

Ziauddin *et al.* (1994) reported higher percentage of cooking loss and thermal shrinkage in *biceps femoris* and *longissimus dorsi* muscles of old buffaloes compared to young animals.

Diles *et al.* (1994) studied the effects of calcium chloride injection time and concentration on the cooking losses of steaks from mature cows and reported that the weights of loin steaks injected with 0.2 M calcium chloride at 24 hour postmortem averaged 106.2 per cent of their initial weight after the first 7 days storage period compared to 97.6 per cent for controls. The percentage losses from cooking did not differ between calcium chloride concentrations or between ageing periods.

Landsell *et al.* (1995) observed that injection of calcium chloride did not affect cooking traits, viz., cooking loss in percentage, internal temperature, cooking time in minutes, cooking rates in g/min, or cooked color score.

Wheeler *et al.* (1997b) studied the effect of postmortem injection time and post injection ageing time on the calcium activated tenderisation process in beef, and observed that the percentage cooking loss was higher for calcium chloride treated steaks regardless of injection time and post injection ageing time than for the control steaks. They attributed this to the added water in calcium chloride treated steaks.

Whether cooking loss will cause an increase or decrease in the tenderness depends on a variety of factors including the temperature to which the meat is raised, the time of heating and the particular muscle, being considered (Lawrie, 1998).

Dikeman *et al.* (2003) observed a very low amount of drip loss, improved dressing percentage from calcium chloride marinated beef steaks, compared to the steaks treated with a solution of saccharides, sodium chloride and phosphates.

2.3.3.4 . Colour

Diles *et al.* (1994) studied the retail display color of loin steaks from mature cows injected at 24 hour with 0.15 M, and 0.2 M. calcium chloride and showed that concentration of calcium chloride had no effect on color on days 1, 2 and 3 of retail display and no differences in colorimeter a or b scores were observed between treatments. They concluded that calcium chloride injection can be used with ageing to improve the palatability of mature cow loin steaks without reducing shelf life, as measured by color traits.

Landsell *et al.* (1995) observed that neither visual color scores during a 5 day display nor the Minolta colorimeter L^*, a^*, b^* readings during a 3 day display of *longissimus lumborum* were affected by injection of calcium chloride in beef steaks.

The influence of storage time on parameters of color stability of beef was studied by Feldhusen *et al.* (1995) and they observed that, in a muscle stored for up to 5 days, the color parameters increased obviously with exposure to oxygen by 3-4 L; a, b units, and meat that has been ripening for longer periods of time showed less intensive oxygenation and L, a, b increased by 1-2 units.

Calcium chloride treatment at 0.2 M concentration did not affect Hunter 'a' values at day 0 or day 1 of retail display. However, after 7 days of display, calcium injected steaks had lower 'a' values than their respective control steaks with the same total ageing time (Wheeler *et al.*, 1997b).

Wulf *et al.* (1997) found out that the correlation of colour measurements were higher than correlations of marbling score with tenderness measurements, b*

value showed the highest correlation with shear force value and taste panel tenderness rating.

Perez *et al.* (1998) conducted a study on the effect of calcium chloride marination on calpain and quality characteristics of meat from chicken, horse, cattle and rabbit. They observed that meat with postmortem calcium chloride treatment improved consistently in colour intensity and redness in all species studied.

2.3.3.5 Myofibril Fragmentation Index (MFI)

Takahashi *et al.* (1967) demonstrated that degree of myofibril fragmentation increased with postmortem storage in chicken pectoral muscle.

Davey and Dickson (1969) showed that turbidity (index of myofibril fragmentation) of myofibril suspension prepared by controlled homogenisation increases with postmortem storage.

Moeller *et al.* (1973) while relating tenderness to various changes occurring in myofibril structure opined that MFI (the extent of fragmentation of myofibrils caused by homogenisation) has been shown to be highly correlated with indices (shear force and sensory panel tenderness) of meat tenderness.

Olson and Parrish (1977) successfully adapted the technique of determining myofibril fragmentation of postmortem muscle and termed this value MFI. Culler *et al.* (1978) recommended MFI as an excellent predictor of broiled beef loin steak tenderness.

Infusion of carcasses with calcium chloride accelerates postmortem proteolysis of myofibrillar proteins as determined by SDS- PAGE, in both control and beta adrenergic agonist fed animals (Koohmaraie and Shackelford, 1991).

When the effects of lamb age, muscle type and 24 hour activity of endogenous proteinases on postmortem proteolysis was assessed by MFI,

Whipple and Koohmaraie (1992a) observed that less postmortem proteolysis occurred in younger and more oxidative muscles. This could be attributed to the greater calpastatin activity.

The *longissimus* shear force values decreased and MFI values increased as postmortem ageing time increased in bull and steer carcasses which were aged for 7 days (Morgan *et al.*, 1993).

Veiseth *et al.* (2001) opined that MFI is a very useful indicator of meat tenderness, particularly for muscles that are not big enough to determine shear force or sensory tenderness. They further stated that the relationship of myofibril fragmentation index to measures of tenderness is unaffected by the freezing status of the experimental muscle.

Gonzalez *et al.* (2001) in their investigation to determine the possibility of using 0.2 M calcium chloride solution in tough muscles to reduce ageing period required to increase tenderness, found that MFI values of calcium chloride treated samples aged 3 days were similar to those obtained for the control samples, but aged 7 days.

Hopkins *et al.* (2000) while studying the effect of freezing on MFI of lamb carcasses aged day 1 and days 3, concluded that the values were lower for frozen samples than for fresh samples at homogenisation speeds of 5000 to 10000 rpm, But they were similar for both fresh and frozen samples at a homogenization speed of 25000 rpm.

Mc Donagh *et al.* (2001) reported a correlation of 0.29, 0.25 and -0.45 for MFI with μ-calpain, m-calpain, caipastatin, respectively.

Veiseth *et al.* (2004) in a study to characterise the changes in the different indicators of postmortem proteolysis, observed that the myofibril fragmentation values increased with storage and detected as early as 12 h postmortem. Further increases in MFI were detected at all sample times from 12 to 360 h postmortem.

2.3.3.6 Warner -Bratzler Shear Force (WBSF)

Koohmaraie *et al.* (1988) reported a reduction in ovine carcasses shear force values from 4.61 to 2.77 kgf at day 6 postmortem, when they were infused through the carotid artery with 0.3 M calcium chloride, while studying the effect of calcium chloride in activating the calcium dependent proteases.

Based on their study in ovine carcasses, Koohmaraie *et al.* (1989) stated that carcasses infused with calcium chloride immediately after slaughter, result in a significant reduction in shear force and postmortem storage beyond 24 h to ensure meat tenderness is no longer necessary.

Koohmaraie *et al.* (1990) could obtain low shear force values at 1 day postmortem in bovine *longissimus* muscle injected with calcium chloride, at 45 min after exsanguination. The shear force values were similar to those of non-injected controls at day 14 postmortem.

Prerigor injection of hot boned cuts with 0.3 M calcium chloride solution (10 per cent by weight) within 30 min of exsanguination improved day 14 WBSF values of steaks from top round, top sirloin, and strip loin by 41 per cent, 40 per cent, and 15.3 per cent, respectively (Morgan *et al.*, 1991b).

The longissimus muscle tenderness at days 1, 4, 7 postmortem as determined by WBSF were improved under 0.3M calcium chloride infusion in both beta adrenergic agonist fed and control carcasses (Koohmaraie and Shackelford, 1991).

Stronger effects on tenderness and Warner-Bratzler shear force were reported by Morgan *et al.* (1991b) using a higher calcium chloride concentration, and by Wheeler *et al.* (1991) in hot boned muscles from *Bos indicus* bulls and late castrate steers.

Whipple and Koohmaraie (1993) conducted a study, where carcasses from beta agonist fed steers and control steers were marinated in 150 mM calcium

chloride solution for 24 hour and 48 hour for five days postmortem, and reported that calcium chloride marination improved tenderness regardless of diet.

Loin steaks from mature cows injected with 200mM calcium chloride at 0.5 hour postmortem and steaks injected with 150mm calcium chloride at 24 hour postmortem found to provide significant improvement in both WBSF and sensory ratings (Diles *et al.*, 1994).

Postmortem injection effects of calcium chloride on beef quality traits when studied by Landsell *et al.* (1995), they found that 86 per cent of control *longissimus lumborum* steaks and 78 per cent of *semimembranosus* control steaks had WBSF values more than 4.5 kgf and the injection of calcium chloride reduced these percentages to 43 and 24, respectively.

Alarcon–Rojo and Dransfield (1995) reported that when the decline of calpain activity during conditioning was compared to the decline in shear force, no difference in the rate of decrease was found and both decreased at a common rate constant of 0.52/day. This implied that both the decline in calpains and toughness are related in the ageing process.

Wheeler *et al.* (1997b) reported that beef *longissimus* steaks injected with 0.2 M calcium chloride on day 2 which were given an additional ageing period of 7 days shared a tendency to have lower shear force than control aged for 21 days.

Data from an investigation on the effect of calcium activated tenderisation on diverse genotypes of cattle indicate that calcium chloride can be used to improve meat tenderness with equal responses shown in cattle containing 0, 50, and 100 percent Brahman inheritance (Pringle *et al.*, 1999).

Polidori *et al.* (2001) in their study on postmortem proteolysis and tenderisation of beef muscle through infusion of calcium chloride noted significant improvement in tenderness assessed by shear force both at two and eight days postmortem.

Beef marinated in calcium chloride solutions had lower WBS values and were much tender than control samples (Aktas *et al.*, 2003).

2.3.3.7 Sensory Attributes

Meat tenderness has been assessed by taste panel sensory analysis or by different instrumental methods. However, for several reasons, sensory analysis still remains the reference method in spite of the difficulties encountered with it (Ouali, 1984).

The National Beef Tenderness Survey conducted by Morgan *et al.* (1991a) concluded that, subprimals from mature cows injected with 0.3 M calcium chloride at 10 per cent level, exhibited higher sensory panel tenderness ratings, and lower amounts of detectable connective tissue. Moreover the flavor of the calcium chloride injected steaks was stronger and increased in intensity during storage compared with that of the control steaks.

Sensory evaluation of the steaks injected with calcium chloride at 0.5 hour and 24 hour post-slaughter revealed no differences in sensory measures attributable to injection time. However, steaks injected at 24 hour were 5 per cent more variable in tenderness and 8 per cent more variable in palatability than steaks injected at 0.5 hour (Diles *et al.*, 1994).

While working on the effect of different concentrations of calcium chloride and different ageing periods on the tenderness of loin steak from mature cows, Diles *et al.* (1994) pointed out that injections of calcium chloride at 200 mM concentrations increased the sensory scores for juiciness, tenderness and overall palatability over control steaks, but only juiciness scores increased with 150 mM injections. Further more, steaks aged for 14 days had significantly higher scores than those aged 7days for all traits except juiciness.

Trained sensory panelists rated the steaks injected with 200 mM calcium chloride at 5 per cent (wt/wt) level, higher for tenderness and juiciness scores. No

difference was observed in flavor intensity or off flavor scores compared with the control. Ease of fragmentation was also rated higher by sensory panelists for steaks injected with calcium chloride; also they could detect less sensory detectable connective tissue in calcium chloride injected steaks compared with the control steaks (Landsell *et al.*, 1995).

A restaurant consumer acceptance study of beef loin strip steaks tenderised with calcium chloride by Hoover *et al.* (1995) revealed that calcium chloride injection is an acceptable means of making beef a more consistently tender product.

Wheeler *et al.* (1997b) studied the effectiveness of calcium chloride treatments at different injection times and post injection ageing time in beef longissimus and observed that cooked color score (more well done) was higher in calcium chloride treated sample. The postmortem injection time and the post injection ageing time did not affect cooked color score.

Gonzalez *et al.* (2001) reported that the consumer panelists preferred calcium chloride treated samples of *cutaneous trunci* muscle aged 3 days to the control ones aged 7 days, and concluded that calcium chloride treatment can be used in *cutaneous trunci* muscle to reduce the ageing time required to increase tenderness.

Ground beef patties from calcium chloride infused carcasses that were freshly cooked and evaluated by the descriptive flavor profile panel were evaluated as having more beef flavor identification, more brown roasted flavor, and less soapy/chemical flavor than the patties from carcasses which were infused a solution of saccharides, sodium chlorides and phosphates. The descriptive attribute sensory panel scored fewer off flavors for the calcium chloride infused beef steaks, compared to those marinated with a solution of saccharides, sodium chloride and control carcasses (Dikeman *et al.*, 2003).

Carr *et al.* (2004) conducted a study on consumer acceptance of calcium chloride marinated top loin steaks and reported that the marinated steaks scored higher in tenderness, juiciness, beef flavour and overall mouth feel than control steaks.

Materials and Methods

MATERIALS AND METHODS

The present study was undertaken to assess the effectiveness of calcium chloride marination in improving the tenderness of buffalo meat. Muscle samples for the study were collected from 14 healthy crossbred Murrah he buffaloes (*Bubalus bubalis*) of four to eight years of age, slaughtered in the Department of Livestock Products Technology, College of Veterinary & Animal Sciences, Mannuthy and local meat stalls in and around Mannuthy.

3.1 COLLECTION OF BUFFALO MEAT SAMPLES AND TREATMENT

Immediately after slaughter and dressing, samples of *longissimus dorsi* (LD) muscle between ninth and twelfth thoracic vertebrae of either side were hot boned from the carcass. The muscle sample was trimmed off visible fat and connective tissue and made into 15 -18 steaks of 2.5 cm thickness. Meat samples hygienically collected from local meat stalls were brought to the laboratory in High Density Polyethylene (HDPE) pouches and processed same as above. The different treatments and further physical and organoleptic studies were initiated within one hour of exsanguination.

3.1.1 Treatments

Sufficient number of LD steaks from each animal was segregated for the following three treatments and further investigations.

1. Muscle samples without ageing and calcium chloride marination (NAM).

2. Muscle samples with ageing only (A).

3. Muscle samples with ageing and calcium chloride marination (AM).

The steaks were aerobically packaged in HDPE pouches (200 μ) and used for the different treatments. These steaks were evaluated for the meat quality

parameters, viz., pH, colour, Water Holding Capacity (WHC), cooking loss, Warner-Bratzler shear force (WBSF), Myofibril Fragmentation Index (MFI) and subjected to sensory evaluation of the eating qualities of meat, viz., juiciness, ease of fragmentation, amount of connective tissue, over all tenderness, flavour intensity and colour.

Two steaks kept at room temperature (26-30° C) were used for the first treatment (NAM) and observations were made at 1h and 6h postmortem.

Eight muscle steaks used for the ageing without calcium chloride marination (A) were kept at 2- 4°C in a chiller for eight days. Observations were conducted at 6h and on days 1, 2, 4, 6 and 8 after exsanguination.

Six muscle steaks assigned for the ageing and calcium chloride marination (AM) and stored at 2 – 4° C were taken out of the chiller after 24 h and weighed. Food grade calcium chloride solution 200mM (5% w/w) was injected into steaks using syringe and needle at multiple sites. The steaks were allowed to equilibrate for another 5 min. They were aerobically packaged in HDPE pouches and aged at 2 - 4° C for 8 days after exsanguination. Various parameters were of the steaks were evaluated before marination at 1h, 6h and on day 1, and after marination on days 2, 4, 6 and 8.

3.2 DETERMINATION OF MEAT QUALITY PARAMETERS

3.2.1. pH

The pH of the muscle samples of the three treatments were recorded at the prescribed intervals using a digital pH meter with combined electrode (μ pH system 362, Systronics, India) following the method of O'Halloran *et al.* (1997). About 50g of sample was kept in a glass beaker. A scalpel incision of 2 inches depth was made in the sample, to insert the combined electrode and temperature probe of the pH meter. Care was taken to avoid trapped air between the electrode

and muscle surface. The mean of the three consecutive readings was taken as the pH of the sample.

3.2.2. Water Holding Capacity (WHC)

The water holding capacity of the samples in each treatment were estimated at 1 h, 6 h and on days 1, 2, 4, 6 and 8 using the filter paper press method of Grau and Hamm (1957). Whatman filter paper qualitative No.1 kept in desiccator for the control of moisture was placed on a Plexiglass plate (10 cm x 10 cm) and 0.3g of meat was placed on its centre. A second Plexiglass plate was put on top and pressed by a weight of 50 kg for 5 minutes (0.5 kg/cm^2). After removing the top plate, the meat film area (M) and total area (T) were marked with a pencil. The filter paper was kept aside for drying in a dessicator. The area of meat film and the total area were measured using the method of Henning (1967) by superpositioning of a grid of clear plastic over the filter paper, and then counting the number of squares of the plastic grid fitting to the zone of interest. The total number of squares counted is multiplied by unit area of a square to the estimate the total area. The water holding capacity was then expressed as the ratio of meat film area (M) to the total area (T).

3.2.3 Cooking Loss

Cooking loss of the longissimus steaks at the prescribed intervals for each treatment was estimated following the method described by Boccard *et al.* (1981).

A meat slice of 80 g was placed in a HDPE pouch. It was then kept in water at 75° C for 50 min. and then placed under running tap water for 40 min., after which the meat was taken from the bag, mopped dry and weighed. The cooking loss was expressed in percentage of the initial sample weight.

3.2.4 Colour

Colour of the fresh meat samples was determined objectively at 1 h, 6 h and on days 1, 2, 4, 6, 8 of storage postmortem with a Hunterlab Miniscan XE Plus spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination. The instrument was set to measure Hunter L, a and b using illuminant 45/0 and 10^0 standard observer with an aperture size of 2.54 cm. It was calibrated using black and white tiles. Definition of the variables; L: 0 = black and 100 = white, a: lower numbers = more green (less red), higher numbers = more red (less green) and b: lower numbers = more blue (less yellow), higher numbers = more yellow (less blue) (Page *et al.*, 2001).

3.2.5 Myofibril Fragmentation Index (MFI)

Fragmentation index values of the longissimus steaks under study at the prescribed intervals in each treatment were determined by the procedure outlined by Davis *et al.* (1980).

Ten grams of 7 mm cubes of longissimus muscle were added to 50 ml of cold Sucrose (0.24M) and potassium chloride (0.02M) solution in a homogenisation cup. After 5 min., each sample was blended for 40s at full speed in a Polytron homogeniser PT 3100 (Kinematica AG, Switzerland). The resulting homogenate was then filtered through the fitter assembly consisting of a pre-weighted Nylon cloth (250 μ pore size) and using a glass funnel and glass stirring rod. The residue and the cloth were blotted twice on an absorbent towel immediately after stirring and then weighed. The fragmentation index was reported as the weight of residue in grams times one hundred. A low fragmentation index is related to increased tenderness.

3.2.6 Warner-Bratzler Shear Force (WBSF)

The Warner-Bratzler Shear force values of the longissimus steaks of all treatments were determined by the method outlined by Wheeler *et al.* (1997a).

The muscle samples after the corresponding period of storage were cooked to an internal temperature of 80° C monitored using a meat thermometer. After cooking, the steaks were chilled over night at 2-5° C before coring. On the following day, 6-8 cylindrical cores of 1.27 cm diameter were removed, parallel to the longitudinal orientation of muscle fibres using a hand held coring device. The cores were kept at 2 – 5° C until they were sheared. The cores were then sheared once at the centre perpendicular to the muscle fibres on a Universal Testing Machine - Shimadzu Texture Analyzer Model EZ Test (Shimadzu Corporation, Kyoto, Japan) with Warner-Bratzler Shear attachment having a crosshead speed of 200mm/min. WBSF was expressed in kgf.

3.2.7 Sensory Evaluation

The sensory panel evaluation of the eating qualities of meat was conducted by a semi trained panel consisting of eight panelists. The steaks for sensory evaluation were cooked to an internal temperature of 80° C, maintained for up to 20 min, then it was trimmed into uniform pieces (1.3 x 1.3 x 1.9 cm) of lean and were served warm (approx. 50° C) to the panelists in labeled plates. Panelists were asked to evaluate the meat pieces and record a score for the samples on an 8-point Hedonic scale, for juiciness (8 = extremely juicy, 1 = extremely dry), ease of fragmentation (8 = extremely easy, 1 = extremely difficult), amount of connective tissue or residue remaining after chewing (8 = None, 1 = abundant), overall tenderness (8 = extremely tender, 1 = extremely tough), flavour intensity (8 = extremely intense, 1 = extremely bland) and color. Flavour description was included to ascertain any off flavour problems that may have been associated with calcium chloride marination. Colour was included to reflect the effect of calcium chloride marination and ageing on cooked colour.

3.3 STATISTICAL ANALYSIS

The data recorded were statistically analysed in order to compare the effect of $CaCl_2$ marination and ageing in different treatment groups during the

SENSORY FORM FOR DESCRIPTIVE ATTRIBUTE PANEL

Panelist ..... Date & Time ..... Expt..... Session No..... (Please examine the samples in the order listed below)

Attribute	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7
Juiciness							
Ease of Fragmentation							
Amount of Connective Tissue							
Overall Tenderness							
Flavour Intensity							
Colour							
Comments							

CODES

Juciness

8. Extremely juicy
7. Very juicy
6. Moderately juicy
5. Slightly juicy
4. Slightly dry
3. Moderately dry
2. Very dry
1. Extremely dry

Ease of Fragmentation

8. Extremely easy
7. Very easy
6. Moderately easy
5. Slightly easy
4. Slightly difficult
3. Moderately difficult
2. Very difficult
1. Extremely difficult

Amount of Connective Tissue

8. None
7. Practically none
6. Traces
5. Slight
4. Moderate
3. Slightly abundant
2. Moderately abundant
1. Abundant

Overall Tenderness

8. Extremely tender
7. Very tender
6. Moderately tender
5. Slightly tender
4. Slightly tough
3. Moderately tough
2. Very tough
1. Extremely tough

Flavour Intensity

8. Extremely intense
7. Very intense
6. Moderately intense
5. Slightly intense
4. Slightly bland
3. Moderately bland
2. Very bland
1. Extremely bland

Signature of the Panelist

period of postmortem storage by Paired *t*-test. Correlation coefficients for MFI and WBSF were calculated using Pearson's correlation coefficient option and their correlation with overall tenderness using Spearman correlation coefficient method (Snedecor and Cochran, 1994).

Results

RESULTS

The present investigation on tenderisation of buffalo meat by calcium chloride marination is envisaged to assess the effect of marination on the various quality parameters of meat especially tenderness and to compare it with those of natural ageing. The muscle samples were subjected to three treatments: 1) samples neither aged nor $CaCl_2$ marinated and kept at room temperature for 6 h (NAM), 2) samples aged only (A) and 3) aged and marinated (AM). Samples of A and AM were stored at 2-4^0 C for 8 days postmortem. The results of the study on the various meat quality parameters, viz., pH, WHC, cooking loss, colour, MFI, WBSF and the sensory quality attributes are subjected to statistical analysis and are given below.

4.1 pH

The effect of $CaCl_2$ marination and ageing on the pH of buffalo longissimus steaks at different postmortem times are shown in Table 1 and the trend of pH is presented in Figure 1.

The mean and SE for pH recorded for the buffalo steaks at 1 h postmortem was 6.7 ± 0.001. The mean pH value at 6 h in treatments NAM and A remained unaltered. The same for the nonmarinated samples kept for ageing at 2^0 C on days 1, 2, 4, 6 and 8 were 5.6 ± 0.01, 5.7 ± 0.001, 6.0 ± 0.001, 6.1 ± 0.001 and 6.1 ± 0.001, respectively. The pH on all days were significantly lower ($P < 0.05$) than at 1 h and 6 h. The ultimate pH of 5.6 was reached on day 1 and thereafter the pH gradually increased till the day 6 and then remained static. But pH on days 1 and 2 only were significantly lower than on the subsequent days ($P < 0.05$).

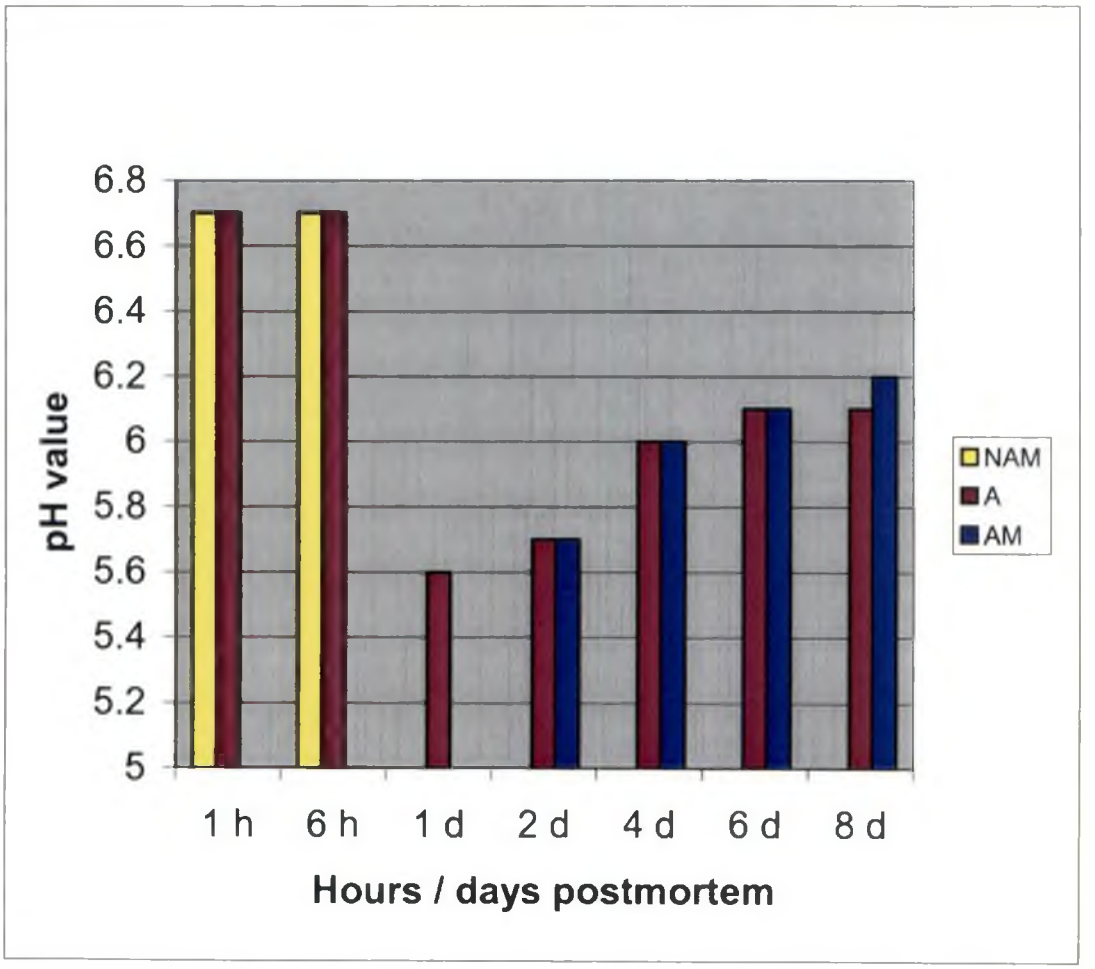
The mean pH value of the marinated samples on days 2, 4, 6 and 8 were 5.7 ± 0.001, 6.0 ± 0.001, 6.1 ± 0.001 and 6.2 ± 0.001, respectively. At the time of marination on day 1 the ultimate pH (UpH) was 5.6 ± 0.001. From day 2 to day 8

Table 1. Effect of $CaCl_2$ marination and ageing on the pH of buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	6.7±0.01[a]	6.7±0.01[c]	6.7±0.01[d]
6 h	6.7±0.01[a]	6.7±0.01[c]	6.7±0.01[d]
1 d	*	5.6±0.01[a]	5.6±0.01[a]
2d	*	5.7±0.01[a]	5.7±0.01[a]
4 d	*	6.01±0.01	6.0±0.01[b]
6 d	*	6.1±0.01[b]	6.1±0.01[b]
8 d	*	6.1±0.01[b]	6.2±0.01[c]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values, which do not differ significantly at 5% level

Fig. 1. Effect of $CaCl_2$ marination and ageing on the pH of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

there was significant increase in pH ($P < 0.05$). The pH remained above 6.0 from day 4 onwards.

The variations in the pH between treatments A and AM revealed no significant difference which indicated that $CaCl_2$ marination has no significant effect on the alterations in pH.

4.2 WATER HOLDING CAPACITY (WHC)

The effect of $CaCl_2$ marination and ageing on the WHC of buffalo longissimus steaks at different postmortem times are shown in Table 2 and the changes are presented in Figure 2.

The mean and SE of WHC for the buffalo *longissimus* steak was 0.37 ± 0.02 at 1 h postmortem. The mean WHC for the treatment NAM at 6 h was 0.35 ± 0.02 and that of A was 0.34 ±0.02 which were not significantly different.

The WHC of the aged but non-marinated samples on days 1, 2, 4, 6 and 8 were 0.31 ± 0.02 , 0.27 ± 0.02 , 0.27 ± 0.48, 0.26 ± 0.01 and 0.27 ± 0.01 , respectively which did not differ significantly. But these values were significantly lower than at 1 h and 6 h ($P < 0.05$), which indicated reduced WHC.

The mean WHC of the steaks at the time of marination on day 1 was 0.31 ± 0.02 which was followed by 0.29 ± 0.02, 0.27 ± 0.02, 0.28 ± 0.02 and 0.25 ± 0.01on days 2, 4, 6 and 8, respectively. These values were also not significantly different.

Paired *t*-test values revealed that WHC of buffalo meat samples did not differ significantly between treatments A and AM.

4.3 COOKING LOSS

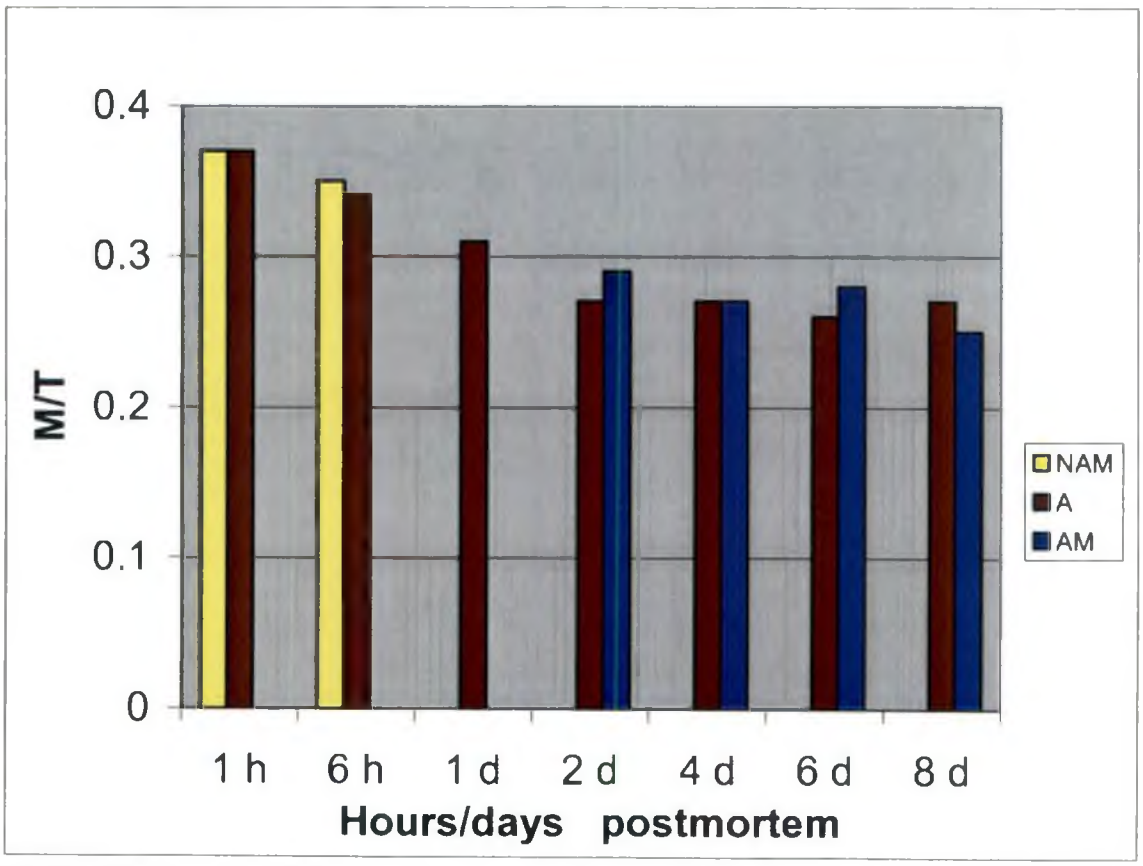
The effect of $CaCl_2$ marination and ageing on the cooking loss (%) of buffalo longissimus steaks at different postmortem times are shown in Table 3 and Figure 3.

Table 2. Effect of $CaCl_2$ marination and ageing on the WHC (M/T) of buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	0.37± .021[a]	0.37± 0.021[c]	0.37± 0.021[b]
6 h	0.35±0.021[a]	0.34 ±0.022[c]	0.34 ±0.022[b]
1 d	*	0.31 ±0.024[b]	0.31 ±0.024[a]
2d	*	0.27± 0.021[ab]	0.29± 0.021[a]
4 d	*	0.27 ±0.048[ab]	0.27± 0.022[a]
6 d	*	0.26± 0.015[a]	0.28 ±0.021[a]
8 d	*	0.27± 0.016[ab]	0.25 ±0.017[a]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level
M/T = Meat film area / total area

Fig. 2. Effect of $CaCl_2$ marination and ageing on the WHC of buffalo steaks at different postmortem times



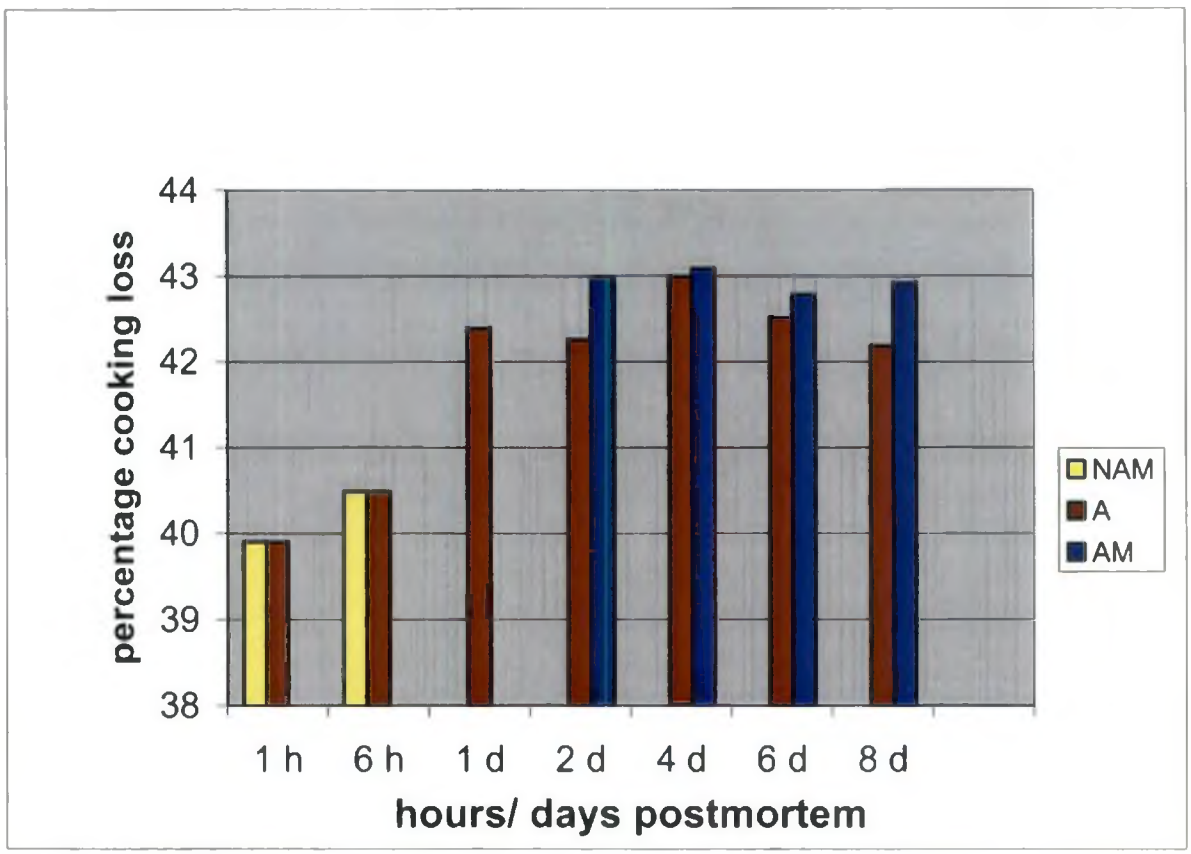
NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

Table 3. Effect of $CaCl_2$ marination and ageing on the cooking loss (%) of buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	39.90 ± 0.35[a]	39.90± 0.35 [a]	39.90± 0.35 [a]
6 h	40.48± 0.33[a]	40.48±0.39 [b]	40.48±0.39 [a]
1 d	*	42.38± 0.31 [cd]	42.38± 0.31 [b]
2d	*	42.25± 0.42 [cd]	42.97±0.38 [b]
4 d	*	42.99± 0.35 [c]	43.08±0.33 [b]
6 d	*	42.51±0.36 [de]	42.78±0.43 [b]
8 d	*	42.18±0.34 [c]	42.91±0.39 [b]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 3. Effect of $CaCl_2$ marination and ageing on the cooking loss of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

The buffalo *longissimus* steaks recorded a cooking loss of 39.90 ± 0.35 at 1 h. At 6 h the cooking loss of the nonmarinated samples kept at room temperature and those aged at 2 - 4^0 C were 40.48 ± 0.33. The mean values of aged samples were 42.38 ± 0.31, 42.25 ± 0.42, 42.99 ± 0.35, 42.51 ± 0.36 and 42.18 ± 0.34 on days 1, 2, 4, 6, and 8, respectively. From day 1 to day 8 cooking loss remained more or less static with the highest loss `on day 4 which differed significantly ($P < 0.05$) from all the other days except day 6.

Trend in cooking loss in treatment AM was the same as that of aged samples with mean values of 42.97 ± 0.38, 43.08 ± 0.33, 42.78 ± 0.43 and 42.91 ± 0.39 on days 2, 4, 6 and 8, respectively. The cooking loss on day 1 was 42.38 ± 0.31 at the time of marination which increased significantly ($P <0.05$) on day 4 only.

Cooking loss in the marinated steaks on day 2 and day 8 only were significantly higher ($P < 0.05$) compared to the aged steaks.

4.4 COLOUR

The effect of $CaCl_2$ marination and ageing on the colour of buffalo longissimus steaks at different postmortem times are presented in Table 4 and Figure 4.

4.4.1. Hunter 'L' values

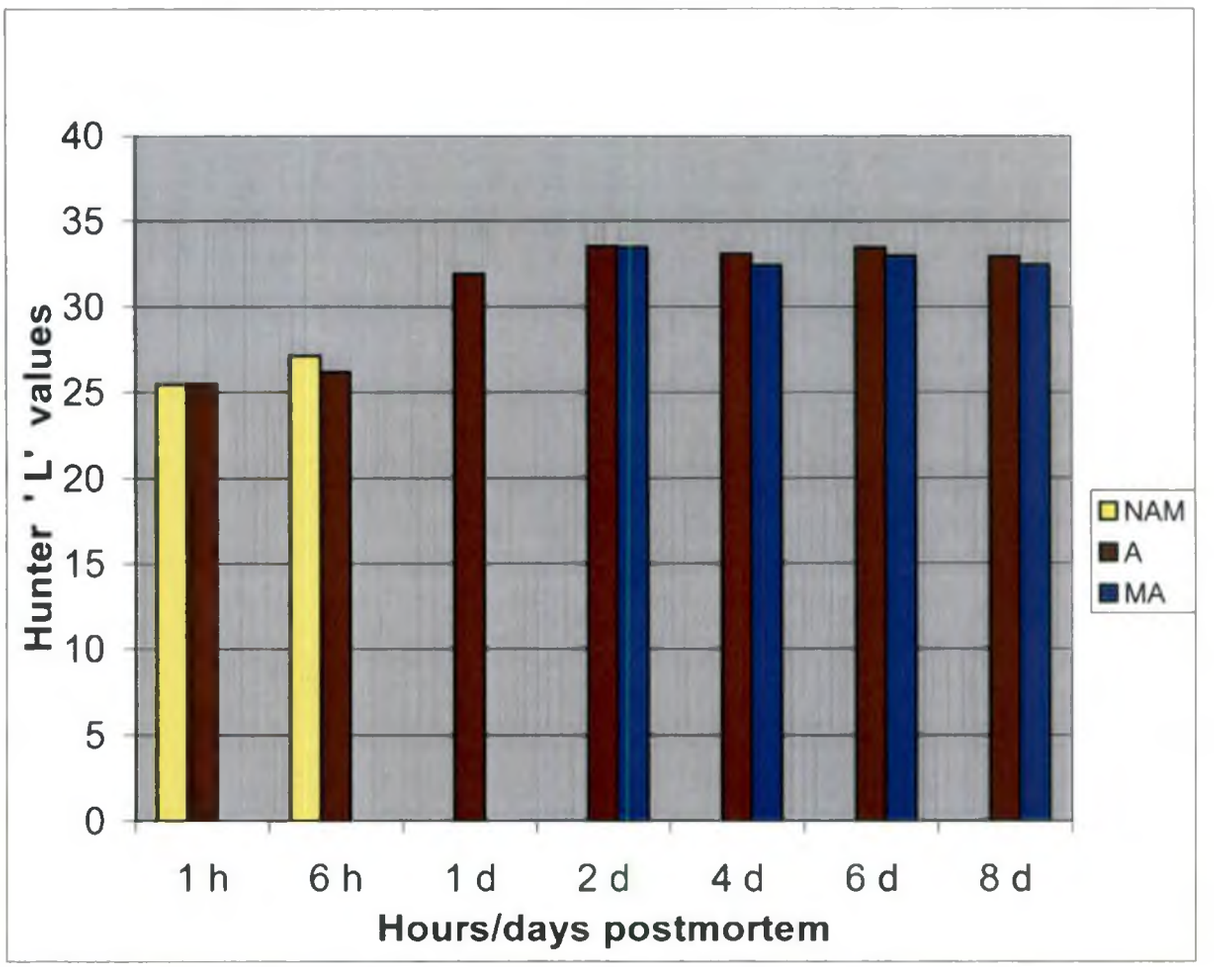
Hunter L values of buffalo *longissimus* steak was 25.42 ± 0.74 at 1 h of postmortem in all treatments. The L values in treatments NAM and A at 6 h were 27.11 ± 0.8 and 26.18 ± 0.84, respectively. The L value in the former was significantly higher ($P < 0.05$) than in the latter. Lightness of aged *longissimus* steaks increased significantly ($P < 0.05$) on day 1 with an L value of 31.90 ± 0.62. The mean values on days 2, 4, 6, and 8 were 33.50 ± 0.53, 33.07 ± 0.28, 33.46 ± 0.20 and 32.90 ± 0.54, respectively. The slight increase in values was not significant. The values recorded for the marinated steaks were 33.46 ± 0.44,

Table 4. Effect of $CaCl_2$ marination and ageing on the colour (Hunter L a b) of buffalo steaks at different postmortem times

Hours/days postmortem	NAM			A			AM		
	L	a	b	L	a	b	L	a	b
1 h	25.42±0.74^{a}	13.02±0.58^{a}	6.94±0.58^{a}	25.42±0.74^{a}	13.02±0.58^{ab}	6.94±0.58^{a}	25.42±0.74^{a}	13.02±0.58^{a}	6.94±0.58^{a}
6 h	27.11±0.8^{a}	13.07±0.55^{a}	7.12±0.55^{a}	26.18±0.84^{a}	13.13±0.57^{ab}	7.12±0.57^{ab}	26.18±0.84^{a}	13.13±0.57^{a}	7.12±0.57^{a}
1 d	*	*	*	31.90±0.62^{b}	18.56±0.49^{c}	9.17±0.19^{d}	31.90±0.62^{b}	18.56±0.49^{d}	9.17±0.19^{a}
2 d	*	*	*	33.50±0.53^{c}	15.5±0.49^{cd}	9.08±0.49^{d}	31.90±0.62^{b}	15.98±0.58^{c}	8.47±0.58^{a}
4 d	*	*	*	33.07±0.28^{bc}	16.04±0.40^{d}	8.33±0.40^{c}	32.41±0.44^{b}	16.46±0.46^{c}	8.41±0.46^{a}
6 d	*	*	*	33.46±0.20^{c}	14.98±0.47^{bc}	8.35±0.47^{c}	32.94±0.30^{b}	15.77±0.44^{c}	8.27±0.44^{a}
8 d	*	*	*	32.90±0.54^{bc}	12.76±0.57^{a}	7.90±0.57^{b}	32.43±0.57^{b}	13.75±0.55^{b}	7.56±0.55^{a}

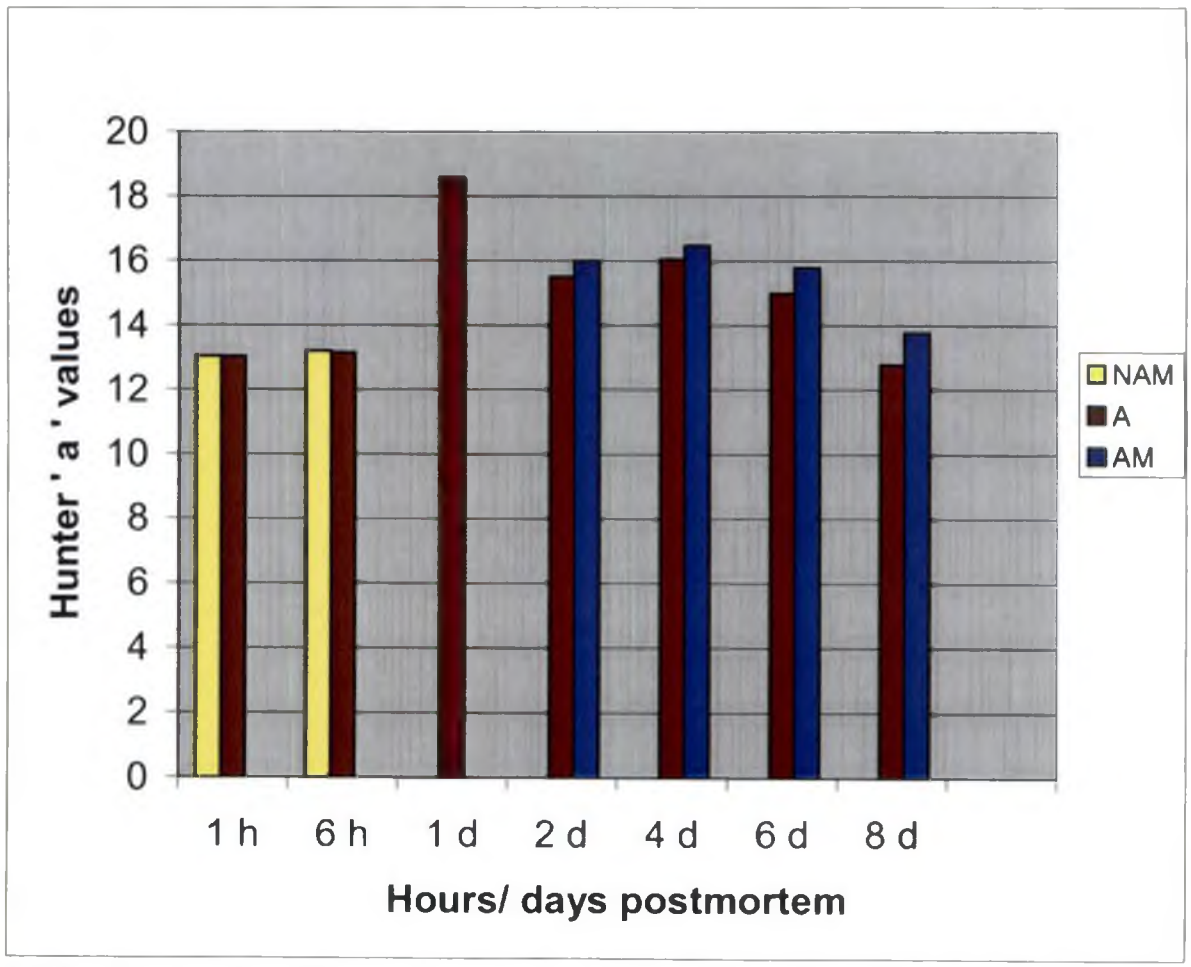
NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 4.1. Effect of $CaCl_2$ marination and ageing on the Hunter 'L' value of buffalo steaks at different postmortem times



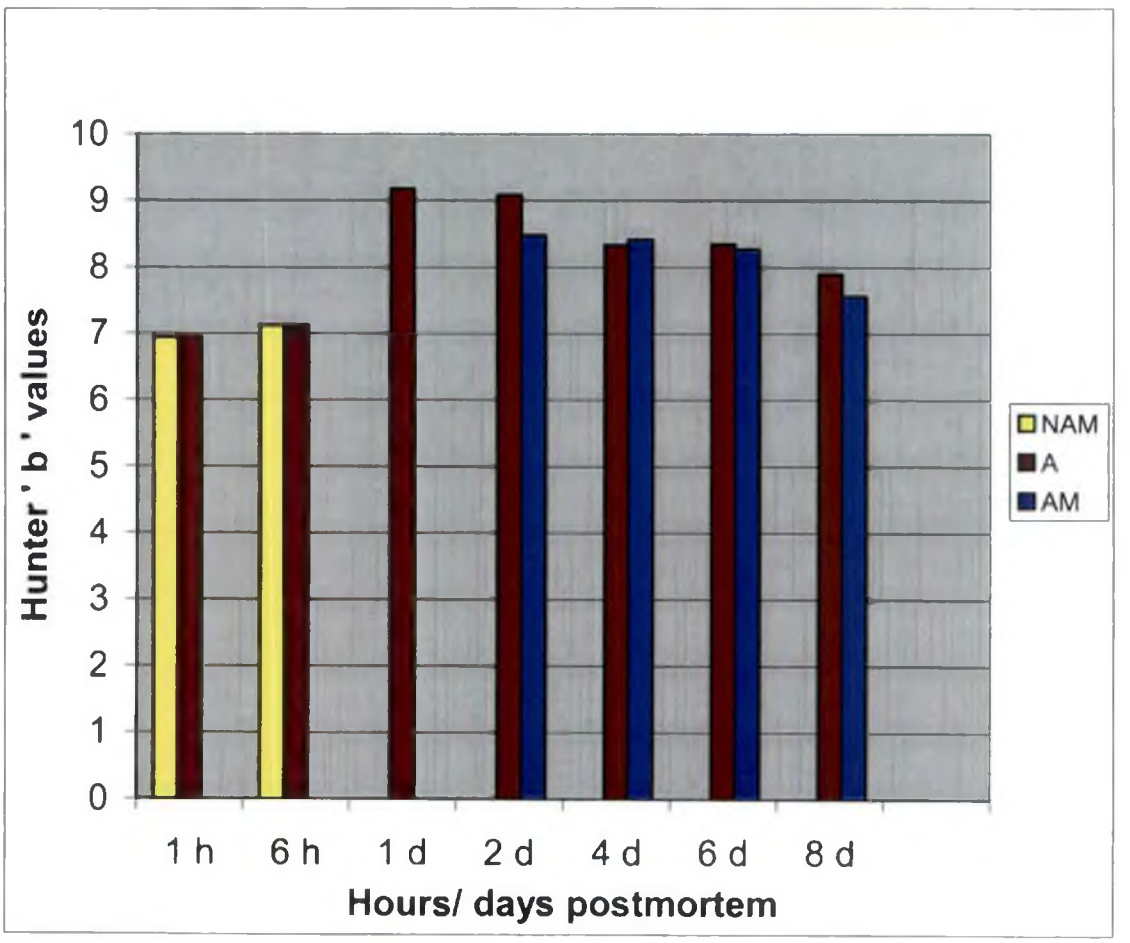
NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

Fig. 4.2. Effect of $CaCl_2$ marination and ageing on the Hunter 'a' value of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

Fig. 4.3. Effect of $CaCl_2$ marination and ageing on the Hunter 'b' value of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

32.41 ± 0.44, 32.94 ± 0.30 and 32.43 ± 0.57 on days 2, 4, 6 and 8, respectively which also did not differ significantly (P > 0.05).

The mean L value on the day of marination was 31.9 ± 0.62 which did not differ significantly from the values on subsequent days. The L values of *longissimus* on days 2, 4, 6 and 8 for the treatments A and AM did not differ significantly which indicated that Hunter 'L' values were not affected by calcium chloride marination. Irrespective of calcium chloride marination of the buffalo *longissimus* steaks lightness increased from day 1 to day 8 on ageing compared to 6 h.

4.4.2 Hunter 'a' values

The mean and SE for Hunter 'a' values at 1 h was 13.02 ± 0.58. The values for the treatments NAM and A at 6h were 13.07 ± 0.55 and 13.13 ± 0.57, respectively. The differences were not significant (P >0.05).

The values for the aged samples on days 1, 2, 4, 6 and 8 were 18.56 ± 0.49, 15.50 ± 0.49, 16.04 ± 0.40, 14.98 ± 0.47 and 12.76 ± 0.57, respectively. This indicated that 'a' values of the steaks were significantly (P < 0.05) higher on day 1 to day 8 compared to 1 h and 6 h but were significantly lesser than (P <0.05) on day 1 with the maximum redness.

In the treatment AM, the steaks had an 'a' value of 18.56 ± 0.49 on day 1 before marination. This got reduced on days 2, 4, 6, and 8 to 15.98 ± 0.58, 16.46 ± 0.46, 15.77 ± 0.44 and 13.75 ± 0.55, respectively. This followed the same trend as in the aged samples but the variations in values were not significant within the treatment. Although not significant there was a slight decrease in redness in second and third treatment from day 2 to day 8 compared to day 1.

Paired *t*-test values indicated that 'a' values between the marinated and aged samples did not differ significantly.

4.4.3 Hunter 'b' values

The Hunter 'b' values recorded at 1 h for the buffalo LD steaks was 6.94 ± 0.58. The values for the treatment NAM and A were 7.12 ± 0.55 at 6 h in aged steaks. The 'b' values recorded for the aged steaks from day 1 to day 8 were 9.17 ± 0.19, 9.08 ± 0.49, 8.33 ± 0.40, 8.35 ± 0.47 and 7.90 ± 0.57, respectively. The values were significantly higher ($P < 0.05$), compared to 6 h. Significantly highest ($P < 0.05$) values were obtained on days 1 and 2.

The 'b' values of the marinated steaks on days 2, 4, 6 and 8 were 8.47 ± 0.58, 8.41 ± 0.46, 8.27 ± 0.44 and 7.56 ± 0.55, respectively which did not differ significantly. But the values in both treatments A and AM were significantly higher than at 1 h and 6 h.

The Paired *t*-test values revealed no significant difference between the treatments A and AM.

4.5 MYOFIBRIL FRAGMENTATION INDEX (MFI)

The effect of $CaCl_2$ marination and ageing on the MFI values of buffalo longissimus steaks at different postmortem times are presented in Table 5 and the decreasing trend of the same is shown in Figure 5.

The MFI value obtained at 1 h was 878.78 ± 48.49. The nonmarinated and aged samples recorded mean values of 872.57 ± 49.93 and 874.71 ± 43.08, respectively at 6 h.

In the aged steaks, MFI values then followed a gradual significant decrease ($P < 0.01$) from day 1 to day 8 with mean values of 768.0 ± 40.52, 714.21 ± 44.54, 679.42 ± 34.62, 613.42 ± 36.6 and 541.78 ± 34.63 on days 1, 2, 4, 6 and 8, respectively.

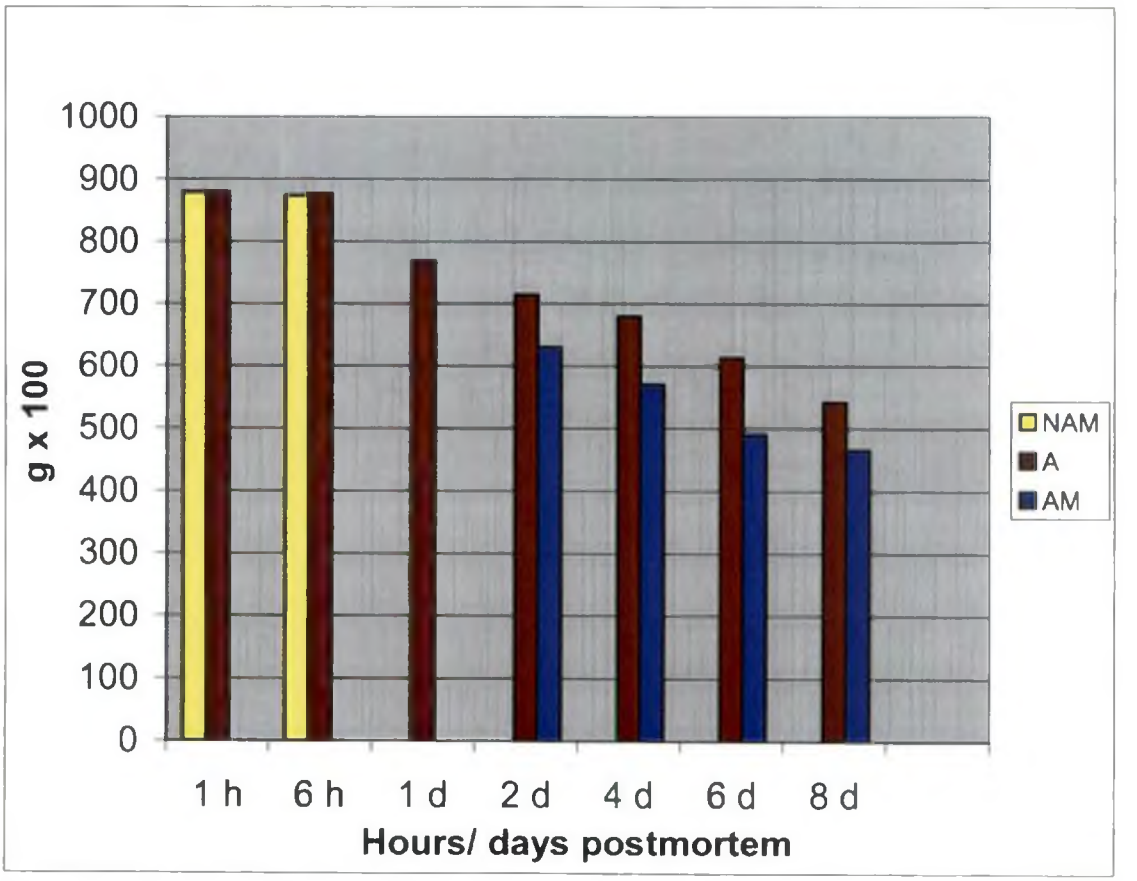
A similar trend was noticed in marinated steaks as well, with MFI values 630 ± 42.88, 570.92 ± 37.5, and 489.64 ± 30.38 and 465.28 ± 29.79 on days 2, 4, 6, and 8.

Table 5. Effect of $CaCl_2$ marination and ageing on the MFI (gx100) of buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	878.78 ±48.49[a]	878.78 ±48.49[e]	878.78 ±48.49[f]
6 h	872.57 ± 49.93[a]	874.71 ±43.08[e]	874.71 ±43.08[f]
1 d	*	768 ±40.52[d]	768 ±40.52[e]
2d	*	714.21 ±44.54[c]	630.21 ±42.88[d]
4 d	*	679.42 ±34.62[c]	570.92 ±37.5[c]
6 d	*	613.42 ±36.6[b]	489.64 ±30.38[b]
8 d	*	541.78 ±34.63[a]	465.28 ±29.79[a]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made MFI = residue in grams x 100
Same superscripts denote values which do not differ significantly at 1% level

Fig. 5. Effect of $CaCl_2$ marination and ageing on the MFI of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

MFI values for treatment AM were significantly lower ($P < 0.01$) than treatment A from day 2 to day 8 which indicated that $CaCl_2$ marination enhanced tenderisation of buffalo steaks more than in ageing..

4.6 WARNER-BRATZLER SHEAR FORCE (WBSF)

The effect of $CaCl_2$ marination and ageing on the WBSF values of cooked buffalo longissimus steaks at different postmortem times are given in Table 6 and Figure 6.

The mean and SE of WBSF (kgf) values of buffalo steaks for all treatments at 1 h was 10.48 ± 0.48. The steaks kept at room temperature recorded a significantly lower ($P < 0.01$). SF of 10.08 ± 0.46 at 6h, compared to the steaks kept for ageing at 2° C which recorded a mean value of 10.61 ± 0.50 at 6 h.

Significantly highest ($P < 0.01$) SF value of 11.37 ± 0.61 was observed on day 1 for the samples aged at 2-4° C compared to all other samples in the three treatments. Thereafter, a gradual significant ($P < 0.01$) fall in SF values were observed in aged samples with mean values of 9.81 ± 0.53, 8.59 ± 0.48, 7.5 ± 0.63 and 6.75 ± 0.56 on days 2, 4, 6 and 8, respectively.

A similar trend of significant decline ($P < 0.01$) in WBSF was noticed from day 2 to day 8 in marinated samples as well. The mean values on days 2, 4, 6 and 8 were 8.25 ± 0.44, 7.01 ± 0.42, 5.6 ± 0.51 and 4.88 ± 0.45, respectively. The lowest SF recorded was on day 8.

On Paired *t*-test of the SF values between treatments A and AM indicated significantly lower ($P < 0.01$) values for the latter from day 2 to day 8.

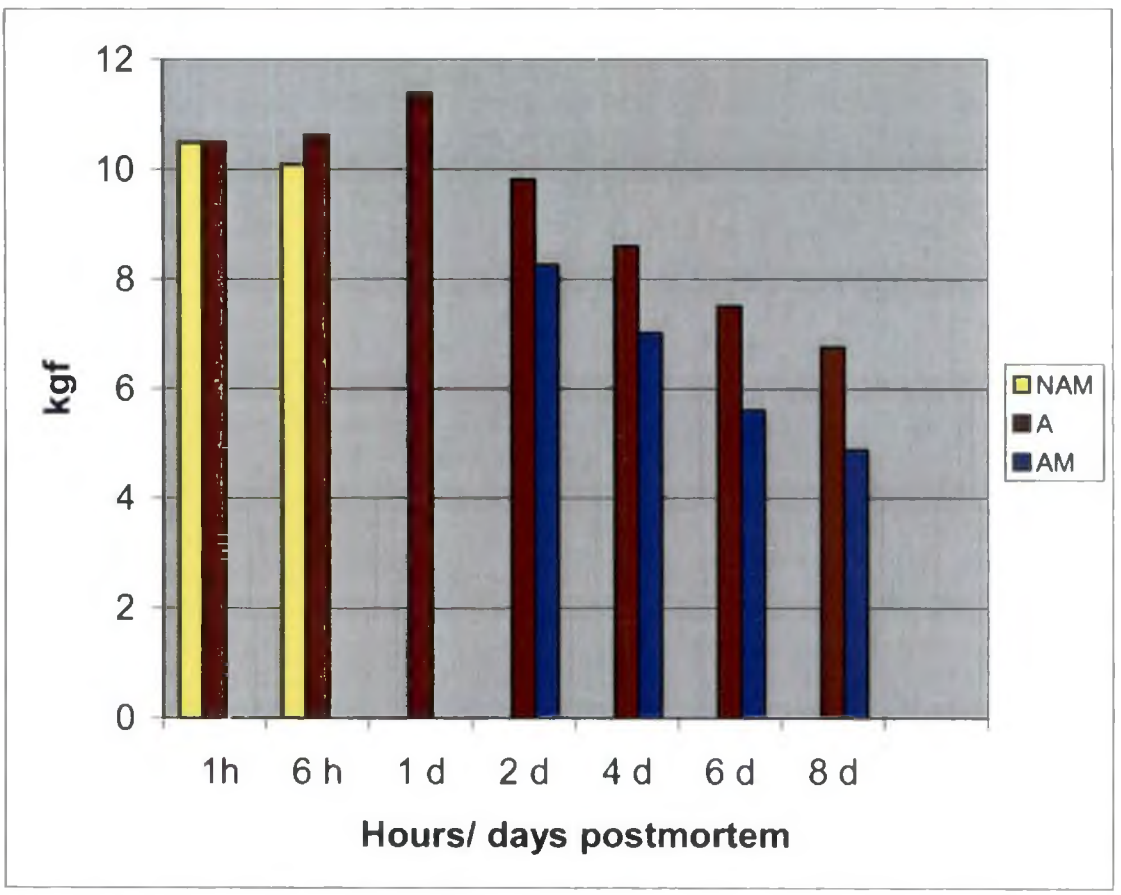
WBSF values of $CaCl_2$ marinated buffalo *longissimus* steaks reduced from 10.48 ± 0.48 at 1h postmortem to 4.88 ± 0.45 in a course of 8 days, which indicated a 53.44% reduction in the values. While in aged meat, SF reduced from 10.48 ± 0.48 to 6.75 ± 0.56 only (35.59%). This indicated that calcium chloride can enhance tenderness of buffalo *longissimus* steaks in combination with ageing.

Table 6. Effect of $CaCl_2$ marination and ageing on the WBSF (kgf) of buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	10.48 ±0.48 [a]	10.48±0.48 [dc]	10.48±0.48 [c]
6 h	10.08±0.46 [a]	10.61±0.50 [e]	10.61±0.50 [e]
1 d	*	11.37±0.61 [f]	11.37±0.61 [f]
2d	*	9.81±0.53 [d]	8.25±0.44 [d]
4 d	*	8.59±0.48 [c]	7.01±0.42 [c]
6 d	*	7.5±0.63 [b]	5.6±0.51 [b]
8 d	*	6.75±0.56 [a]	4.88±0.45 [a]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 1% level

Fig. 6. Effect of $CaCl_2$ marination and ageing on the WBSF of buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

4.7 SENSORY EVALUATION

4.7.1 Juiciness

The effect of $CaCl_2$ marination and ageing on the juiciness scores of buffalo longissimus steaks at different postmortem times are presented in Table 7.1 and Figure 7.1.

The mean taste panel score for juiciness at 1 h was 6.03 ± 0.02. The treatments NAM and A scored mean values of 6.01 ± 0.01 at 6 h.

From d 1 to d 8, the *longissimus* steaks kept at 2^0 C for ageing improved in scores with the mean values of 6.20 ± 0.001, 6.35 ± 0.01, 6.61 ± 0.01, 6.57 ± 0.02 and 6.76 ± 0.02 on days 1, 2, 4, 6 and 8, respectively. The increase in scores was significant ($P < 0.05$) from day 4 to day 8 compared to day 1.

The mean juiciness scores for marinated samples were 6.67 ± 0.13, 6.72 ± 0.08, 6.84 ± 0.17 and 7.11 ± 0.14, respectively on days 2, 4, 6 and 8. The variations were not significant. The juiciness scores on all days from day 2 were significantly higher ($P < 0.05$) compared to the mean score on day 1.

On comparing the scores for treatments A and AM, significantly higher scores ($P < 0.05$) were noticed at all times except on day 4.

4.7.2 Ease of Fragmentation

The effect of $CaCl_2$ marination and ageing on the scores for ease of fragmentation of buffalo longissimus steaks at different postmortem times are presented in Table 7.2 and Figure 7.2.

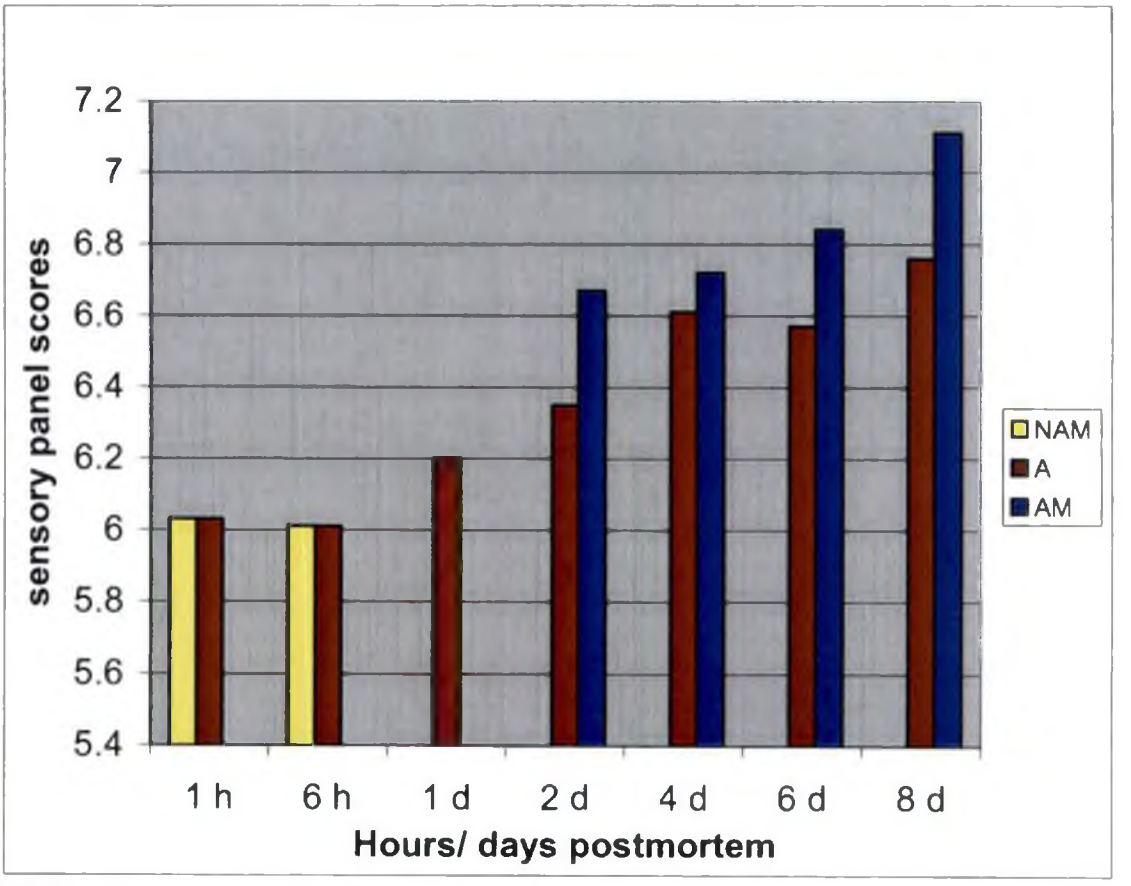
The buffalo *longissimus* steaks scored a mean value of 5.47 ± 0.02 for ease of fragmentation at 1 h. At 6 h, the mean values for the non-marinated as well as the aged samples were 5.79 ± 0.02.

Table 7.1 Effect of $CaCl_2$ marination and ageing on the juiciness of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	6.032 ± 0.11 [a]	6.03±0.10 [a]	6.03±0.10 [a]
6 h	6.01±0.11 [a]	6.01±0.10 [a]	6.01±0.10 [a]
1 d	*	6.2±0.19 [a]	6.2±0.19 [a]
2d	*	6.35±0.15 [a]	6.67±0.13 [b]
4 d	*	6.61±0.12 [b]	6.72±0.08 [b]
6 d	*	6.57±0.17 [b]	6.84±0.17 [b]
8 d	*	6.76±0.16 [b]	7.11±0.14 [c]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 7.1. Effect of $CaCl_2$ marination and ageing on the juiciness of cooked buffalo steaks at different postmortem times



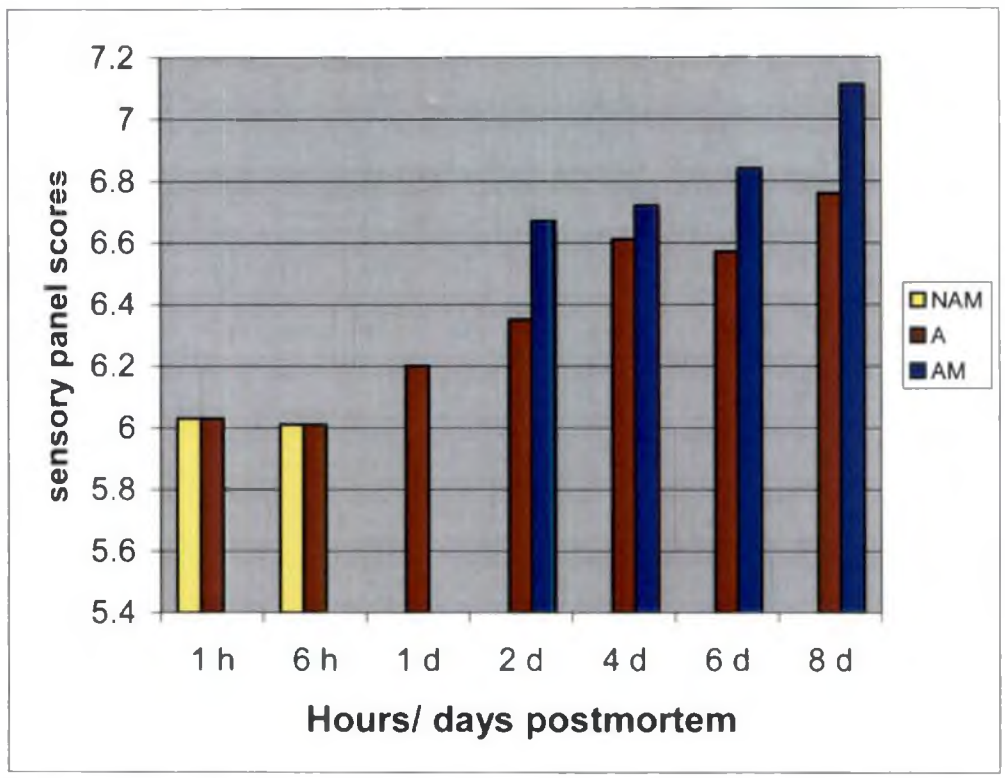
NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

Table 7.2 Effect of $CaCl_2$ marination and ageing on the ease of fragmentation of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	5.47 ±0.28 [a]	5.47±0.19 [a]	5.47±0.19 [a]
6 h	5.79±0.28 [a]	5.79±0.19 [ab]	5.79±0.19 [a]
1 d	*	6.14±0.22 [abc]	6.14±0.22 [a]
2d	*	6.16±0.18 [bc]	6.63±0.17 [b]
4 d	*	6.4±0.15 [c]	6.9±0.12 [b]
6 d	*	6.42±0.19 [c]	6.97±0.15 [c]
8 d	*	6.56±0.18 [c]	7.19±0.16 [c]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 7.2. Effect of $CaCl_2$ marination and ageing on the ease of fragmentation of cooked buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

For the aged samples, the mean taste panel scores were 6.14 ± 0.22, 6.16 ± 0.18, 6.4 ± 0.15, 6.42 ± 0.19 and 6.56 ± 0.18 on days 1, 2, 4, 6, and 8, respectively. This indicated an increase in the scores in treatment A, which was not significant.

The mean scores for the marinated samples were 6.63 ± 0.17, 6.9 ± 0.12, 6.97 ± 0.15 and 7.19 ± 0.16 on days 2, 4, 6 and 8, respectively. The scores increased significantly ($P < 0.05$) from day 2 to day 8 compared to day 1.

The Paired *t*-test showed significantly higher scores ($P < 0.05$) for the ease of fragmentation on all days from day 2 to day 8 in the treatment AM compared to A.

4.7.3 Amount of Connective Tissue

The effect of $CaCl_2$ marination and ageing on the scores for the amount of connective tissue of buffalo longissimus steaks at different postmortem times are presented in Table 7.3 and Figure 7.3.

The mean and SE of the scores for the attribute amount of connective tissue at 1 h was 5.58 ± 0.28 for the treatments of buffalo *longissimus* streaks. At 6 h, treatments NAM and A showed mean values of 6.02 ± 0.19 and 6.02 ± 0.24, respectively.

The mean scores on days 1, 2, 4, 6 and 8 for the samples kept for ageing were 6.08 ± 0.24, 5.85 ± 0.27, 6.37 ± 0.16, 6.37 ± 0.26 and 6.65 ± 0.18, respectively. This showed a decline in the score on day 2 and then remained almost static on days 4 and 6. However, these variations were not significant.

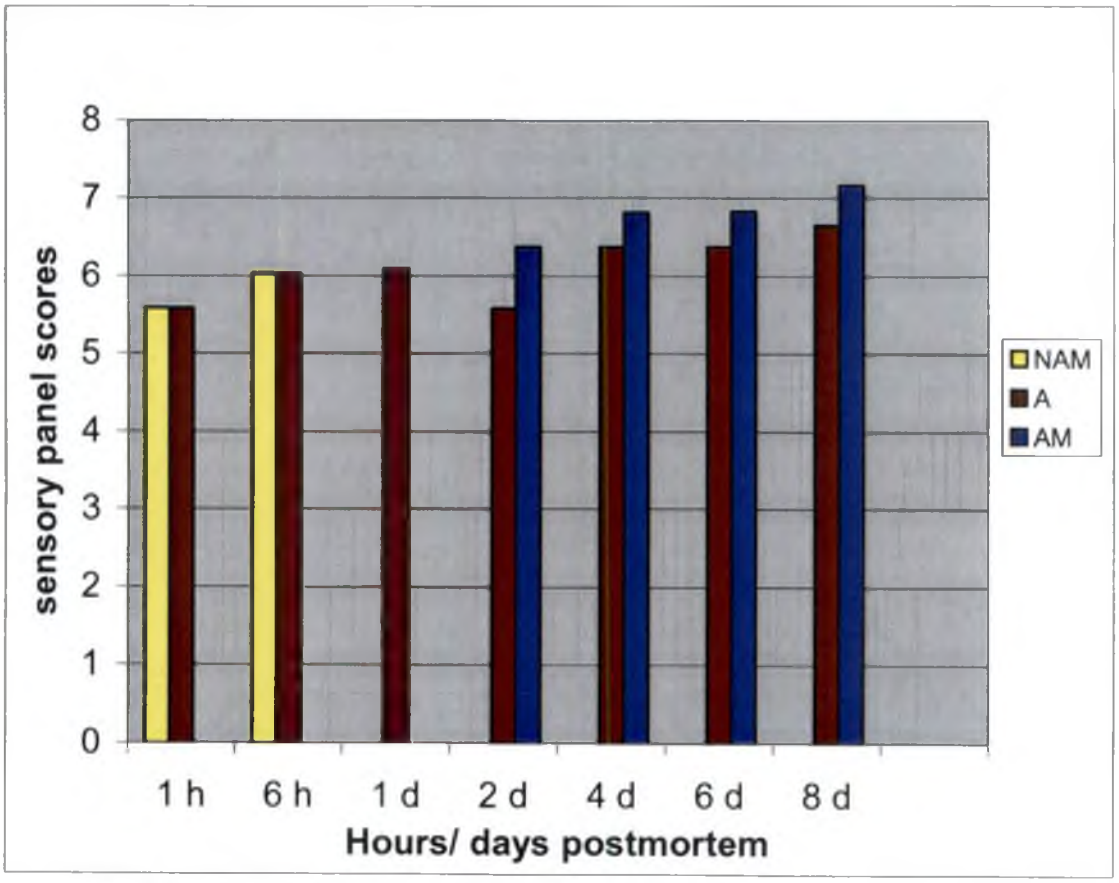
The mean scores for the marinated samples were 6.37 ± 0.19, 6.81 ± 0.16, 6.82 ± 0.12 and 7.16 ± 0.16 respectively on days 2, 4, 6 and 8. Unlike in the case of aged samples, in marinated samples the scores improved significantly ($P < 0.05$) from d 2 to day 8, compared to day 1.

Table 7.3 Effect of $CaCl_2$ marination and ageing on the amount of connective tissue of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	5.58 ±0.28 [a]	5.58±0.1 [a]	5.58±0.1 [a]
6 h	6.02±0.19 [a]	6.02±0.24 [ab]	6.02±0.24 [a]
1 d	*	6.08±0.24 [ab]	6.08±0.24 [a]
2d	*	5.85±0.27 [a]	6.37±0.19 [b]
4 d	*	6.37±0.16 [bc]	6.81±0.16 [b]
6 d	*	6.37±0.26 [bc]	6.82±0.12 [b]
8 d	*	6.65±0.18 [c]	7.16±0.16 [c]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 7.3. Effect of $CaCl_2$ marination and ageing on the amount of connective tissue of cooked buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

The scores of the marinated steaks were significantly higher ($P < 0.05$) on all days from day 2 to day 8 except on day 6 compared to aged samples.

4.7.4 Overall Tenderness

The effect of $CaCl_2$ marination and ageing on the overall tenderness of buffalo longissimus steaks at different postmortem times are presented in Table 7.4 and Figure 7.4.

The steaks at 1 h showed a mean score of 5.71 ± 0.34 for overall tenderness. The score recorded for both treatments NAM and A was 6.01 ± 0.23.

On day 1, the mean score was lower than at 6 h, which was non significant. Then the scores for the aged samples gradually improved up to day 8 with the mean values of 5.93 ± 0.29, 6.11 ± 0.19, 6.32 ± 0.20, 6.5 ± 0.16 and 6.47 ± 0.19 on days 1, 2, 4, 6 and 8, respectively. Variations in scores were not significant.

The mean and SE of the scores on days 2, 4, 6 and 8 for the marinated samples were 6.61 ± 0.19, 7.03 ± 0.15, 7.1 ± 0.16 and 7.55 ± 0.14, respectively. This showed a significant improvement ($P < 0.05$) in the mean scores of overall tenderness from day 2 to day 8. However the variations between day 4 and day 6 were not significant.

The overall tenderness scores were significantly higher ($P < 0.05$) for marinated samples on all days from day 2 to day 8 than aged samples. The overall tenderness scores for treatments A and AM on all days were significantly higher ($P < 0.05$) than on day 1.

4.7.5 Flavour

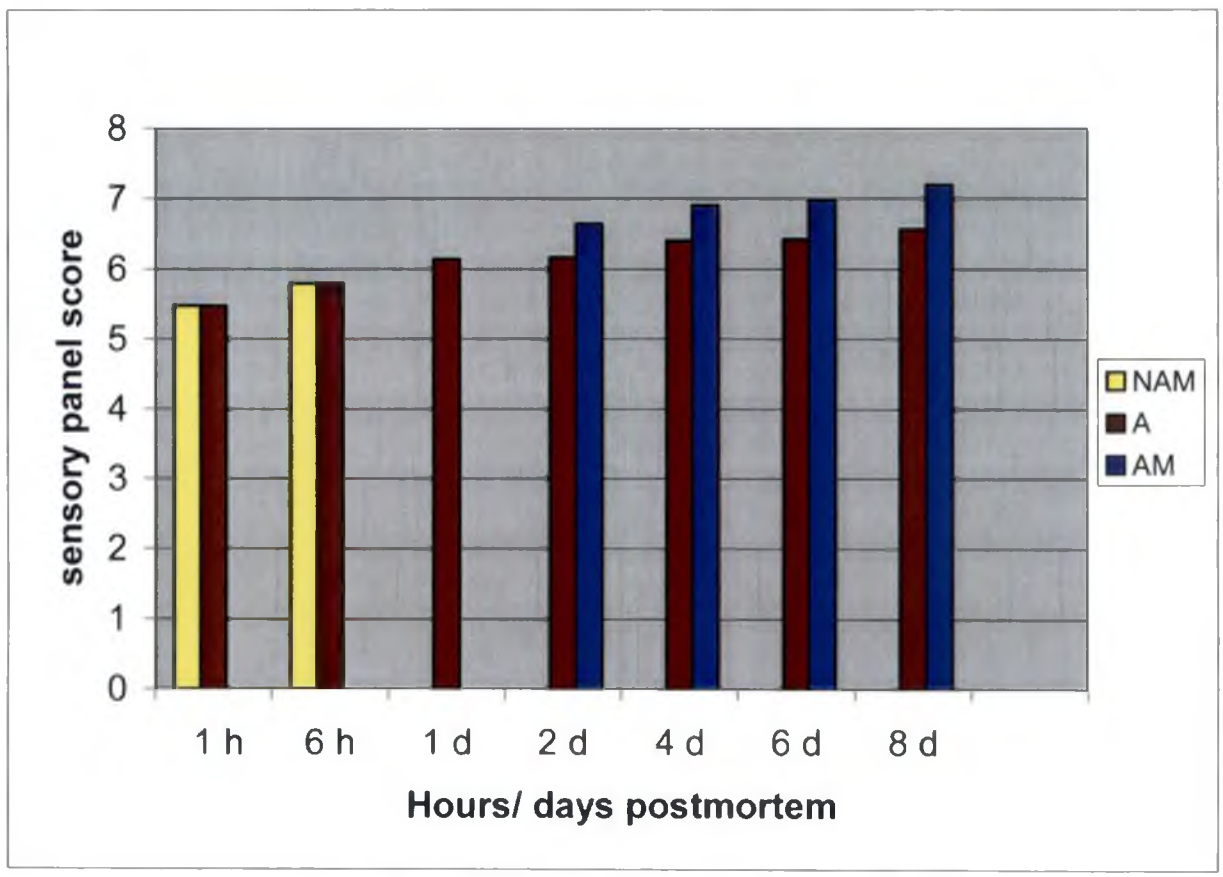
The effect of $CaCl_2$ marination and ageing on the flavor intensity scores of buffalo longissimus steaks at different postmortem times are presented in Table 7.4 and Figure 7.5.

Table 7.4 Effect of $CaCl_2$ marination and ageing on the overall tenderness of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	5.71 ±0.34 [a]	5.71±0.34 [a]	5.71±0.34 [a]
6 h	6.01±0.23 [a]	6.01±0.23 [ab]	6.01±0.23 [a]
1 d	*	5.93±0.29 [ab]	5.93±0.29 [a]
2d	*	6.11±0.19 [abc]	6.61±0.19 [b]
4 d	*	6.32±0.20 [bcd]	7.03±0.15 [c]
6 d	*	6.5±0.16 [d]	7.1±0.16 [c]
8 d	*	6.47±0.19 [cd]	7.55±0.14 [d]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 1% level

Fig. 7.4. Effect of $CaCl_2$ marination and ageing on the overall tenderness of cooked buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

A mean flavour score of 5.06 ± 0.81 was observed at 1 h for the buffalo *longissimus* steak. At 6 h, for both the first and second treatments the flavour scores were 5.57 ± 0.91.

The samples kept for ageing, showed mean scores of 5.73 ± 1.17, 5.72 ± 0.59, 6.13 ±1.47, 6.0 ± 1.92 and 6.28 ± 01.91 on days 1, 2, 4, 6 and 8, respectively. The variations were not significant.

Marinated samples recorded mean scores of 5.89 ± 1.69, 6.36 ± 1.36, 6.24±1.87 and 6.52 ± 1.95 on days 2, 4, 6 and 8, respectively. In this case also the variations were not significant.

Paired *t*-values revealed that only the variations in mean values on day 2 and day 8 differed significantly ($P < 0.05$) between treatments A and AM.

4.7.6 Colour

The effect of $CaCl_2$ marination and ageing on the colour score of buffalo longissimus steaks at different postmortem times are presented in Table 7.6 and Figure 7.6.

The mean and SE of colour scores of cooked steaks at 1 h was 6.03 ± 0.16. The mean value for the non marinated and aged samples at 6 h was the same, 6.17 ± 0.13.

The samples kept for ageing recorded scores of 6.11 ± 0.13, 6.48 ± 0.09, 6.53 ± 0.11, 6.44 ± 0.13 and 6.87 ± 0.57 on days 1, 2, 4, 6, and 8, respectively. The scores on day 6 and day 8 were significantly higher ($P < 0.05$) than on day 1.

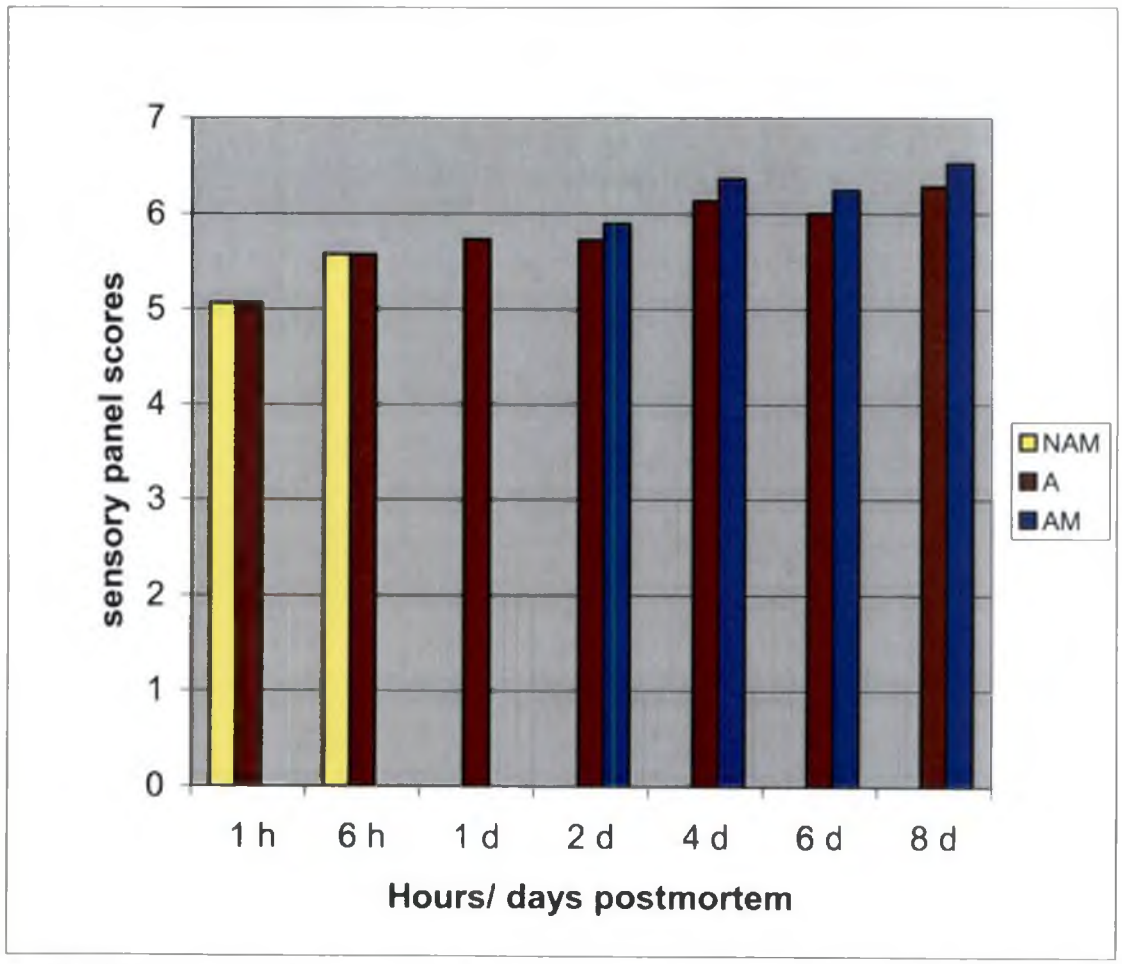
Marinated samples showed a slight improvement in scores on each day. The mean scores were 6.5 ± 0.10, 6.68 ± 0.08, 6.66 ± 0.04 and 6.98 ± 0.15 on days 2, 4, 6 and 8 respectively. The higher score on day 8 compared to day 2 was significant ($P < 0.05$).

Table 7.5 Effect of $CaCl_2$ marination and ageing on the flavour of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
1 h	5.06±0.81 [a]	5.06±0.91 [a]	5.06±0.91 [a]
6 h	5.57±0.91 [a]	5.57±0.91 [ab]	5.57±0.91 [a]
1 d	*	5.73±1.17 [b]	5.73±1.17 [b]
2d	*	5.72±0.59 [b]	5.89±1.69 [b]
4 d	*	6.13±1.47 [b]	6.36±1.36 [c]
6 d	*	6.0±1.92 [b]	6.24±1.87 [c]
8 d	*	6.28±1.91 [b]	6.52±1.95 [d]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 7.5. Effect of $CaCl_2$ marination and ageing on the flavour of cooked buffalo steaks at different postmortem times



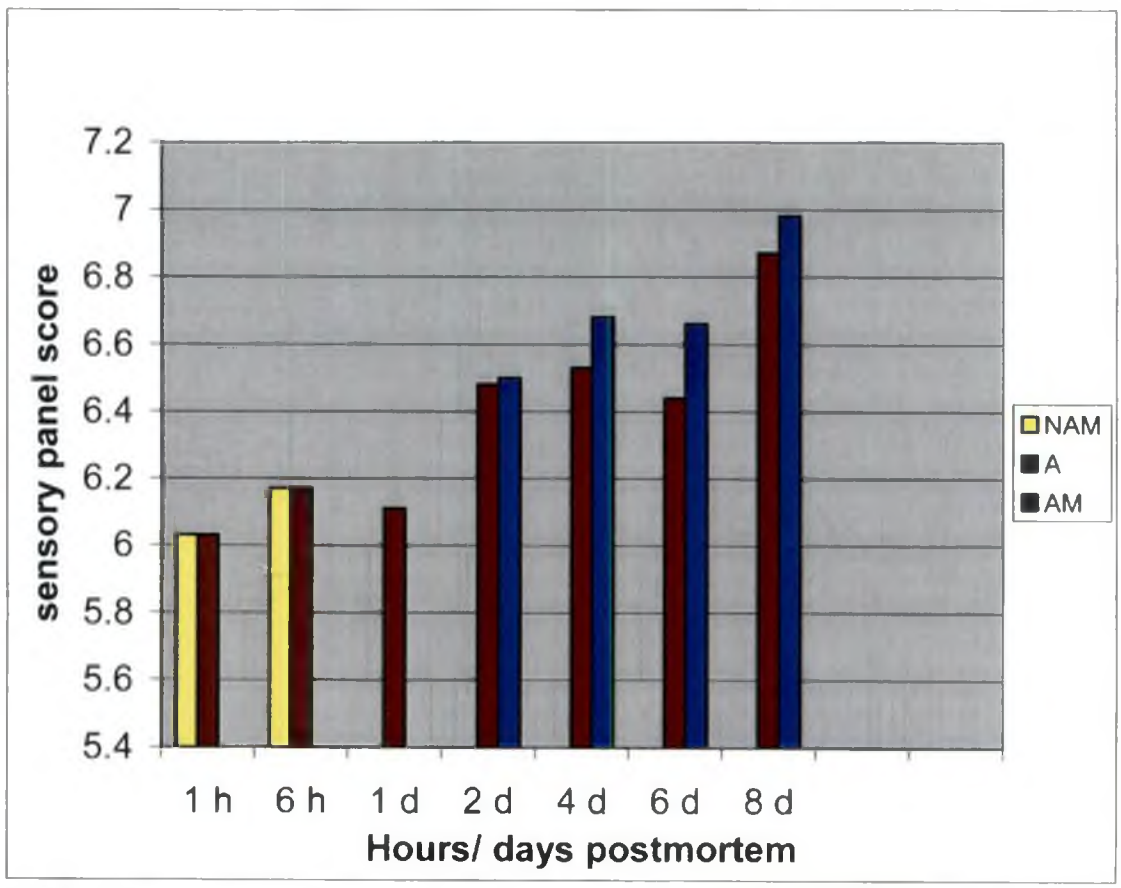
NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

Table 7.6 Effect of $CaCl_2$ marination and ageing on the colour of cooked buffalo steaks at different postmortem times

Hours/days postmortem	NAM	A	AM
	6.03 ±0.16 [a]	6.03±0.16 [a]	6.03±0.16 [a]
6 h	6.17±0.13 [a]	6.17±0.13 [ab]	6.17±0.13 [a]
1 d	*	6.11±0.13 [ab]	6.11±0.13 [a]
2d	*	6.48±0.09 [b]	6.5±0.10 [b]
4 d	*	6.53±0.11 [bc]	6.68±0.08 [b]
6 d	*	6.44±0.13 [b]	6.66±0.04 [b]
8 d	*	6.87±0.15 [c]	6.98±0.15 [c]

NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated
* Observations not made
Same superscripts denote values which do not differ significantly at 5% level

Fig. 7.6. Effect of $CaCl_2$ marination and ageing on the colour of cooked buffalo steaks at different postmortem times



NAM = Not Aged and Marinated; A = Aged only; AM = Aged and Marinated

The mean colour scores recorded on day 2 to day 8 did not differ significantly between the treatments A and AM, which indicated that calcium chloride marination did not affect the cooked colour scores.

4.8 CORRELATION COEFFICIENTS OF MFI, WBSF AND OVERALL TENDERNESS

The correlation coefficients (r) amoung myofibril fragmentation index, Warner-Bratzler shear force and overall tenderness of buffalo LD steaks at different postmortem times is presented in Table 8.

The Pearson's test revealed significant positive correlations ($P < 0.01$) between MFI and WBSF from day 1 to day 8 in treatments A and AM. The r-values on days 1, 2, 6 and 8 were 0.78, 0.81, 0.88 and 0.92, respectively in aged samples. In marinated samples the correlation coefficients were 0.78, 0.92, 0.88, 0.84 and 0.79, respectively on days 1, 2, 4, 6 and 8.

When the correlation between MFI values and scores for overall tenderness was tested by Spearman's test, the mean values from day 1 to day 8 were negatively correlated ($P < 0.05$) in both treatments A and AM. The r-values were -0.63, -0.56, -0.71 and -0.54 on days 1, 2, 6 and 8, respectively. While in marinated steaks, significant correlation was observed on days 1, 2, 4, 6 and 8 with r-values of -0.63, -0.58, -0.54, -0.78 and -0.73, respectively.

The SF values on all days were negatively correlated ($P < 0.01$) with the overall tenderness scores when tested by Spearman's correlation test. The r- values at 1 h, 6 h and on days 1, 2, 4, 6 and 8 were -0.80, -0.64, -0.66, -0.63, 0.91, -0.79 and -0.68 in treatment A, while those in AM were -0.80, -0.64, -0.66, -0.67, -0.84, -0.60 and -0.69 on the corresponding postmortem times.

Table 8. Correlation coefficients (r) among MFI, WBSF and overall tenderness of buffalo steaks at different postmortem times

Hours/days postmortem	MFI vs WBSF		MFI vs Overall Tenderness		WBSF vs Overall Tenderness	
	A	AM	A	AM	A	AM
1 h	0.49	0.49	-0.43	-0.43	-0.80**	-0.80**
6 h	0.50	0.50	-0.42	-0.42	-0.64**	-0.64**
1 d	0.78**	0.78**	-0.63*	-0.63*	-0.66*	-0.66*
2 d	0.81**	0.92**	-0.56*	-0.58*	-0.63*	-0.67*
4 d	0.32	0.88**	-0.15	-0.54*	-0.91**	-0.84**
6 d	0.88**	0.84**	-0.71**	-0.78**	-0.79**	-0.60*
8 d	0.92**	0.79**	-0.54*	-0.73**	-0.68**	-0.69**

MFI = Myofibril Fragmentation Index. WBSF = Warner- Bratzler shear force
A = Aged only; AM = Aged and Marinated
* $P < 0.05$; ** $P < 0.01$

Discussion

DISCUSSION

The current study on the tenderisation of buffalo meat by calcium chloride marination was designed to assess the effects of marination on the tenderness and other organoleptic quality of meat and compare it with those of natural ageing. The muscle samples were subjected to three treatments: 1) samples not aged and marinated and kept at room temperature for 6 h (NAM), 2) aged at 2 – 4° C for 8 days (A) and 3) aged and marinated at 2-4° C for 8 days (AM).

The effect of calcium chloride marination and ageing on the various meat quality parameters of buffalo *longissimus* steaks were assessed by making observations on pH, water holding capacity, cooking loss, colour, myofibril fragmentation index, Warner-Bratzler shear force and sensory quality attributes.

5.1 pH

The initial pH of the buffalo *longissimus* steaks in all treatments at 1h postmortem was 6.7 ± 0.001. The sample aged at 2-4^0 C attained the UpH 5.6 on day 1. Thereafter, pH gradually increased till day 6 and remained more or less static in both treatments A and AM. But pH on day 1 and 2 were significantly lower than on subsequent days. From the time of marination on day 1, the pH significantly increased from 5.6 to 6.2 on day 8. The pH remained above 6 from day 4 onwards in both the treatments. The trend in the change of pH was the same in both treatments A and AM which indicate that calcium chloride marination has no significant effect on the alterations in pH.

The significant difference in pH values pre-rigor and post-rigor stages are attributed to glycolysis. Postmortem anaerobic glycolysis and subsequent accumulation of lactic acid lead to the fall in pH. This supports the findings of Pearson and Young (1989). The increase in pH from day 2 to day 8 in the present study was probably due to the accumulation of the products of postmortem

proteolysis and changes in H_2 ion concentration. This is in agreement with the observations of Boakye and Mittal (1993).

Ziauddin *et al.* (1994) also reported an initial pH of 6.95 in buffalo *longissimus* muscle as in this investigation and an UpH of 5.6 at the end of 10 h in meat held at 28 ± 2^0 C. Soares, G. J. D *et al.* (1995) also reported an UpH of 5.6 at 24 h postmortem in buffalo LD muscle aged at 2^0 C. Hamm (1986) observed a decrease in the pH of beef up to 1.5 day postmortem and then a steady increase up to day 13. Boakye and Mittal (1993) also observed an increase in pH of beef LD between days 4 and 16 which is in agreement with the findings of this study.

The decline in the postmortem pH to 5.6 in 24 h is an indication of normal rigor development with a significant amount of calpain remaining after rigor. This level of calpain will become more active during the subsequent rise in pH thereby enhancing tenderness. Therefore, the rise in pH from day 2 in the present study would possibly favour the activity of calpain system. Moreover, the calcium chloride marination at 24 h postmortem would augment m-calpain activity in buffalo meat which enabled in increasing the tenderness.

5.2 WATER HOLDING CAPACITY

The WHC of the buffalo *longissimus* on the day of slaughter at 1 h was 0.37 ± 0.021. At 6 h of exsanguination the WHC was 0.35 ± 0.021 for the non marinated samples at room temperature and 0.34 ± 0.022 for those at 2 - 4° C. On the subsequent days, a declining trend was noticed on day 1, which remained static through the next two days and a no significant variation on days 6 and 8 in both the treatments. The values on all days from day 1 were significantly lower than at 1 h and 6 h, which implied lower WHC.

Though the WHC of the buffalo steak did not exactly agree with the postmortem trend in pH, they followed the same overall pattern with a decrease on the first two days postmortem followed by more or less constant values on the

subsequent days. These results are expected because the positive relationship between WHC and muscle pH is well established (Bouton *et al.*, 1971).

The initial reduction in the values of WHC is attributed to loss of water from the muscle fibres driven by pH and calcium induced shrinkage of myofibrils during rigor development (Honikel *et al.*, 1986). Later in ageing the degradation of cytoskeleton by proteolysis reduced or removed the linkage between the rigor induced lateral shrinkage of myofibrils and that of the whole muscle fibre which would improve the WHC (Kristensen and Purslow, 2001).

Results obtained in the present study is in agreement with the findings of Kristensen and Purslow (2001) who observed that WHC decreased during the first 2 - 7 days postmortem and eventually increased. Boakye and Mittal (1993) also provided substantial evidence on the dip in WHC up to the second ageing day and then gradual increase up to day 13.

Further more the present study indicated that $CaCl_2$ marination of the *longissimus* steaks did not significantly affect the WHC. Therefore, marinated meat could be utilized for further processing without affecting processing yield.

5.3 COOKING LOSS

The cooking loss of buffalo *longissimus* steak in the various treatments at 1 h postmortem was 39.90 ± 0.35 This showed a slight increase up to 40.48 ± 0.39 at 6 h in both treatments NAM and A. Cooking loss significantly increased ($P < 0.05$) in aged samples on day 1 and remained almost unaltered until day 8. But the highest value was observed on day 4 which differed significantly ($P< 0.05$) from all the other days except day 6. The values in the case of marinated samples followed the same pattern and the cooking loss on the day of marination was significantly lower than on day 4 only.

The cooking loss observed in the study is in accordance with the reports of Ziauddin *et al.* (1994) and O'Halloran *et al.* (1997). The higher cooking loss recorded in the less marbled buffalo longissimus steaks was in agreement with the findings of Dransfield *et al.* (1984) who also reported significantly higher evaporative losses in bulls with lower fat content.

Cooking loss remained more or less the same throughout the ageing period which is in conformation to the observations of Honikel *et al.* (1981), who obtained similar cooking losses both on days 2 and 7 in beef muscles.

Following the postmortem pattern of pH and WHC, the cooking loss also did not differ significantly between the treatments implying that $CaCl_2$ marination at 200mM, 5 per cent (w/w) in the study did not affect cooking loss percentages of buffalo meat. The results were similar to the findings of several researches (Koohmaraie *et al.*, 1990; Morgan *et al.*, 1991b; Diles *et al.*, 1994; Landsell *et al.*, 1995 and Dikeman *et al.*, 2003).

5.4 COLOR

The L, a, b values remained almost the same through the 6 hour of postmortem, although the samples at room temperature recorded a slightly higher 'L' value. The L, a, b values increased significantly on day 1 and thereafter the 'L' values registered a non significant increase in the values up to day 8 in both A and AM. The Hunter 'a' values also followed an increase from day 1 to day 8. The 'b' values remained more or less the same in both aged only and aged and marinated samples.

The Hunter 'L' values in the study is in agreement with the findings of Boakye and Mittal (1996) who obtained 29.0, 32.1, 32.2 and 33.1 on 0, 2, 4, 6 and 8 days, respectively in beef LD, so also for the 'a' and 'b' values.

The findings of the study revealed that 'L' values were higher and 'a' values were lower on days 2, 4, 6 and 8 compared to day 1 which is in accordance with the observations of Wulf and Wise (1999).

The 'L' values of samples at room temperature were found to be higher than the samples at 2° C because at a higher rigor temperature of 26-30° C, the protein denaturation tended to increase the scattering of light, indicating higher L values. Similar observation was already reported by Offer *et al* (1989).

The significantly higher Lightness on day 1 is attributed to the ultimate pH of 5.6 reached on day 1. At this pH, the lactic acid produced from glycolysis together with the rate of temperature decrease during ageing and the degree of protein denaturation would have affected the light scattering power of meat and thereby increased lightness. This observation is in agreement with the findings of Orcutt *et al.* (1984); Mac Doughall (1999) and Page *et al.* (2001) also reported that muscle pH is negatively correlated with Hunter L, a, b values.

The redness and yellowness were maintained throughout the ageing period with significant increase on day 1 which could be attributed to the antioxidant action of amino acid and dipeptides formed during postmortem proteolysis, which preserve the metmyoglobin reducing activity (Lee and Hendricks, 1997 and Farouk and Swan, 1998).

Ageing is found to improve the color of fresh meat from day 1 onwards. But $CaCl_2$ marination did not have any additional effect on colour characteristics of aged meat measured in terms of Hunter L a b values in buffalo steaks. Similar findings were reported by Diles *et al.* (1994); Landsell *et al.* (1995); Feldhusen *et al.* (1995) and Wheeler *et al.* (1997b) who employed different concentrations of calcium chloride to tenderise beef carcasses and found that marination did not exert any effect on the color compared to the controls.

5.5 MYOFIBRIL FRAGMENTATION INDEX

In treatments A and AM the MFI followed a gradual significant decrease ($P < 0.01$) from day 1 to day 8. MFI values of AM were significantly lower ($P < 0.01$) than those of A at all times postmortem. The reduction in the MFI was due to enhanced proteolysis by calpains and the resulting myofibril fragmentation and increased tenderness.

The decline in the MFI postmortem in this study is in agreement with observations of Koohmaraie *et al.* (1987) and Veiseth *et al.* (2004) which they attributed to the μ-calpain proteolytic activity.

The MFI values of marinated samples on day 2 were similar to those obtained for the aged samples on day 4. This is in agreement with the findings of Gonzalez *et al.* (2001). This indicated the early achievement of tenderness in marinated samples compared to normally aged samples.

5.6 WARNER-BRATZLER SHEAR FORCE

The steaks at room temperature recorded a significantly ($P < 0.05$) lower shear force value at 6 h compared to the steaks kept at 2° C. The mean value on day 1 was significantly higher ($P < 0.01$) than on subsequent days. Thereafter, a gradual significant ($P < 0.01$) fall in shear force values were observed on all days postmortem in treatments A and AM. Of all the treatments, the lowest shear force value of 4.88 ± 0.45 was recorded on day 8 in marinated samples.

WBSF values of the marinated steaks were significantly lower ($P < 0.01$) than those of the aged steaks on all days postmortem from day 2 to day 8. It reduced from 10.48 ± 0.48 at 1 h postmortem to 4.88 ± 0.45 in a course of 8 days which indicated 53.44 per cent reduction in values. While in aged meat, SF reduced to 6.75 ± 0.56, i.e., 35.59 per cent reduction only. This substantiates that $CaCl_2$ marination can enhance tenderness of buffalo meat.

The lower values obtained for the sample at room temperature at 6 h is in corroboration with the findings of Petaja *et al* (1985) who showed that high temperature conditioning for relatively shorter times (4 or 6 h) materially improved meat tenderness over holding at temperatures below 10° C.

The decreasing trend in the SF values from day 2 to day 8 observed in the current investigation was the same noticed by earlier researchers (Whipple *et al*, 1990; Wheeler and Koohmaraie, 1994 and Pringle *et al.* 1999) who attributed the increased shear force at 24 h to rigor induced shortening. The improvement on the subsequent days could be attributed to the activation of μ- and m-calpain by calcium ions which brought about enhanced tenderisation of buffalo meat. Moreover, the autolysis of calpastain at 2 - 4° C of ageing could be another reason for the enhanced tenderness. This supports the findings of Doumit and Koohmaraie (1999) in lamb LD. The improvement in tenderness on calcium chloride marination had been documented by several researchers (Koohmaraie and Shackelford, 1991; Diles *et al.*, 1994; Wheeler *et al.*, 1997b; Pringle *et al.*, 1999 and Carr *et al.*, 2004)

5.7 SENSORY EVALUATION

The pattern in the scores was more or less the same for the different attributes. For juiciness, ease of fragmentation and amount of connective tissue, there noticed a significant ($P < 0.01$) improvement in scores from day 2 onwards in marinated samples compared to day 1, the same was observed in treatment A from day 4 only. The scores for juiciness, ease of fragmentation, amount of connective tissue and overall tenderness of the cooked steaks improved by 17.9 per cent, 31.4 per cent, 28.3 per cent and 32.2 per cent, respectively on day 8 compared to 1 h. in marinated samples as against 12 per cent, 19.9 per cent, 19.1 per cent and 13.3 per cent for the non marinated steaks. In between the treatments A and AM, the scores were significantly ($P < 0.01$) higher for the marinated samples on all days which revealed the tenderising potential of calcium chloride marination.

The flavour and colour scores were not affected by calcium chloride marination which was evidenced by a nonsignificant variation between treatments A and AM on all days from day 2 to day 8.

The taste panel scores obtained in this study, is in agreement with the findings of Diles *et al.* (1994); Hoover *et al.* (1995); Landsell *et al.* (1995), Gonzalez *et al.* (2001); Dikeman *et al.* (2003) and Carr *et al.* (2004) who reported higher scores for tenderness and juiciness in calcium chloride marinated beef steaks.

5.8. CORRELATION COEFFICIENTS OF MFI, WBSF AND OVERALL TENDERNESS

In between MFI and WBSF, a significant positive correlation ($P < 0.01$) with mean correlation coefficients 0.85 in A and 0.84 in AM on all days from 1 to 8 were recorded in this study. This is in agreement with the reports of several researchers (Davis, 1980; Wulf *et al.*, 1997; Wulf and Page, 2000). This gives a fair indication that MFI can be used as an indicator of tenderness of fresh meat rather than cooked which would enable in selecting tender meat for retail sale and processing.

The overall tenderness was more negatively correlated with SF rather than MFI. The mean correlation coefficients of overall tenderness with MFI and WBSF ($r = 0.61$ and 0.65 for MFI in treatments A and AM, respectively and $r = 0.73$ and 0.70 for SF in A and AM, respectively) was also in agreement with the findings of Rhee and Kim (2001) and Riley *et al.* (2003). This confirms the enhanced rate of myofibrillar proteolysis under calcium chloride marination in combination with ageing. WBSF and MFI could be used as objective methods of determination of tenderness of buffalo meat as they are strongly correlated with the overall tenderness measured by taste panel evaluation.

Summary

SUMMARY

The present study on the tenderisation of buffalo meat by calcium chloride marination was designed to assess the effect of marination on tenderness and other organoleptic qualities of meat and to compare the effects with those of natural ageing. In India consumers prefer buffalo meat to other meats. Buffalo meat production contributes to a gross 26 per cent of the total meat production in India. Most of the animals are being slaughtered at the end of their productive life. Besides the older age, several pre-slaughter and post-slaughter factors render the meat tougher and less palatable. Moreover, the general habit is to consume fresh muscle before its tenderisation and conversion into meat by ageing. In this context the present study is relevant.

Muscle samples were collected from 14 healthy crossbred Murrah buffaloes of 4 – 8 years old. The LD muscle between 9^{th} and 12^{th} thoracic vertebrae was collected with in 1 h postmortem were made into steaks. They were assigned for three treatments, viz., steaks with out ageing and calcium chloride marination held at room temperature for 6 h postmortem (NAM), samples aged at 2° - 4° C for 8 days (A) and samples with ageing and calcium chloride marination held at 2 - 4° C for 8 days (AM). The aerobically packaged samples for the treatment AM after 24 h of storage were marinated by injection with 200 mM calcium chloride solution (5% w/w) at multiple sites.

The various meat quality parameters, viz., pH, WHC, cooking loss, color, MFI, WBSF and sensory quality attributes such as juiciness, ease of fragmentation, amount of connective tissue, overall tenderness, flavour intensity and colour of the steaks were assessed. The sensory evaluations of the steaks were conducted by a semi trained panel on an 8-point Hedonic scale. The samples for the treatment NAM were subjected to the study at 1 h and 6 h and the steaks from A and AM at 1 h, 6 h and on days 1, 2, 4, 6 and 8.

The pH did not alter under calcium chloride marination. It followed the normal postmortem pattern, with a pH of 6.7 ± 0.01 at 1 h in all treatments. The ultimate pH of 5.6 reached on day 1 was significantly lower ($P < 0.05$) than on all days and then gradually increased to 6.1 and 6.2 on day 8 in treatments A and AM, respectively. The samples followed a normal rigor with sufficient amount of proteolytic potential in the form of residual m-calpain. It was activated by calcium chloride marination resulting in myofibrillar proteolysis on subsequent days to tenderise buffalo meat. The increase in pH from day 2 – 8 was due to accumulation of products of postmortem proteolysis.

The WHC at 1 h was 0.37 ± 0.021 which declined to a significantly lower ($P < 0.05$) value of 0.31± 0.021 at the ultimate pH on day 1. This remained almost static without registering much variation till day 8 in both treatments A and AM. Although, WHC was not significantly affected by calcium chloride marination, the trend showed normal postmortem changes in the muscle. The pH and calcium induced shrinkage on the initial postmortem period contributed to the water loss from the tissue. The WHC remained static till day 8 which may improve on further storage in response to the changes in cytoskeleton. This would remove the rigor induced shrinkage of myofibrils.

The percentage of cooking loss increased significantly ($P < 0.05$) on day 1 and thereafter it remained static till day 8. The highest loss of 42.99 ± 0.35 recorded on day 4 which differed significantly ($P < 0.05$) from all the other days in both A and AM. Calcium chloride was not found to affect the percentage of cooking loss. The comparatively higher loss observed in the study could be attributed to the lack of external fat covering and marbling in the buffalo LD steaks.

Ageing is found to improve the colour of fresh meat from day 1 onwards. But calcium chloride marination did not have any additional effect on the colour characteristics of aged meat measured in terms of Hunter L a b values in steaks. The L values of samples at room temperature were higher than the samples at 2° C because at 26 - 30° C the protein denaturation tended to increase the scattering

of light. With the decline in postmortem pH to 5.6 on d 1, the L, a, b values registered an increase signifying an increase in lightness, redness and yellowness. This could be explained based on the changes in pH, which determine the water holding properties of meat and hence the light scattering properties. On subsequent days, L a b values remained without significant changes. This could be due to the antioxidant action of amino acids and dipeptides formed during postmortem proteolysis, which preserve the methmyoglobin reducing activity.

In treatments A and AM the MFI followed a gradual significant decrease from day 1 to day 8. The significantly lower ($P < 0.01$) MFI values of marinated samples on day 2 postmortem compared to day 4 in aged samples was obviously the consequence of the activation of calpain proteolytic system under calcium chloride treatment. This indicated the early achievement of tenderness in marinated samples compared to the normally aged samples. On day 1 postmortem, unlike the other meat quality parameters under study, MFI varied little with ultimate pH. MFI is deemed as a very useful indicator of meat tenderness particularly when sample size is smaller for the determination of shear force or sensory evaluation.

The steaks at room temperature recorded significantly lower ($P < 0.05$) WBSF value at 6 h compared to those at 2° C which seems to be due to high temperature conditioning. Significantly highest ($P < 0.01$) value of 11.37 ± 0.61 was obtained on day 1. This could be attributed to rigor induced shortening. The values declined significantly in treatments A and AM. The values obtained for the marinated steaks were significantly lower ($P < 0.01$) than the aged samples on all days postmortem. It reduced from 10.48 ± 0.48 at 1 h postmortem to 4.88 ± 0.45 in a course of 8 days. This indicated a 53.44% reduction in values from 1 h to day 8, while in aged sample only a 35.59% reduction was noticed in a course of 8 days. The improvement on the subsequent days could be attributed to the activation of μ- and m-calpain by calcium ions which brought about enhanced tenderisation of buffalo meat. Moreover, the autolysis of calpastain at 2 - 4° C of ageing could be another reason for the enhanced tenderness.

The sensory panel evaluation of organoleptic qualities showed that calcium chloride marinated steaks significantly improved ($P < 0.01$) in the scores for the different attributes studied on each day of ageing. No flavor problems or alterations in cooked color were noticed at 200mM $CaCl_2$.

Correlation studies in between MFI and WBSF revealed a significant positive correlation ($P < 0.01$) with $r = 0.85$ in A and 0.84 in AM on all days from 1 to 8. This gives a fair indication that MFI can be used as an indicator of tenderness of fresh meat rather than cooked which would enable in selecting tender meat for retail sale and processing. The overall tenderness was more negatively correlated with SF ($r = 0.70$) rather than MFI ($r = 0.65$). This confirms the enhanced rate of myofibrillar proteolysis under calcium chloride marination with ageing. WBSF and MFI could be used as objective methods of determination of tenderness of buffalo meat as they are strongly correlated with the overall tenderness measured by taste panel evaluation.

The tenderness and other organoleptic qualities of buffalo *longissimus* steaks could be improved significantly by post-rigor marination with 200mM $CaCl_2$ (5 % w/w) on day 1 and subsequent ageing at 2 – 4° C for 4 – 8 days. The tenderness improved by 53.44 per cent in marinated steaks as against 35.59 per cent in those aged without marination. The improvement in tenderness could be attributed to the activation of μ- and m-calpain by calcium ions and the autolysis of calpastain at 2 - 4° C of ageing.

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TENDERISATION OF BUFFALO MEAT BY CALCIUM CHLORIDE MARINATION

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ABSTRACT

The present study on the tenderisation of buffalo meat by calcium chloride marination was designed to assess the effect of marination on tenderness and other organoleptic qualities of meat and to compare the effects with those of natural ageing.

Samples of *longissimus dorsi* muscle between 9th and 12th thoracic vertebrae from 14 healthy crossbred Murrah buffaloes of 4 – 8 years old were collected. They were assorted for three treatments, viz., samples neither aged nor calcium chloride marinated; and stored at room temperature for 6 h postmortem (NAM), samples aged only (A), samples which are marinated (AM). Samples of A and AM were stored at 2-4^{o} C. The aerobically packaged samples for the treatment AM after 24 h of storage were marinated by injection with 200 mM calcium chloride solution (5% w/w) at multiple sites.

The pH, WHC, cooking loss, color, MFI, WBSF and sensory quality attributes such as juiciness, ease of fragmentation, amount of connective tissue, overall tenderness, flavour intensity and colour of the steaks were assessed. The samples for the treatment NAM were subjected to the study at 1 h and 6 h and the steaks from A and AM at 1 h, 6 h and on days 1, 2, 4, 6 and 8.

The pH of the steaks was not affected by $CaCl_2$ marination. It followed the normal postmortem pattern, with a pH of 6.7 ± 0.01 at 1 h in all treatments. The ultimate pH of 5.6 reached on day 1 was significantly lower ($P < 0.05$) than on all days, which gradually increased until day 8 in treatments A and AM. The samples followed a normal rigor with sufficient amount of m-calpain and the increase in pH was due to the accumulation of products of proteolysis.

The WHC at 1 h was 0.37 ± 0.021 which declined to a significantly lower ($P < 0.05$) value of 0.31± 0.021 at the ultimate pH on day 1. This remained almost static till day 8 in both treatments A and AM. On day 1 the pH and calcium induced shrinkage caused loss of water. Later changes in the cytoskeleton improved WHC by removing rigor induced shrinkage of myofibrils. WHC was not significantly affected by calcium chloride marination.

The percentage of cooking loss increased significantly ($P < 0.05$) on day 1 and thereafter it remained static till day 8. Calcium chloride was not found to affect the percentage of cooking loss. The comparatively higher loss observed in the study could be attributed to the lack of external fat covering and marbling in the buffalo LD steaks.

Ageing is found to improve the colour of fresh meat from day 1 onwards. But calcium chloride marination did not have any additional effect on the colour of aged meat. The decline in postmortem pH to 5.6 on day 1 contributed to higher L, a, b values which increased the light scattering properties. On subsequent days, L, a, b values remained without significant changes. This could be due to the antioxidant action of amino acids and dipeptides formed during postmortem proteolysis, which preserve the methmyoglobin reducing activity.

In treatments A and AM the MFI followed a gradual significant decrease ($P < 0.01$) from 768.0 ± 40.52 to 541.78 ± 34.63 on day 1 and day 8, respectively in aged steaks. While in marinated it declined to 465.28 ± 29.79 on day 8. Significantly lower ($P < 0.01$) value of 630 ± 42.88 on day 2 in marinated samples was comparable to that in the aged samples on day 4, indicating the early achievement of tenderness in marinated samples. MFI varied little with ultimate pH. MFI is deemed as a very useful indicator of meat tenderness particularly when sample size is smaller for the determination of shear force or sensory evaluation.

Significantly highest ($P < 0.01$) WBSF value of 11.37 ± 0.61 obtained on day 1 could be attributed to rigor induced shortening. The values declined significantly in treatments A and AM. The values obtained for the marinated steaks were significantly lower ($P < 0.01$) than the aged samples on all days postmortem. It reduced from 10.48 ± 0.48 at 1 h postmortem to 4.88 ± 0.45 in a course of 8 days. This indicated a 53.44% reduction in values from 1 h to day 8, while in aged sample only a 35.59% reduction was noticed in a course of 8 days.

The sensory panel evaluation of organoleptic qualities showed that calcium chloride marinated steaks significantly improved ($P < 0.01$) in the scores for the different attributes studied on each day of ageing. No flavor problems or alterations in cooked color were noticed at 200mM $CaCl_2$.

Correlation studies in between MFI and WBSF revealed a significant positive correlation ($P < 0.01$) with $r = 0.85$ in A and 0.84 in AM on all days from 1 to 8. This indicates that MFI could be used as an indicator of tenderness of fresh meat rather than cooked. WBSF and MFI were strongly correlated with the overall tenderness measured by taste panel evaluation. The overall tenderness was more negatively correlated with SF ($r = 0.70$) rather than MFI ($r = 0.65$).

The tenderness and other organoleptic qualities of buffalo *longissimus* steaks could be improved significantly by post-rigor marination with 200mM $CaCl_2$ (5 % w/w) on day 1 and subsequent ageing at 2 – 4° C for 4 – 8 days. The tenderness improved by 53.44 per cent in marinated steaks as against 35.59 per cent in those aged without marination. The improvement in tenderness could be attributed to the activation of μ- and m-calpain by calcium ions and the autolysis of calpastain at 2 - 4° C of ageing.