1.3. MUSCLE AND MEAT

Muscle contains water, proteins, lipids , carbohydrates and minerals. Table 1.3.1 summarises the range of different chemical constituents found in a muscle with information of the forms in which they are present.

Table 1.3.1 Proximate composition of muscle. Forms present and changes during development (Pearson and Young, 1989).

Constituent	Range (%)	Forms Present
Water	70–78	Immobilized and free, mainly in association with the proteins
Protein	15–22	Sarcoplasmic, myofibrillar, and stromal proteins
Lipid	1–13	Triacylglycerides, phosphoglycerides, glycolipids, proteolipids, and depot fat
Lipid	0.5–3.0	Mainly in membranes as glycolipids and proteolipids, some as fat droplets in sarcoplasm
Carbohydrate	1–2	Glycogen, monosaccharides and other metabolic intermediates, glycolipids, and acid mucopolysaccharides
Minerals	1–2	Constituents of extracellular and intracellular fluids, also may be bound to tissue
Vitamins	μg % range	Largely found bound as coenzymes or constituents of tissue
Nitrogenous nonprotein extractives	1.5–1.8	Free amino acids, creatine, carnosine, anserine, glutathione, and various hormones

1.3.1. Muscle Structure

A muscle is usually completely enclosed by a thick sheath of connective tissue, the epimysium (Figure 1.3.1), and is divided into bundles of fibres by a connective tissue network, the perimysium. The individual muscle fibres are bounded by a plasma membrane itself surrounded by a thin connective tissue network, the endomysium. This consist of a basement membrane surrounded by a reticular layer, in which a meshwork of fine collagen fibrils is embedded in a matrix. This basal lamina may act as a diffusion barrier for large proteins (20 to 25 nm diameter).

The skeletal muscle fibres show very regular transverse striations along their length, the protein-dense A-bands alternating with the less dense I-bands. At moderate to long muscle lengths there is a lighter zone, the H-zone, within the A-band, and in the centre of the A-band there is a dense line, the M-line. The I-band is bisected by the very dense Z-disc. The structure is not continuous across the width of the muscle fibre but is divided up into roughly cylindrical elements, the myofibrils, which are aligned along the fibre axis. These are separated from one to another by gaps containing membrane-lined channels (the sarcoplasmatic reticulum) whose function is to store Ca^{2+} ions until they are released to trigger muscular contraction. In some muscle fibres mitochondria are additionally present in these gaps. The structural unit which is repeated between successive Z-discs is called the sarcomere. The striations of the myofibrils are caused by a highly organised array of two kinds of longitudinally-oriented filaments: the thick filaments, confined to the A-band and joined together at their centres by the M-line, and the thin filaments, extending from either side of the Z-disc to the edge of the H-zone and joined together by the Z-disc (Figure 1.3.2).

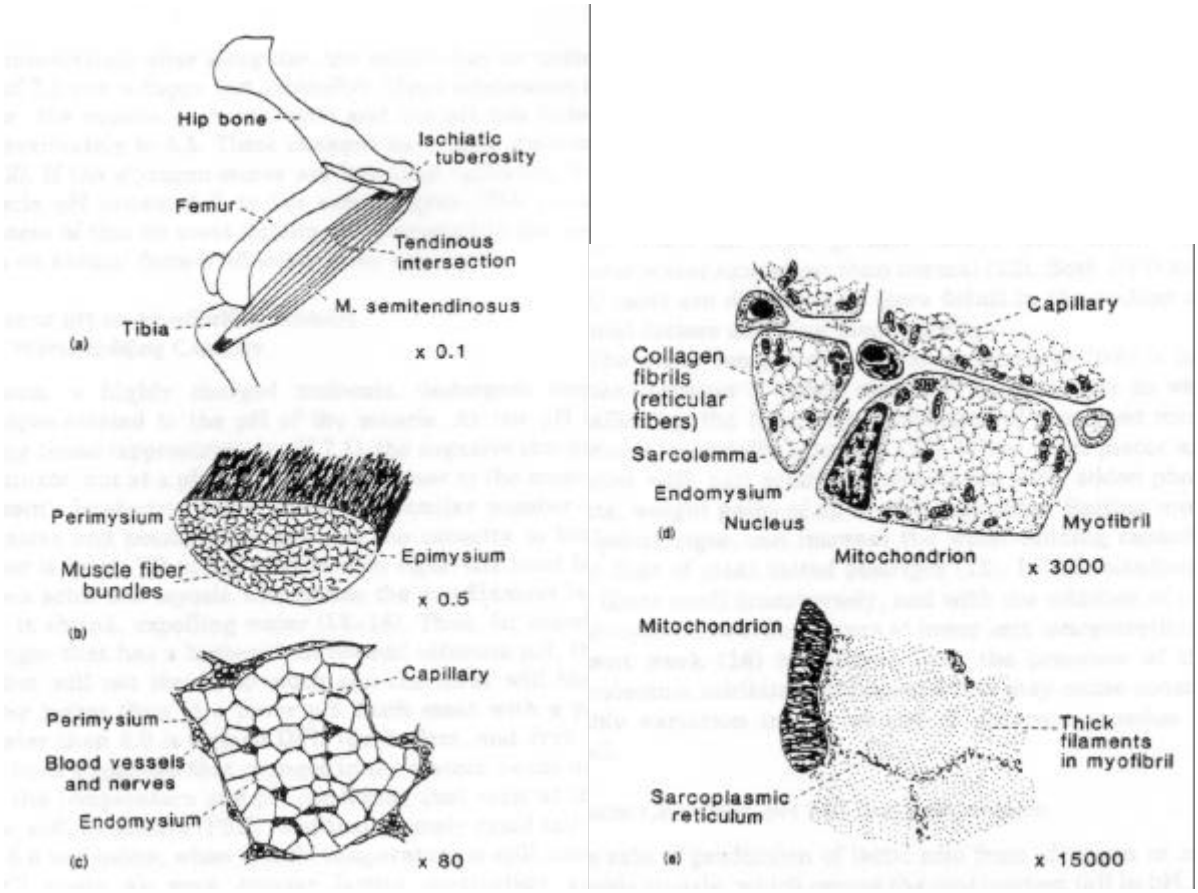


Figure 1.3.1 Gross and ultrastructural anatomy of a mammalian semitendinosus muscle. (a) The semitendinosus muscle lies caudally in the thigh region. (b) Seen grossly in transverse section, perimysial strands course through the muscle and enclose muscle fiber bundles. (c) Within a bundle, an endomysial connective tissue network is revealed by light microscopy to surround individual muscle fibers. (d) Electron-microscopic examination shows a network of sarcoplasmic reticulum enclosing myofibrils within the fibers. The two complete fibers drawn sectioned here are (e). At a still higher magnification, a patter of thick filaments is visible within each myofibril (Devine, 1992).

1.3.2. Postmortem biochemistry of muscle

The changes that occur when muscle in a living animal becomes meat are initiated from the moment the circulation stops, i.e., slaughter. From this moment, nutrients and oxygen are no longer available to the muscle from outside sources, so the energy stores present within the muscle start to be used up. Muscle adenosine triphosphate (ATP) is quickly used up, and upon depletion of this source, glycogen is metabolized. This glycogen allows the muscle to contract until the pH falls from around 7.1 to below 6.02.

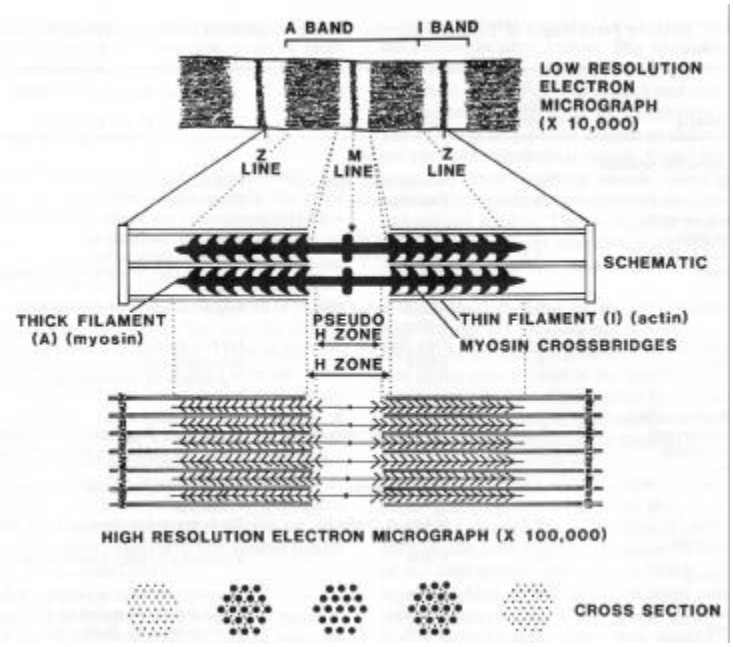


Figure 1.3.2 Fine structure of a single sarcomere along with portions of two adjacent sarcomeres (Pearson and Young, 1989).

The sliding of the myofilaments relative to each other can occur in muscle from living animals with the contraction initiated by the nervous system. Contraction occurs by the actin and myosin filaments sliding with respect to each other. This contractile activity, initiated via release of calcium from the sarcoplasmic reticulum, is activated and sustained by ATP. In the presence of calcium and absence of ATP, the muscles are in rigor, usually as a consequence of death. Upon attainment of rigor, the muscle is inextensible.

The depletion of muscle energy stores leads eventually to rigor mortis and a change in status, i.e., the muscles becomes meat.

The ageing of meat involves the breakdown of the myofibrillar muscle proteins by endogenous enzymes, primarily at the junction of the A-I band, as well as fragmentation of the Z-lines. Among the enzymes involved, calcium-dependent proteases seem to be the best candidates. However, a synergistic contribution by lysosomal and calcium-dependent proteinases has been proposed, with no direct relationship between aging rate and protease content found in the muscles (Devine, 1992).

1.3.3. Water and meat

As stated before, the water content in ham muscles may differ from 70 to 78%. This differences are due basically to variations in fat content.

In other systems, water is also present. For instance, the plant leaves contain about 95% water. This water does not readily escape and the surfaces of the leaves are dry to the touch because the water is contained in many enclosed packets (cells), the walls of which do not readily let the water pass. However, if the leaf rots, the cell walls break down and copious amounts of water ooze out. Another example, is when water is taken up by blotting paper in the irregular spaces between the feltwork of cellulose fibres forming a continuous phase. The water is taken up in place of air by capillary flow. Also in gels of polymers, the water is held in the narrow spaces between the individual chains of the polymer network and again forming a continuous phase. A closer analogy to a muscle is the collagen fibres of connective tissue, where water is held in longitudinally-oriented channels of a range of sizes: between polypeptide chains, between molecules, between fibrils and between fibres. When a collagen fibre is dried, shrinkage takes place mainly by a reduction in the width, rather than the length, of the fibres.

Water is held in the muscle; if the membrane around the muscle cell is cut, water does not come out under gentle centrifugation. Thus, the water is held by the internal structure of the cell (Offer and Knight, 1988).

1.3.3.1. Nature of water in the living muscle fibres

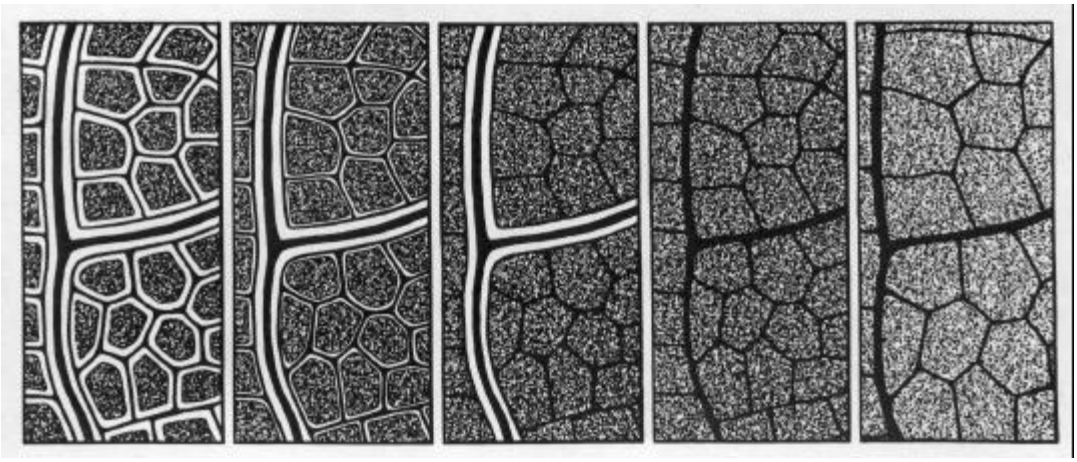
A protein molecule in an aqueous solution interacts with water, and when it moves through the solvent it carries some water with it. Part of this water is believed to be hydrogen-bonded to the surface of the protein molecule and part may be present in clefts or pockets, and both are in dynamic exchange with free water. It was concluded that the bulk of the water inside a muscle fibres is free and that the less mobile water bound to the muscle proteins is only about 4% of the total (Offer and Knight, 1988). Motarjemi (1988) reported from other

studies that the bound water can reach 20% of total, although she states that results obtained by different measurement techniques might not be valid for comparison. Nevertheless, it is thus important to appreciate that very little water is held by the myofibrillar proteins themselves: it is the structure they make up, the myofibrils, which holds the water. A rough analogy is a rubber sponge. It is not the hydrophobic rubber itself that holds the water but the channels present in the rubber (Offer and Knight, 1988).

1.3.3.1.1. Relation between fibre volume and muscle volume

The swelling or shrinkage of myofibrils or muscle fibres alters the distribution of water within the muscle but does not necessarily cause swelling or shrinkage of the muscle as a whole. The structural relation between fibre volume and muscle volume is illustrated in Figure 1.3.3. It shows in diagrammatic form transverse sections through a muscle with varying degrees of expansion (or shrinkage) of the fibres. In Figure 1.3.3 (b) the fibres are sufficiently shrunken for there to be appreciated extra-cellular spaces between the muscle fibres and the endomysil network and also between fibre bundles and the perimysial network. If, as a result of treatment, for example, by immersing a piece of meat in a salt solution, the fibres swell, they will first fill the endomysial network (Figure 1.3.3 (c)). If they swell further, forcing the endomysial network to expand, the fibre bundles will eventually fill the perimysial network (Figure 1.3.3 (d)), but the volume of the meat will not have changed up to this point. Only if swelling goes beyond this point must swelling of the meat as a whole necessarily occur (Figure 1.3.3 (e)). If the fibres become more shrunken than in normal rigor muscle, i.e. in the PSE state (Figure 1.3.3 (a)) the extra-cellular space would be increased but the volume of the meat does not necessarily fall; that can happen only if water is lost form the extra-cellular space.

The uptake or loss of water from meat depends most directly on changes in the volume enclosed by the perimysial connective tissue networks. There is not a tight coupling between myofibrillar (or fibre) volume and muscle volume.



(a) (b) (c) (d) (e)

Figure 1.3.3 The relation between fibre volume and muscle volume. Each diagram depicts a transverse section of muscle including parts of three fibre bundles separated by perimysiom (thick lines). The fibres (stippled) are separated by endomysium (thin lines). (a) to (e) show varying degrees of expansion (or shrinkage) of the fibres (Offer and Knight, 1988).

1.3.3.2. Nature of water in meat

As stated by Motarjemi (1988), numerous terms in literature are employed to describe the state of water in meat, but none of the definitions is completely satisfactory. Water binding capacity is often used and mean two different properties of the material. One is the capacity of the material to bind water by chemical interactions, bound water capacity (BWC) and the other is the capacity of the material to entrap water within its structure and retain it upon the application of forces such as centrifugation, pressure or heating. which is called water retention capacity (WRC) or water holding capacity (WHC).

1.3.3.2.1. Bound water capacity

Motarjemi (1988) reported that bound water could be defined in terms of its thermodynamic properties, such as, freezing point, boiling point, heat of sorption, enthalpy, entropy, etc. From her review the following conclusions were obtained:

- Bound water is the water which does not freeze at very low temperatures (-40ºC).

- Bound water can be based on the determination of the sorption isotherm of the material, i.e., is the water which yields a given water activity.
- Bound water is related to the ability of water to act as a solvent.
- Bound water is also based on the heat of evaporation studied by calorimetry.
- Bound water is related to the restriction of molecular motion of water (translational or rotational) due to the interaction of water with the biomolecules in the vicinity of colloids (NMR techniques).
- Bound water is the water which shows an extra resistance to the diffusion process and exhibits a lower diffusion coefficient than the bulk water.
- Bound water is related to the different periods of falling rate period (section 1.6.1.2.1). The first period is related to the drying of the bulk water. The second period, exhibits a drastic decrease in the drying rate, which is attributed to the drying of the bound water. The break-point between these two periods, has been correlated to the bound water determined by other methods (NMR, sorption isotherms).
- Bound water is that water in the vicinity of macromolecules whose properties differ detectably from those of the bulk water in the same system.

1.3.3.2.2. Water holding capacity

The WHC, expresses the ability of the material to retain water in its spatial structure. Thus, any factor which may alter the spatial arrangement of the fibrillar network of the meat may also influence its WHC. For example, the characteristics of the animal (age, sex, breed, etc.), the handling of the animal before and after slaughter, the pre- and post-rigor metabolism (which in turn affects the pH), storage, heating, drying, freezing, etc. influence the WHC of the meat.

Mechanism of gain and losses in meat

The nature and location of water in living muscle have been considered in section 1.3.3.1. In this state, there is little extra-cellular water and about 85% of the water in a mammalian fibre is located within the myofibrils and is therefore firmly held. Since myofibrils contain most of the water of a muscle, a reasonable general hypothesis for explaining the gains and losses of water in meat is that they originate from volume changes of the myofibrils. In rigor muscle a myofibril is unlikely to change volume by a change in sarcomere length, since the attached cross-bridges would prevent sliding of thick and thin filaments. In contrast, the side-to-side spacing of filaments is able to vary substantially, depending on the ionic conditions to which a fibre is subjected. The simplest hypothesis, depicted in Figure 1.3.4 is that lateral expansion of the filament lattice results in more water being incorporated into the myofibrils in the spaces between filaments and, since this water is firmly held, the meat is able to retain more water. Conversely, lateral shrinkage or the filament lattice expels water from the myofibrils and, since this expelled water is less-firmly held, it can be lost more readily from the meat (Offer and Knight, 1988).

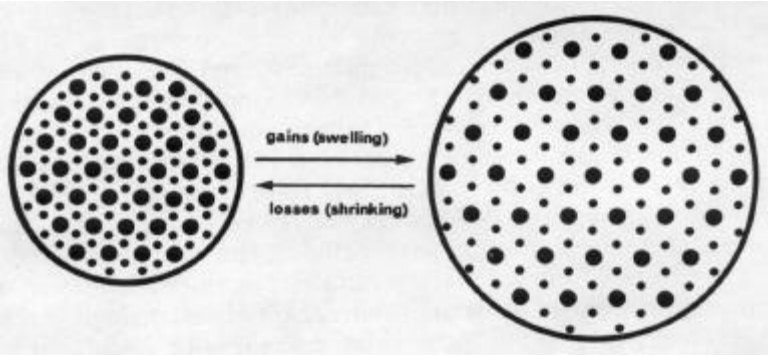


Figure 1.3.4 Hypothesis explaining the origin of changes in water-holding of meat. On the left hand side it is shown schematically a transverse section of a myofibril, with thick filaments (large circles) and thin filaments (small circles), forming a hexagonal lattice. On the right hand side, the same myofibril is shown with an expanded lattice (Offer and Knight, 1988).

Effect of pH on myofibrilar proteins and water-holding capacity

The myosin, a highly charged molecule, undergoes some changes related to the pH of the muscle. At the pH of living tissue (approximately 7.1), the negative charges dominate, but at

a pH of 5.5, which is closer to the muscle protein isoelectric point, there is a similar number of negative and positive charges, and the capacity to bind water is minimal. When the muscle is in rigor, the bond between actin and myosin also causes the myofilament lattice to shrink, expelling water. It has been suggested that changes in the myosin heads under the temperature and pH conditions that exist at the PSE condition (namely rapid fall to pH below 6, when muscle temperature is still about 35ºC) cause an even greater lattice contraction and greater water exudation than normal (Hamm, 1986).

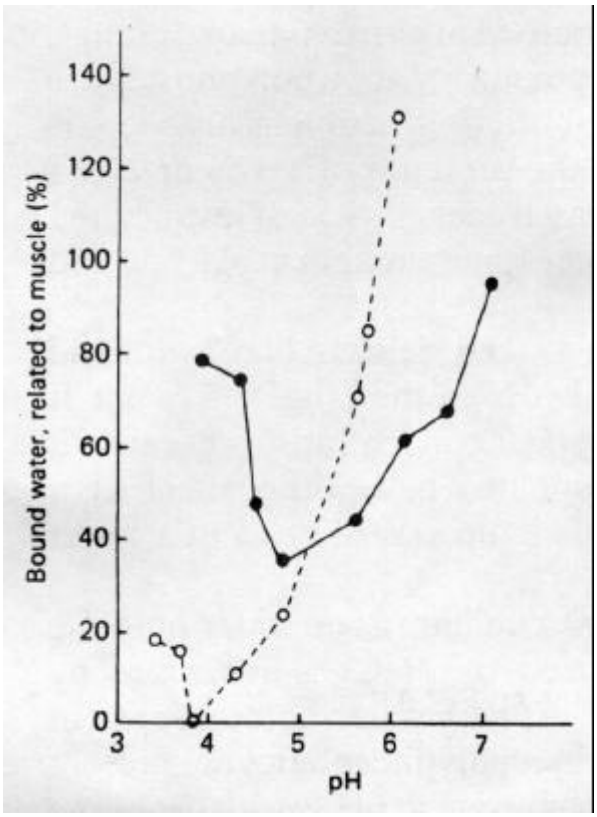


Figure 1.3.5 Effect of pH on water retention by homogenised beef muscle. (●)Non salted meat. (◦) Salted meat (0.22 M NaCl) (Hamm, 1986).

At pH 5.0, which corresponds to the isoelectric point, the WHC is a minimum. At this pH the positively charged groups neutralize the negatively charged groups of the proteins and confer to the protein a minimum net charge. Thus, due to these ionic interactions the protein matrix exhibits a minimum swelling and consequently a minimum WHC. Upon increasing

or decreasing pH, the net charge increases (Figure 1.3.6). The mutual repulsion of fibrillar proteins causes the swelling of the matrix and the WHC increases. At very low and high pH, the WHC decreases again due to the denaturation of the proteins.

Effect of sodium chloride on WHC

At pH values above the isoelectric point the addition of sodium chloride (2%) appreciably increases the WHC. Whereas at pH values below the isoelectric point the WHC decreases. The explanation given for this effect is that proteins interact preferentially with the Cl^- ions (Figure 1.3.6). Thus, below the isoelectric point the positive charge of the proteins is neutralized by Cl^- ions (Hamm, 1975). The repulsive force decreases, the matrix shrinks, and the WHC is reduced.

At pH values above the isoelectric point Cl^- ions neutralize the few positive charges, the net negative charge increases and causes swelling of the matrix. the WHC then increases. At high ionic strength (above 5% NaCl) the opposite effect (i.e. the dehydration) on the meat can be expected, due to the denaturation and precipitation of the proteins. The volume of salt-treated meat depends on the history of salt treatment. Thus a meat piece placed in 5 M NaCl loses water, but if instead it is placed in a series of solutions of increasing NaCl concentration, the swelling occurring at 1 M NaCl is fully retained at 5 M (Offer and Knight, 1988).

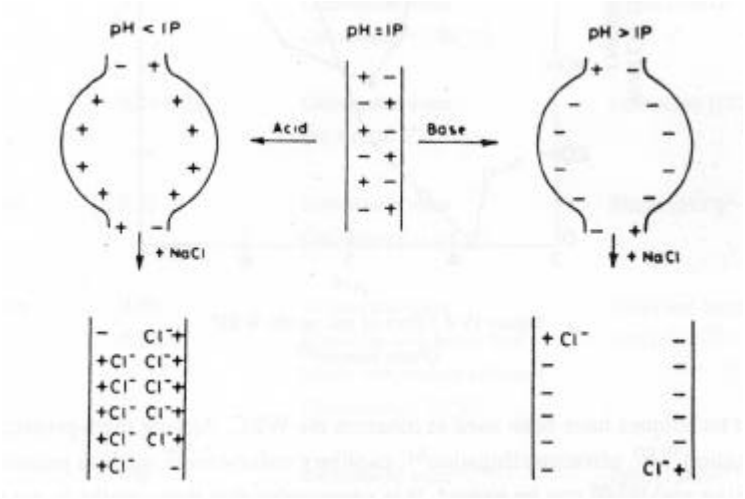


Figure 1.3.6 Mechanism of the effect of pH and NaCl on the water retention capacity of meat.

The NaCl also induces the extraction of proteins in meat. There are two classes of proteins involved: one class, the sarcoplasmic proteins, are soluble at physiological ionic strength and diffuse out of rigor muscle into isotonic saline. The other class, the myofibrilar proteins, stay in polymerised form within the muscle in isotonic saline, but become soluble at higher ionic strength (Offer and knight, 1988).