Biochemistry of Lean Muscle Tissue as Related to Water-Holding Capacity

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Introduction

The literature on water-holding contains many references to the term "water-holding capacity." Water-holding capacity (WHC) has been defined as the ability of meat to retain its own water despite the application of force. We will use the term WHC as it relates to drip losses from fresh uncooked meat. Drip loss and WHC will be used as alternative terms for the same phenomenon.

The amount of drip lost from carcasses is negligible, but after breaking, drip losses are of the order of 0.1 to 1% during 2 days. When the meat is further fabricated (steaks etc.), drip losses increase and may exceed 10%.

Water-holding is of great importance, because moisture losses affect the weight and therefore the financial value of the meat. Moreover, exudation of drip produces an unsightly pool of liquid around the meat which adversely affects the appearance and consumer appeal. As drip has about two-thirds the protein concentration of whole meat, its loss is a costly waste of animal protein. The content of water and its distribution have a profound influence on the sensory properties of meat.

Information on the mechanisms responsible for drip formation will provide better means of controlling and predicting WHC. Also, knowledge of the processes involved may allow for the prediction of the impact of new technologies on this quality attribute.

In the following, we give a short overview of the current knowledge on WHC and indicate which aspects need more attention. It was not the purpose of the authors to provide an extensive review. For more detailed information, the reader is referred to reviews by Offer and Knight (1988) and Hamm (1972; 1986).

Muscle Structure

A muscle is completely enclosed by a sheath of connective tissue, the epimysium. Muscle is divided into bundles of fibres by a connective tissue network, the perimysium. The muscle bundles, in turn, consist of long, contractile fibres (muscle cells) bounded by a plasma cell membrane, surrounded by a thin connective tissue network, the endomysium. Each contractile fibre is composed of myofibrils 1 to 2 μm in diameter. These myofibrils are separated from one another by spaces containing sarcoplasmic reticulum and mitochondria. Myofibrils consist of contractile filaments ordered in a regular way; this arrangement is responsible for the striated appearance of skeletal muscle. The smallest contractile unit, a sarcomere, consists of thick myosin filaments, thin actin filaments and a network of longitudinal and transverse elements; the cytoskeletal network.

Water in the Muscle: How Much, Where?

From the point of view of quantity, water is the most important constituent of meat. At the time of slaughter, muscle contains about 75% water. Hamm (1975) estimated that approximately 85% of this water is located intracellularly, primarily in the spaces between thick and thin filaments, but also in the sarcoplasm and in connective tissue. The remaining 15% is located in extracellular spaces. According to Hamm (1972), the different muscle components contribute to water-holding capacity as follows: myofibrillar proteins 50%, sarcoplasmic proteins 3%, non-protein components of sarcoplasma 47%.

A portion of water (4 to 10g/100g protein) is bound very tightly. A much larger amount, 20 to 60g/100g protein, of water is located in a less organized fashion between the protein molecules. This loosely (electrostatically) bound water is affected by molecular changes brought about by protein denaturation.

Since proteins form about one-fifth of the wet-weight of meat, and the weight of bound water is about half the weight of the protein (10g), water bound (firmly or loosely) to the protein in meat amounts maximally to about 10% of the muscle weight. The remaining water (70%) must be held within the meat structure by capillary forces. Since myofibrils occupy approximately 70% of the volume of lean muscle, most of the tissue water must be located in the myofibrils. The myofibrils are well suited to retain water because of the three-dimensional network of the filaments. The amount of water immobilized depends on the space available between the filaments.

The Origin of Drip Losses

Considering the amount of water bound to proteins and the amount of water lost from meat, it appears unlikely that WHC is related to the bound water. Any large changes in the distribution of water within the meat structure must, by necessity, originate from changes in myofilament structure. Swell-

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ing or shrinkage of the muscle fibre is caused by expansion or shrinkage of the myofilament lattice and the resultant water movement between intracellular and extracellular spaces. It should be emphasized that swelling or shrinkage of myofibrils alters the distribution of water within the muscle but does not necessarily affect the volume of the muscle as a whole (Offer and Knight, 1989; Fig. 1).

The hypothesis that WHC is determined by myofibrillar shrinkage and movement of water from the myofilament space into the sarcoplasmic space and subsequently into extra- cellular space is supported by experimental data:

NMR:

^{1}H-pulse-NMR is a powerful, non-invasive tool for studying water in tissues. Water protons in muscle have a shorter transverse relaxation time than water protons in bulk water. A long relaxation time suggests a long distance of the free water protons to the site where these can exchange with hydration water. This means that larger "pockets" of water within the structure have a greater chance of obtaining relaxation times similar to that of free water than water in small pores. Post-rigor, there is a larger percentage of water with long (100-150ms) relaxation times, indicating more water in large pores (extracellular spaces) than in pre-rigor meat (Renou et al., 1985; Fjelkner and Tornberg, 1986; Tornberg et al., 1993).

Fibre Diameter Measurements with Light Microscopy

These indicate that the pre-rigor fibre diameter is larger than the post-rigor, e.g. the post-rigor myofibrils are shrunk (Offer and Knight, 1988). Using light microscopy, Penny (1977) and Larsson and Tornberg (1988) observed a larger extracellular volume for PSE (Pale Soft Exudative) meat than for normal meat. Diesbourg et al. (1988) demonstrated that the distance between filaments, measured with X-ray diffraction, becomes smaller during rigor onset, indicater shrinkage of fibre diameter.

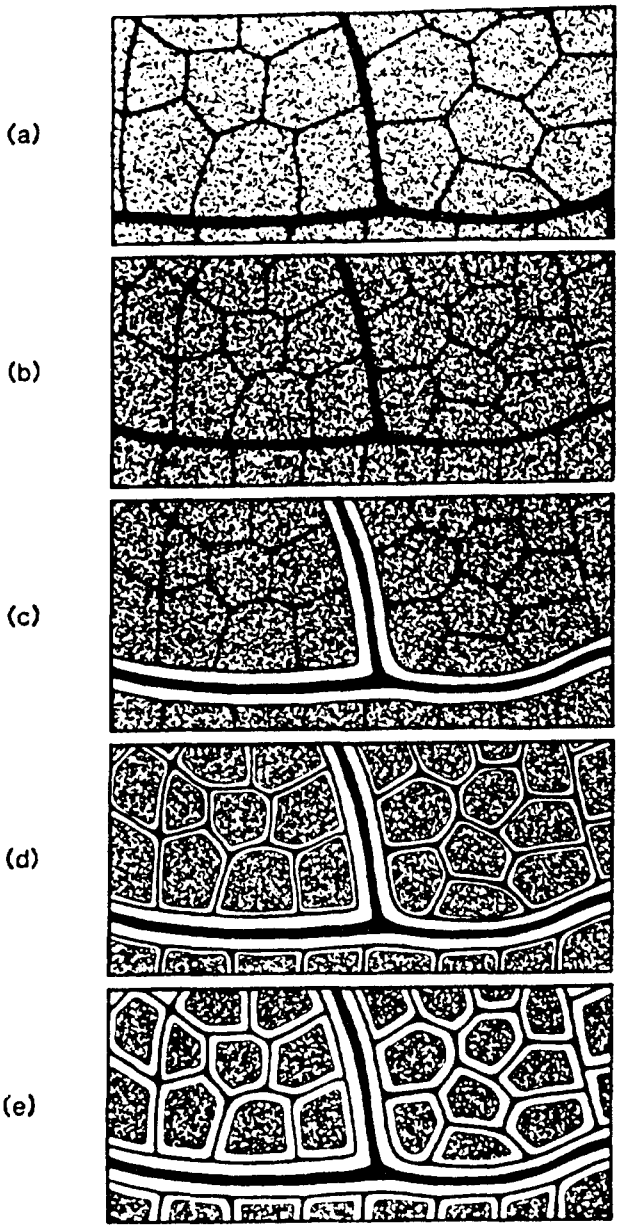
Measurements with Inulin

The extracellular space can be studied by incubating a thin muscle with non-metabolic sugars, such as inulin, which are presumed not to enter the muscle. After equilibration, the amount of the substance in the muscle gives an indication of the size of the extracellular space. Currie and Wolfe (1980; 1983) showed that the post-rigor muscle takes up more inulin than pre-rigor muscle, indicating that post-rigor muscle has a larger extracellular compartment than pre-rigor muscle.

Offer and Cousins (1992) have shown that fibre and fibre bundles shrink post-mortem when their constituent myofibrils shrink, thus resulting in the formation of two extracellular compartments around the fibres and the fibre bundles. Swatland et al. (1989) suggested the following path for drip loss from the muscle:

the myofilament lattice
↓
the interfibrillar sarcoplasm
↓
the interfibre space
↓
the interfascicular space
↓
drip loss

Figure 1



The relationship between fibre volume and muscle volume; (a) to (e) show varying degrees of swelling and shrinkage.
Offer and Knight, 1989.

Offer et al. (1989) confirmed that the drip arises predominantly from the longitudinal channels through the meat between the fibre bundles. The main force for drip formation seems to be gravity.

Presuming that myofibrillar shrinkage (the interfilament spacing) is indeed the main determinant of WHC, we have to focus on the factors that may affect myofibrillar shrinkage. There are three main factors involved in the shrinkage and/or swelling of myofibrils:

1. onset of rigor mortis
2. the extent of pH decline
3. protein denaturation
   a. myosin denaturation
   b. sarcoplasmic protein denaturation

Each of these factors will be discussed in the following paragraphs.

Onset of Rigor Mortis

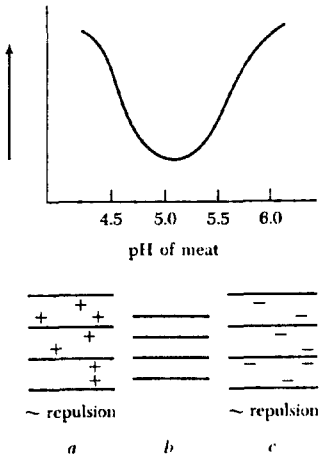
At rigor onset, there is a rapid decline (about 4.4%) in filament spacing due to attachment of myosin heads to actin (Offer et al. 1989). The amount of shrinkage will be smaller at longer sarcomeres (see sarcomere length).

Extent of pH Decline

The importance of the size of the total space between filaments on the amount of immobilized water is illustrated by the effect of pH on water-holding capacity. Around pH 5.0-5.1, WHC is minimal. This pH corresponds approximately to the iso-electric point (I.E.P.) of the myofibrillar proteins. At the I.E.P., the number of positive and negative charges is equal, resulting in a net charge of zero. When pH is higher than the I.E.P., proteins have a surplus of negative charges and there will be repulsion between filaments resulting in a larger interfilament space which can contain more water (Fig. 2).

During post-mortem glycolysis, the pH decreases from 7.0 to approximately 5.5. Thus, the electrostatic repulsion between negatively charged myofilaments decreases and the myofibrils will shrink.

Figure 2



Effect of pH on amount of immobilized water in meat through its impact on the distribution of charged groups on the myofilaments and size of space between them. (*a*) Excess positive charges on the filaments. (*b*) Balance of positive and negative charges. (*c*) Excess negative charges on the filaments.

Effect of pH on water-holding capacity of meat. Wismer-Pedersen, 1978.

Protein Denaturation

Approximately 95% of total muscle protein is located within the myofibre. Depending on their nature and function, intracellular proteins can be classified as sarcoplasmic proteins or myofibrillar proteins. Sarcoplasmic proteins are dissolved in the sarcoplasm, and play an important role in cellular metabolism. These proteins are soluble in low ionic strength solutions. Myofibrillar proteins are insoluble in water or low ionic strength solutions.

A protein in an aqueous solution interacts with water. Part of this water is electrostatically bonded to the surface of the molecule and part may be entrapped. Denaturation of the protein will affect the structure and charge of the protein and therefore the binding of water and solubility of the protein.

Myosin Denaturation

There is good evidence that myosin is involved in WHC; specifically in the loss of WHC in PSE meat.

> Salt solubility of myofibrillar proteins and ATP-ase activity are lower in PSE meat than in normal meat (Bendall and Wismer-Pedersen, 1962; Penny, 1969).
>
> Myosin denaturation is related to the rate of glycolysis (Penny, 1969).
>
> Differential calorimetry of PSE meat indicates myosin denaturation (Starburvik et al., 1984).

The heads of the native myosin molecule are 19 nm long, but when myosin is 'heated' under conditions resembling those in a PSE carcass, the heads shrink to 17 nm. Shorter myosin heads imply that thick and thin filaments are closer and consequently, more water will be expelled into extracellular spaces. This small change in filament distance is sufficient to account for the increased myofibrillar shrinkage observed in PSE meat (Offer and Knight, 1989).

According to Offer (1991), the denaturation of myosin is the decisive event in determining WHC. Based upon this assumption, and the assumption that loss of ATP-ase and head shrinkage involve the same event, Offer (1991) developed a model to predict and explain the effect of pH decline and temperature on the WHC of meat.

Based on in-vitro studies (Penny, 1967a; 1967b), we know that myosin denaturation is influenced by several factors:

Factor 1. pH decline: A fall in pH of 1 pH unit causes the rate of inactivation to increase 20-fold.

Factor 2. Temperature: An increase of 10°C results in a 12-fold increase of myosin denaturation.

Factor 3. ATP: The presence of ATP reduces the inactivation of ATP-ase activity (e.g. myosin denaturation) to about 9% of the values observed in absence of ATP. This means that although pre-rigor temperatures may be high, myosin is protected against denaturation by the presence of ATP.

Factor 4. Actin: The combination of myosin with actin, e.g. formation of actomyosin, protects myosin against denaturation; the rate of inactivation is reduced to less than 1% of that of free myosin. This means that after rigor onset, the myosin denaturation is negligible; events up to rigor are likely to be crucial for the WHC of meat.

During post-mortem glycolysis, factors influencing myosin denaturation do not 'operate' independently. For instance, often a high temperature not only accelerates myosin denaturation, but also accelerates post-mortem glycolysis, resulting in a low pH in the early post-mortem period. Thus, a high temperature results in increased denaturation directly (Factor 2) and indirectly (Factor 3).

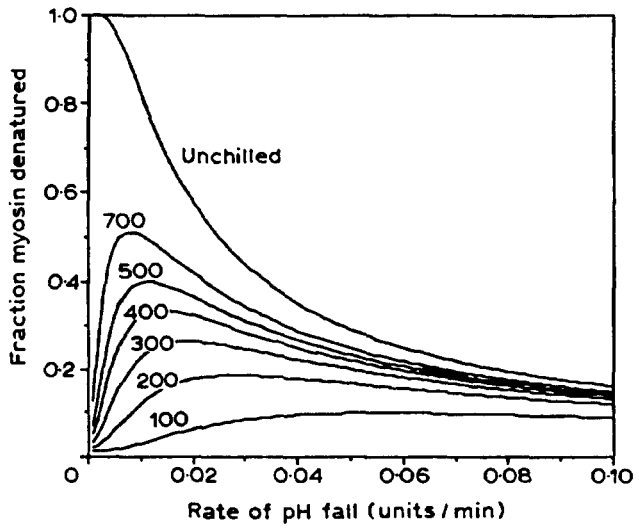
ATP protects myosin against denaturation. A high rate of glycolysis implies a rapid breakdown of ATP. Thus, myosin will be exposed to lower pH, high temperature and low concentration of ATP — conditions very favorable for myosin denaturation.

Offer (1991) emphasized that it is not only the severity of the conditions, but also the ***duration*** of these adverse conditions that determines the impact on WHC. In the past, insufficient emphasis has been given to the importance of ***time*** (duration) during which a muscle experiences these adverse conditions. Based upon calculations, it appears that in a carcass experiencing a relatively moderate pH (decline 0.02 pH units/min) and temperature for a long time, the amount of denaturation might be greater than that seen in a carcass experiencing severe conditions (pH decline 0.1 units/min) for any period of time.

It has generally been assumed that the faster the rate of glycolysis, the greater the degree of protein denaturation and the lower the WHC. However, depending on chilling conditions, there is a rate of glycolysis at which maximal myosin denaturation would be expected. The amount of myosin denaturation would be expected to decline at either slower or faster glycolysis rates. This is illustrated in Fig. 3 (Offer, 1991).

Using the model, one can predict that the benefits of rapid chilling are much greater with the slower and intermediate rates of pH decline than with very fast rates. With very fast rates of glycolysis, the main effect of the faster chilling will be a delay in the onset of rigor mortis, meaning that the muscle will be exposed to adverse conditions for a longer period of time than when it was chilled slowly.

Figure 3



Dependence of the fraction of myosin denatured at rigor on the pH_{45} value. Half cooling times 100, 200, 300, 400 and 700 min.
Offer, 1991.

The model also provides a theoretical explanation for the absence of a linear relationship between rate of glycolysis and WHC. Warriss and Brown (1987) and van Laack et al. (1994) observed that rate of glycolysis (based upon pH_{45} min post-mortem, pH_{45}) has a strong effect on WHC above a certain pH_{45}. Above this value (5.9 in van Laack et al. 1994 and 6.1 in Warriss and Brown, 1987) a higher pH_{45} results in higher WHC (Fig. 4). However, below this critical pH_{45}, the effect of pH_{45} on WHC is minimal. It seems most likely that below a certain pH_{45} (the value depending on chilling conditions) the fast rate of glycolysis is accompanied by an accelerated rigor onset, thus protecting the myosin against denaturation induced by low pH.

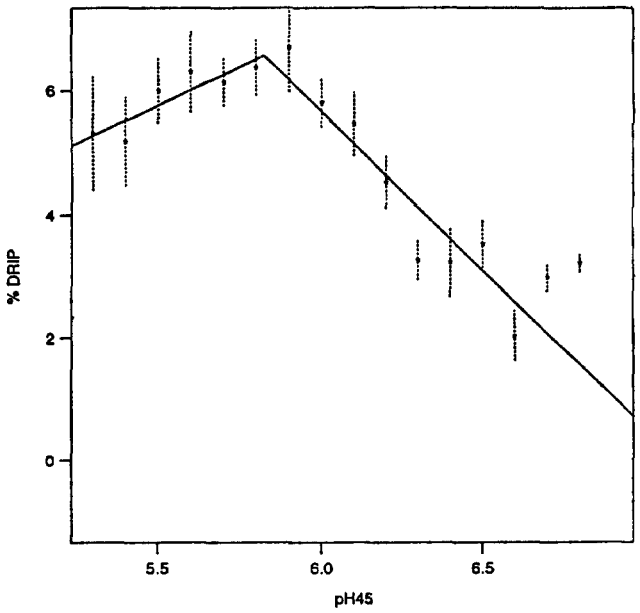
It should be realized that the model is based upon several assumptions. Further research, under strictly controlled conditions, is necessary to establish the validity of the model. Several questions remain:

What level of myosin denaturation will result in the shrinkage observed in PSE meat? Each half of a thick filament contains about 150 myosin molecules and is surrounded by six thin filaments. Hence, the distance between a thick filament and a thin filament is determined by the length of 25 pairs of myosin heads, as well as by the pH. It remains to be determined how the filament spacing depends on the fraction of heads denatured, and if further denaturation causes further shrinkage of the filament lattice.

Does loss of ATP-ase and head shrinkage involve the same event?

We need to distinguish between denaturation of myosin in the H-zones and denaturation of myosin bound to actin, as only the latter may be expected to affect shrinkage of the lattice.

Figure 4



Dependence of drip loss from pork loin (during 2 days storage) on the pH_{45} value.
van Laack et al. 1994.

Sarcoplasmic Proteins

In meat with a low WHC, the solubility of sarcoplasmic proteins is lowered, indicating denaturation of these proteins. Based upon the correlation between sarcoplasmic protein denaturation and WHC, researchers concluded that sarcoplasmic proteins are responsible for changes in WHC. However, the fact that denaturation of sarcoplasmic proteins correlates with lower WHC does not necessarily indicate a cause-effect relationship. Sarcoplasmic proteins only hold about 3% of the water (Hamm, 1972), and can thus not be directly responsible for drip losses in excess of 2.4%. Wismer-Pedersen (1963) suggested that the effect of sarcoplasmic denaturation on WHC is due to precipitation of denatured sarcoplasmic proteins onto myofibrillar proteins, thereby limiting water-holding by the myofibrillar proteins.

Lopez-Bote et al. (1989) studied the extent of denaturation of myofibrillar and sarcoplasmic proteins in PSE, normal and DFD (Dark Firm Dry) meat. They found that there was a better correlation between sarcoplasmic proteins and drip loss (in PSE meat) than between myofibrillar protein denaturation and drip loss. However, they did not determine myofibrillar protein solubility but calculated this by subtracting the sarcoplasmic proteins from the so-called "total protein solubility." We (van Laack and Solomon, unpublished results) found that sarcoplasmic proteins generally denature under the same conditions as myofibrillar proteins. It was impossible to denature sarcoplasmic proteins independently from the myofibrillar proteins, preventing assessment of the contribution of sarcoplasmic protein denaturation to water-holding capacity.

Tornberg et al. (1993) suggested that denaturation of both myofibrillar and sarcoplasmic proteins is important in PSE meat, whereas in normal meat, sarcoplasmic protein denaturation is responsible for drip loss. They suggest the following: Sarcoplasmic proteins cannot cross the cell membrane as long as it is intact, which is presumably most of the time during the rigor process. Being polyelectrolytes, these proteins give rise to an osmotic effect. When the sarcoplasmic proteins start to aggregate, the osmotic effect declines. This will induce a flow of water out of the cell before completion of rigor. When rigor is completed, the cell membranes rupture and the sarcoplasmic proteins fill the extracellular space created during rigor. The aggregated proteins most probably remain in the cell and the extra water outside the cell does not flow back into the cell. The main reason for Tornberg and coworkers' assumption of a role of sarcoplasmic proteins instead of myofibrillar proteins was the higher correlation observed between sarcoplasmic proteins and light scattering and the absence of a significant correlation between drip loss and transverse relaxation times (Tornberg et al. 1993). Winger and Pope (1980-81) estimated the osmotic pressure imparted by sarcoplasmic proteins to be 1.4-1.5 mOs. Osmotic pressure of the whole muscle is ± 500 mOs. It seems unlikely that any change in the sarcoplasmic proteins would have a significant effect on the osmotic pressure. Clearly, more research is necessary to test the hypothesis of Tornberg et al. (1993).

Monin and Laborde (1985) observed a positive effect of sarcoplasmic extract on the WHC of myofibrils and they suggested that sarcoplasmic proteins were involved in this. Hamm (1962) also observed an increase in WHC upon addition of a sarcoplasmic extract to myofibrillar proteins. However, this extract was free of sarcoplasmic proteins. He ascribed this effect to a decrease of the I.E.P. resulting from the anions (phosphate, lactate, chloride and sulphate) in the extract. (Thus, at the same pH there will be more negatively-charged groups, more repulsion and a larger space to contain water).

Other Factors Possibly Involved

There are a few other factors which have been implicated in WHC. These factors do not directly affect myofibrillar shrinkage.

1. sarcomere length
2. aging
3. ions
4. osmotic pressure

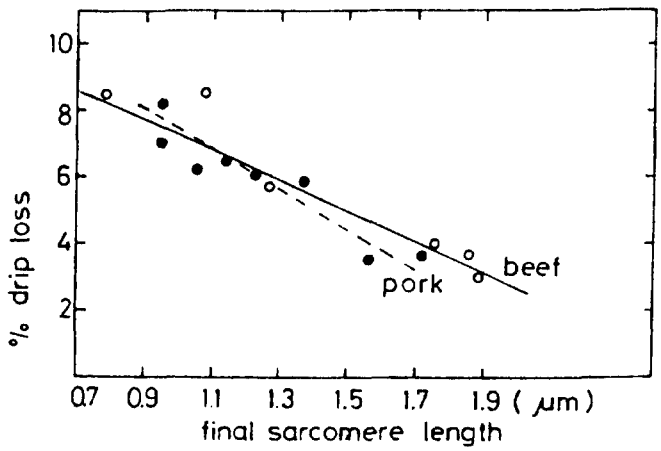
Research on the contribution of these factors to WHC of meat has been very limited.

Sarcomere length

Muscles shorten when they enter the rigor state. The degree of shortening depends on the temperature; from 10° to 37°C, shortening increases with increasing temperatures. However, below 10°C, lower temperatures result in more severe shortening (cold shortening).

Powell (1978) and Smulders et al. (1992) showed that in certain muscles, drip loss could be reduced by hanging the carcasses in such a way that the muscles were restrained from shortening. Honikel et al. (1986) investigated the effect of shortening (induced by temperatures in the range from –2°C to 38°C) on drip production. Drip losses within the first 24h post-mortem were hardly affected by shortening. Even muscles with strongly shortened sarcomeres showed little or no drip formation. The effect of sarcomere shortening became evident only during storage of the meat; drip formation after 7 days at 0° to 4°C was found to decrease linearly with increase of sarcomere length in the range 0.8-1.9 μm (Fig. 5). It was concluded that the higher drip loss in shortened meat probably arose from a reduction in the sarcomere volume.

At longer sarcomere lengths (>2.0 μm), the effect of sarcomere length on drip loss may be due to the relationship



Relationship between sarcomere length and drip loss after 7 days of storage.
van Laack et al. 1994.

between myofilament spacing and sarcomere length. The number of rigor bonds between myofilaments depends on the degree of contraction of the muscle fibre. When the fibres are stretched, and the filaments have little overlap, only a few rigor bonds will result. Thus the effect of rigor on myofilament distance will be limited.

Aging

When meat is aged, a slow increase in water-holding occurs. However, the increase in WHC does not result in WHC levels similar to those of pre-rigor meat. Possibly, this increase in WHC is a consequence of progressive weakening of the linkages between filaments, such as the Z-line.

Ions

Hamm (1972) described the effect of cations and anions on the WHC of meat. Ions affect the water-holding of proteins by affecting the I.E.P. of the proteins. For instance, binding of divalent cations such as Ca^{+2} and Mg^{+2} lowers the WHC. The binding of cations reduces the electrostatic repulsion between the negatively-charged groups by screening effects. Therefore, the protein's structure is tightened and shrinkage occurs. Sequestering of these cations or exchange against monovalent ions resulted in an increase in WHC. The effect of ions on WHC will be small as water bound to proteins accounts for a small percentage of the total water.

Osmotic Pressure

Winger and Pope (1980-81) studied the osmotic properties of beef. Post-rigor beef had an osmotic pressure of about 480-540 mOs. This is almost twice the osmotic pressure of pre-rigor muscle (about 300 mOs). Similar values for post-rigor beef were reported by Geesink (1993) and Ouali (1990). The osmolality of a muscle increases according to contraction speed; osmolality of slow-twitch muscles ranged from 480-500 mOs, whereas values for fast-twitch muscles were ± 550 mOs (Ouali, 1990). The osmolality difference between pre- and post-rigor would imply that water losses would be easier in pre-rigor than in post-rigor meat. Possibly, forces generated by myofibrillar shrinkage occurring during the rigor process compensate for this increase in osmolality. More information is needed before the role of osmotic pressure in formation of drip losses can be established.

Conclusion

We may conclude that there is some understanding of the causes and processes involved in drip formation. However, our knowledge is not complete. We do not know the effect of long-term storage, cannot predict the influence of packaging and cannot explain the variation in WHC observed in different muscles and between species.

The model developed by Offer (1991) is based upon assumptions that need further attention. Also, the validity of the model needs to be tested under practical conditions. Nevertheless, we feel that the model promises to be a useful 'tool' for quality control. The knowledge of the importance of myosin denaturation and rigor for drip formation should be used in development of equipment to predict and select PSE meat.

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