16. Water Holding Capacity

Diana Perry

Learning objectives

On completion of this topic you should be able to:

- Understand what underpins water holding capacity
- Describe factors which alter water holding capacity post-mortem
- Relate these changes to industry practice

16.1 Introduction

The ability of meat to retain its water during application of external forces such as cutting, heating, grinding and pressing, as well as during storage, is called the water holding capacity (WHC) of meat. Water holding capacity is an important quality attribute. It affects appearance of raw meat, its behaviour during cooking, and juiciness when chewed. This is especially so in processed meats such as sausages or mince, where muscle structure has been destroyed and water released from proteins can no longer be held within the muscle structure.

Loss of water holding capacity is evident by exudation of fluid from the meat: “weep”, “purge” or “drip” in uncooked meat, and “shrink” in cooked meats (where it is caused by loss of both water and fat). Product weight losses due to drip can normally range from 1-3% in fresh retail cuts and as high as 10% in PSE (pale, soft exudative) products. There are several areas of muscle from which drip can originate – the space within the myofibril, the intracellular space outside the myofibril and the extracellular space, including the space between the muscle bundles. Loss of water from each of these areas may involve slightly different mechanisms, and occur at different times post mortem.

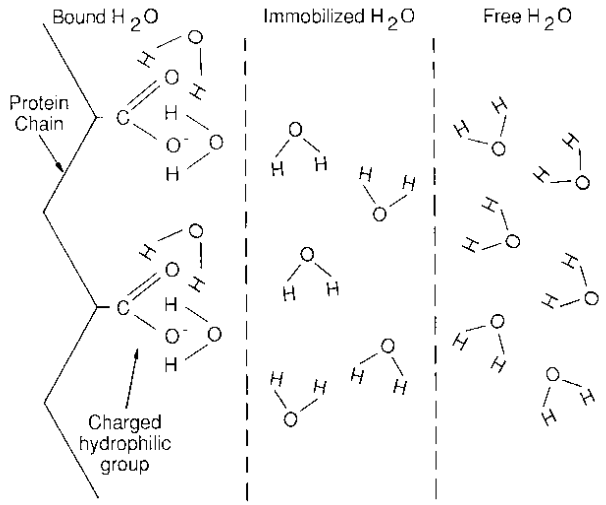
16.2 Location of water in muscle

Lean muscle consists of approximately 75% water. Most of this water is held within the muscle cells (within the myofibrils, between the myofibrils, and between the myofibrils and the cell membrane), between muscle cells and between muscle bundles. Within the myofibrils, it is found between the thick myosin filaments and the thin actin filaments, particularly in the I-band rather than the more protein dense A-band. The size of this space varies three-fold with pH, sarcomere length, ionic strength, osmotic pressure and whether the muscle is pre- or post-rigor (Offer and Trinick, 1983).
Water is a dipolar molecule and as such is attracted to charged species like proteins.

There are three classifications of water in muscle (Figure 16.1).

- **Bound** water is closely bound to protein and has reduced mobility – i.e. does not easily move to other areas. This water is very resistant to freezing and to being driven off by conventional heating. True bound water is a very small fraction (less than a tenth) of the total water in muscle cells.
- **Immobilised (or entrapped)** water may be held by either steric (space) effects and/or by attraction to the bound water. This water is held within the structure of the muscle but is not bound to protein itself. In early post-mortem muscle this water does not flow freely from the tissue, but can be removed by drying, and can be easily converted to ice by freezing. Immobilised water is most affected by the rigor process and the conversion of muscle to meat. With the alteration of muscle cell structure and decline in pH this water can be exuded as drip.
- **Free** water is water which can flow from the muscle unimpeded. Weak surface forces hold this fraction of water in meat. Free water is not easily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structure where it is found.

Figure 16.1 The three forms of water found in muscle. Source:http://labs.ansci.uiuc.edu/meatscience/Library/free%20water%20fig.gif



The major part of the water that is affected by the process of converting muscle to meat is the immobilised water. It is important to maintain as much as possible of this water in the meat. Some of the factors that can affect this are:

- Net charge of myofibrillar proteins
- Intra-myofibrillar space
- Structure of muscle cell and its components
- Amount of extracellular space within the muscle itself

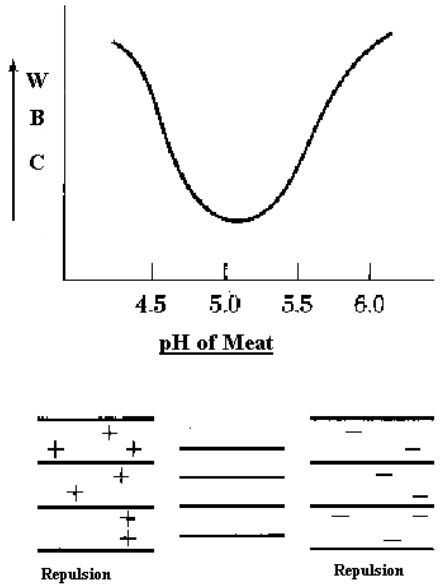
16.3 Net charge effect

There are more negative charges, relative to positive charges, on the protein filaments at high pH levels. As pH declines post slaughter, these charges are reduced. At an ultimate pH of 5.3-55 the isoelectric point of the major proteins (especially myosin) is reached and the net charge of the protein is zero – i.e. the number of positive and negative charges on the proteins are essentially equal. These positive and negative groups within the protein attract each other, which means that less water can be attracted and held by that protein.

More importantly, since like charges repel, as the net charge of the proteins that make up the myofibril approaches zero, repulsion of structures within the myofibril is reduced, allowing these structures to pack together and thus reduce the volume of intra-myofibrillar space (Figure 16.2). Thus at normal ultimate pH, the water holding capacity of meat is lower than that of high pH meat. In high pH meat there is little reduction in intra-fibrillar space, and little loss of attraction between the protein and water molecules. Theoretically, ph values below the isoelectric point of the muscle proteins will also enhance WHC, as shown in Fig. 16.2. This does not occur naturally due to post-mortem glycolysis, but can be induced by latter marination in acidic conditions.

Partial denaturation of the myosin head at low pH levels also causes shrinkage in the volume of myofibrillar spacing. This denaturation is exacerbated if the low pH is combined with high temperatures.

Figure 16.2 Relationship of pH to water holding capacity. The bottom bars show how proteins within the myofibril pack together tighter when net charge on the proteins is zero. Source: http://labs.ansci.uiuc.edu/ meatscience/Library/net%20charge%20effect%20curve.gif



16.4 Rate of pH decline

Accelerated pH decline and low ultimate pH are related to the development of poor water holding capacity and high drip loss. Rapid pH decline resulting in ultimate (or near ultimate) pH while the muscle is still hot causes the denaturation of many proteins, including those involved in binding water. This denaturation causes a loss of functionality and water binding ability. This can result in PSE (pale, soft exudative) product.

Genetics Pigs may have an inherited mutation in the ryanodine receptor/calcium release channel (halothane gene) in the sarcoplsmic reticulum. This mutation results in the impairment of the ability of this channel to properly control calcium release into the sarcoplasm, particularly under periods of physical stress. Accelerated release of calcium causes rapid contraction and an increase in the rate of muscle metabolism and in the rate of pH decline in the carcases of pigs which have been stressed pre-slaughter. Ultimate pH can be lower than normal (<5.3) in these animals.

Pre-slaughter stress Short-term stress in normal animals immediately pre-slaughter can accelerate their metabolism enough that the post-mortem metabolism in the muscle is accelerated, causing a more rapid decline in pH than is seen in non-stressed animals. The condition is not usually as severe as that caused by the halothane gene, but protein denaturation does occur and drip loss can be greater then in muscle that has a slower rate of pH decline. The ultimate pH in these cases is not below normal ranges.

Other post-slaughter effects The loss of ATP and the consequent formation of actomyosin as muscles go into rigor will cause loss of water holding capacity at any pH. This is because:

- WHC of actinomyosin is less than that of myosin and actin from which it forms
- Lower ATP levels initiates denaturation in those proteins whose functionality is most dependent on the provision of energy.
- Extent of sarcomere shortening contributes to loss of WHC.
- Fluid from myofibrils is released, dilutes the sarcoplasm, lowers the intracellular osmotic pressure and thereby increase extracellular space.

16.5 Steric effect

Myofibrils constitute almost 90% of muscle cell volume. Much of the water inside living muscle (about 85%) is located within the muscle cell. Much of that water is held by capillary forces generated by the arrangement of the myofilaments (actin and myosin) within the myofibril. In living muscle, the volume of the sarcomeres remains constant, even during contraction/relaxation (Millman *et al* 1981, 1983). This means that the amount of water within the myofibrils probably doesn't change either. This can change, however, as the volume of intra-myofibrillar space changes during rigor.

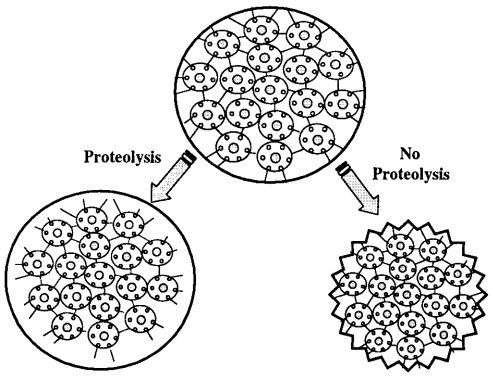
During rigor sarcomeres usually shorten to some extent – this also reduces the available space for water within the myofibril. Drip loss can increase linearly with a decrease in sarcomere length (Honikel et al 1986). As muscle goes into rigor, the net charge effect described above causes a decrease in available space to store water within the myofibril. This forces the water from between the myofilaments (intra-myofibrillar) to the extra-myofibrillar space. It is likely that the gradual mobilization of water from the intra-myofibrillar spaes to the extra-myofibrillar spaces may be the key to providing a source of drip.

The diameter of the muscle cell as a whole may also decrease, as the result of the transmission of lateral shrinkage of the myofibrils to the entire cell via the intact costameres which attach the myofibres to the cell wall. If this happens channels between cells, and between bundles of cells, will form that can funnel drip out of the meat.

Proteolysis If shrinkage of the myofibrillar space is transmitted to the whole muscle cell via the intact proteinaceous linkages between the myofibrils and the cell wall, then it follows that post-mortem changes that affect these linkages will have a consequence on muscle fibre space. Experimental evidence (Melody et al. 2004) supports the idea that the proteolysis of key cytoskeletal proteins such as desmin, talin and vinculin, may be related to drip reduction. These proteins have been shown to be degraded as early as 45 min to 6 hrs post-mortem in some muscles (Melody et al 2004).

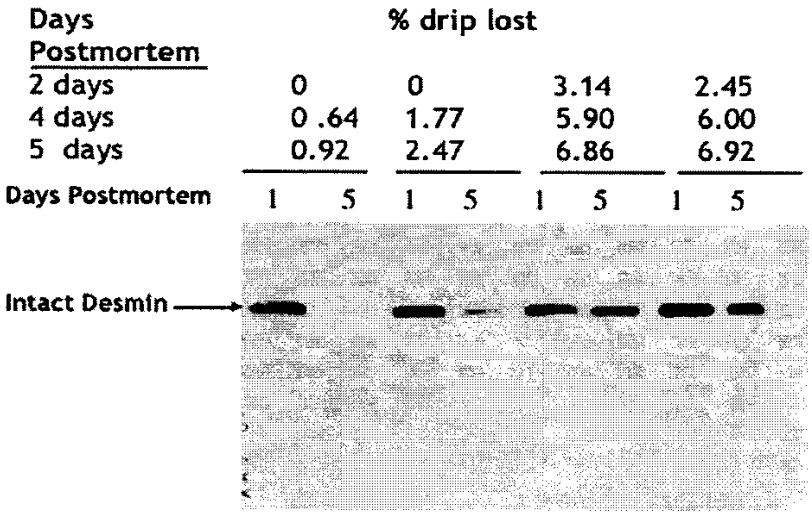
Degradation of these proteins at such an early time post-mortem would allow water that is expelled from the intra-myofibrillar spaces to remain in the cell for a longer period of time, because any shrinkage of myofibrillar space is NOT transmitted to the cell as a whole (Figure 16.3). Conversely, reduced degradation of proteins that attach the myofibril to the cell wall results in increased shrinkage of the muscle cell, which is ultimately translated to drip loss.

Figure 16.3. Schematic view of the potential changes in muscle cell diameter during post mortem ageing as influenced by proteolysis. Source: Huff-Lonergan and Lonergan, (2005). Printed with permission from Elsevier.



Differences in drip loss associated with decreased proteolysis can be seen as early as 24-48 hr post mortem. Figure 16.4 shows that drip loss is higher in the 2 loins which do not display desmin degradation in the first 5 days post mortem.

Figure 16.4. Relationship between desmin degradation and percentage drip loss over the first 5 days post-mortem in 4 pork loins. Desmin is shown at 1 and 5 days post mortem. Lack of a band indicates desmin has been degraded. Source: Melody et al (2004).



Differences in WHC between muscles

- The meat from different species has different WHC.
- Pork (normal) has higher WHC than beef.
- Age of the animal does not affect WHC in pigs, but does in beef (WHC decreases with age).

To some extent this reflects differences in ultimate pH, which is higher in pork and veal than in beef. WHC can also differ between muscles, sometimes for the same reason as above (different ultimate pH), though not all.

- Muscles with a high IMF% tend to have a high WHC
- Longissimus dorsi has lower WHC than psoas in both pork and beef – maybe because the types of proteins are present in different amounts.

16.6 Freezing meat

All the factors affecting WHC apply equally well to frozen or unfrozen meat. With frozen meat, however, removal of water from the muscle during normal freezing provides an additional potential reservoir of fluid which appears as drip on thawing, although this can be largely avoided by very fast rates of freezing. Slow freezing can also cause the formation of ice crystals which pierce the cell walls, so that intracellular water can easily be lost to the extracellular space during thawing. Freezing pre-rigor meat is likely to cause excessive drip as a consequence of thaw rigor.

16.7 Cooked meat

The factors affecting water loss from uncooked meat also apply to the WHC of cooked meat – the relative differences are retained when heat is applied. The losses due to shrinkage during cooking will be greater, and related to such things as temperature and time of cooking, since these affect protein denaturation and the lowering of WHC. Some loss during cooking is also the loss of fat.

- High pH meat has less cooking loss than normal pH meat.
- A fast pH decline will increase moisture loss on cooking

Method of cooking can be important. Water loss when stewing a large piece of meat can be explained in 3 stages (Bendall and Restall 1983).

1. a slow loss of fluid into the extracellular spaces as sarcoplsmic and myofibrillar proteins denature between 40-53oC
2. rapid fluid loss from myofibres as temperature rises to 60oC as the collagen of the basal membrane shrinks
3. heat shrinkage of the endo-, peri- and epimysial collagens between 64-90oC causes shortening, decrease in muscle cell diameter and increased cooking loss

16.8 Juiciness and WHC

The degree of shrinkage during cooking is directly correlated with loss of juiciness (Siemers and Hanning 1953). Juiciness in cooked meat is due to:

- the first impression of wetness produced by the rapid release of meat fluid
- the stimulatory effect of fat on salivation

Juiciness reaches a minimum when the pH level of the meat is about 6 – this possibly reflects the greater ability of the muscle proteins to bind water in this pH region. However this can not be the whole explanation, otherwise juiciness would decrease even more at higher pH levels. Freezing itself does not affect juiciness (unless poorly done and an excessive amount of drip occurs during thawing). However time of storage whilst frozen does, with beef held at -10oC for 20 weeks being less juicy than that held at 0oC for only a few days. This is also apparent during ageing, with roasts and grills being most juicy about 24 hr post-slaughter, and decreasing in juiciness as they are age.

Readings

The following readings are available on CD:

1. Huff-Lonergan, E. and Lonergan, S.M., 2005. Mechanisms of water-holding capacity of meat: The role of post-mortem biochemical and structural changes. Meat Science, vol 71 pp 194-204. Printed with permission from Elsevier.

2. Lawrie, R.A. 1991. Meat Science. Fifth ED. Ch. 10. Pergamon Press. Oxford, New York, Seoul, Tokyo. Printed with permission from Elsevier.

Activities	@ Available on WebCT
Multi-Choice Questions	@ Submit answers via WebCT
Useful Web Links	@ Available on WebCT
Assignment Questions	@ Choose ONE question from ONE of the topics as your assignment. Short answer questions appear on WebCT. Submit your answer via WebCt

Summary

Summary Slides are available on CD

- Water is held in 3 ways within and between muscle cells.
- Immobilised water is the one most affected post-rigor
- Fast rate of pH decline affects WHC because it denatures proteins
- Pre-slaughter stress can increase rate of pH decline
- The halothane gene can increase rate of pH decline

- High ultimate pH meat has higher WHC because the net charge of the proteins is not isoelectric
- Water is squeezed from the intra- to extra-fibrillar space as the space around the myofilaments shrinks
- Where proteolysis does not occur this shrinkage is transmitted to reduction in muscle cell diameter – water is squeezed to extracellular space
- If proteolysis occurs, cell diameter is not so drastically reduced
- WHC is reduced with aging because of denaturation of proteins (a separate and contrary effect to the point above)
- Drip loss increases as sarcomere length decreases

References

Bendall, J.R. and Restall, D.J., 1983. The cooking of single myofibres, small myofibre bundles and muscle strips from beef m. psoas and m. sternomandibularis muscles at varying heating rates and temperatures. Meat Science, vol 8 pp 93-117.

Honikel, K.O., Kim, C.J., Hamm, R. and Roncales, P., 1986. Carcomere shortening of prerigor muscle and its influence on the drip loss. Meat Science, vol 16, pp 267-282.

Hugg-Lonergan, E. and Lonergan, S.M., 2005. Mechanisms of water-holding capacity of meat. The role of post-mortem biochemical and structural changes. Meat Science, vol 71 pp 194-204.

Meloy, J.L., Lonergan, S.M., Rowe, L.J., Huiatt, T.W., Mayes, M.S. and Huff-Lonergan, E., 2004. Early post-mortem biochemical factors influence tenderness and water holding capacity of three porcine muscles. Journal of Animal Science, vol 82, pp 1195-1205.

Millman, B.M., Racey, T.J. and Matsubara, I., 1981. Effects of hyperosmotic solutions on the filament lattice of intact frog skeletal muscle. Biophysical Journal, vol 33 pp 189-202.

Millman, B.M., Wakabayashi, K. and Racey, T.J., 1983. Lateral forces in the filament lattice of vertebrate striated muscle in the rigor state. Biophysical Journal, vol 41, pp 259-267.

Offer, G. and Trinick, J., 1983. On the mechanism of water-holding in meat: the swelling and shrinking of myofibrils. Meat Science, vol 8, pp 245-281.

Siemers, L.L. and Hanning, F., 1953. A study of certain factors influencing the juiciness of meat. Food Research, vol 18 p 113.

University of Illinois at Urbana-Champaign, Department of Animal Science, Meat Science Laboratory, Urbana, Illinois. Retrieved 20th October 2006 from http://labs.ansci.uiuc.edu/meatscience/Library/free%20water%20fig.gif and from http://labs.ansci.uiuc.edu/meatscience/Library/net%20charge%20effect%20curve.gif.