Selected characteristics of fresh meat and influencing factors: a review

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Abstract

The basic characteristics of meat include its colour, water holding capacity and tenderness. These properties are influenced by the chemical composition of the meat (muscle), the proportions of different types of muscle fibres, the physical and chemical condition of the muscle, and the integrity of the internal structures of muscle fibres. The most important variables that influence many of the properties of meat include the pH value, which falls in muscles *post mortem*. The speed and depth of this fall then plays a key role in determining the characteristics of the meat.

Colour, myofibrils, pH, tenderness, water-holding capacity of meat

Introduction

Skeletal muscles consist of highly organised tissue and, along with the skeleton, ensure body movement. Two types of tissue are involved in the construction of skeletal – striated muscles: muscular tissue and connective tissue. The muscles work together with the nervous system and blood vessels in one functional complex. Over the life of an individual, the skeletal muscles perform many functions, not merely the conversion of chemical energy into mechanical energy and heat. In view of the demands placed on these tissues, skeletal muscles must be both strong and elastic. Muscles become meat after the death of animals for slaughter (and game). Consumers, however, have different requirements in terms of meat as food and meat as a living muscular system. They require the meat to be tender, juicy, and of course tasty. How are these properties related to the muscle tissue and how can we influence them? The answer is provided by this review article.

Properties of retail cuts in the eyes of the consumer

Joo et al. (2013) published an extremely apt division of fresh meat characteristics; they defined 3 areas of meat traits: appearance, eating and reliance quality traits.

The list of characteristics of retail cuts that interest consumers may vary slightly depending on the time phase at which the 'meat – consumer' relationship exists. These phases include the moment of buying (selecting) the meat at the retail outlet, the time of meat preparation in the kitchen, and then the final stage – the actual consumption of the meat.

When buying meat, consumers are mainly interested in the following meat characteristics:

- Colour
- Extent of trimming
  - proportion of connective tissue (tendons, fascia, cartilage)
  - fat content
- Drip loss (water-holding capacity)

The colour of the meat informs of its freshness and is determined by the content of myoglobin and its oxidation-reduction status. The resulting colour in fresh meat is always determined by the proportion of oxymyoglobin, deoxymyoglobin and metmyoglobin. The extent of meat trimming also significantly determines how attractive it will be to customers.

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Nowadays, consumers require lean meat with a minimum content of surface fat and adjacent connective tissues such as fasciae and tendons.

The water-holding capacity is another important trait. It expresses the ability of fresh meat to retain its own water during cutting, warming, grinding and pressing, and also during transportation, storage and heat processing (Hughes et al. 2014a). Excessive loss of meat juice is not desirable for retail cuts (or for processing meat either). Not only water is lost with meat juice, but also proteins. Around 112 mg of proteins, mainly sarcoplasmic proteins, is lost from the meat in 1 ml of meat juice (Huff-Lonergan and Lonergan 2005).

The moisture loss (often described as purge or drip loss) can also be influenced by the packaging methods used. Conventional vacuum packaging increases the proportion of purge loss; conversely, skin vacuum packaging (e.g. Darfresh®) has the lowest percentage in comparison to other types of packaging (Kameník et al. 2014). Meat traits that are evaluated by the customer when selecting the meat immediately before purchase have been called appearance quality traits by Joo et al. (2013).

When preparing meat in the kitchen, the cook pays attention to the following traits:
- Meat flavour
- Meat colour
- Extent of trimming

The meat flavour and colour are related to its freshness which is dependent on the condition of the meat at the time of purchase and, of course, on the length of its storage, the temperature of meat storage and the type of packaging. Bacteria begin to multiply on the meat with an increasing period of storage. Their populations can affect the aroma of the meat, and if the number of microorganisms reaches (exceeds) a value of 10^7 CFU·g^{-1} or cm^2, signs of spoilage usually appear, such as variations in the aroma (sour or putrid according to the microbial groups present), discolouration or slime production. In such cases, the meat no longer meets the criteria of food safety, and should be excluded from the food chain.

During the preparation of meat in the kitchen, the water-holding capacity of meat influences the amount of heat loss; the dependence, however, is influenced by the temperature used (Hughes et al. 2014a).

While eating, consumers assess in particular:
- Meat tenderness
- Meat juiciness
- Meat flavour (taste and odour)

The aforementioned meat characteristics have different origins. Most of them (colour, tenderness, water-holding capacity) are based on the chemical composition and physicochemical condition of the meat. Other characteristics are largely, if not exclusively, related to product handling (extent of trimming, signs of meat freshness such as colour and flavour) and the actual culinary preparation. Joo et al. (2013) referred to these properties as eating quality traits.

The influences on meat traits

Hughes et al. (2014a) consider the most important traits of meat its colour, tenderness and water-holding capacity. These traits of meat are influenced by the following factors:
1. The chemical composition of the meat
2. The proportion of muscle fibre types
3. The physicochemical condition of the muscle (meat), especially its pH value
4. The integrity of the internal structure of the muscle fibres

1. Chemical composition of the meat

Meat consists of many substances, though most water and proteins (Table 1). In lean meat, water represents approximately 70 – 75% by weight, proteins around 20%. The basic structural and functional unit of skeletal muscles is the muscle fibre. Proteins in the muscle fibre are represented primarily by two types – sarcoplasmic and myofibrillar proteins. In muscles (i.e. meat), a third group of proteins is present, forming what is known as intramuscular connective tissues (Nishimura 2015). This is mainly collagen which is the basis for the sheaths surrounding each muscle fibre (the endomysium), muscle fascicles (perimysium) and entire muscles (epimysium).

Table 1. The basic chemical composition of skeletal muscles (Kauffman 2012)

Component			
Nitrogenous substances - 21%		Nitrogen-free substances - 79%	
Proteins (19%)	Non-protein nitrogenous substances (2%)	Water (72%)	Fat (5%)
Myofibrillar (11%)	Water-soluble vitamins (< 1%)	Free water (63%)	Minerals(1%)
Sarcoplasmic (6%)	Dipeptides (< 1%)		
IMCT (1%)	Free amino acids (< 1%)	Bound water (9%)	Saccharides and their metabolites (1%)
Proteins of cellular organelles (1%)	Nucleotides (< 1%)		Fat-soluble vitamins (< 1%)

IMCT - intramuscular connective tissue; cell organelles: sarcoplasmic reticulum, mitochondria, T - tubules and others (these proteins are sometimes referred as “granular”)

2. Proportion of muscle fibre types

The formation of the muscle fibre, the “muscle architecture”, is important for the properties of the muscles over the individual’s life and for selected characteristics of meat *post mortem*. The proportion of individual types of muscle fibres in various muscles of the carcass is also important (Anderson et al. 2012). The muscle fibres vary in their morphological, contractile and metabolic capabilities (Ruusunen et al. 2012).

Muscle fibre structure

Up to 87% of the volume of muscle fibres consists of myofibrils (Huff-Lonergan and Lonergan 2005). Their basic components comprise thin actin filaments and thick myosin filaments. The bond between actin and myosin is essential to muscle contraction. The proportion of myosin in muscles is around 26% – the most of all proteins (43% of myofibrillar proteins) (Kauffman 2012).

The myosin molecule constitutes the basic unit of thick filaments (Puolanne and Halonen 2010). It consists of a “head” (S1), “neck” (S2) and “rod”. The rod consists of two polypeptide chains forming an α-helix. Myosin rods are 2 nm x 160 nm in size. The rods aggregate and form the “backbone” of thick myosin filaments, from which units S2 and S1 protrude. The S1 is a contact point with thin actin filaments.

The thick myosin filament is bipolar and contains approximately 200 – 300 myosin molecules. There is a bare zone 150 – 200 nm in length in the central part where there are no myosin “heads”. The core of the thick filaments is probably hollow; the internal diameter of the cavity is 2 nm. The myosin filament is connected by three other proteins (proteins C, M and H) (Puolanne and Halonen 2010).

The basic functional contractile subunit that forms myofibrils is known as the sarcomere. It is a section between two Z-lines (Z-disks) in the myofibrils of muscle fibres (Huff-Lonergan et al. 2010). Titin is another myofibrillar protein that acts as a molecular spring providing the passive elasticity of muscles (Wu et al. 2014). It is the largest known protein with a molecular weight of about 3 000 kDa. It belongs among the proteins creating the cytoskeleton of muscle cells (muscle fibres). Changes to this protein during *post mortem* aging of meat contribute to its tenderness.

3. The physicochemical condition of the muscle (meat)

According to Hughes et al. (2014a), the basic traits of meat, such as its colour, tenderness and water-holding capacity, are mainly influenced by the pH of the meat. England et al. (2015) believe that the quality of pork is determined by two basic factors: the rate of fall of pH in the muscles and the final pH value reached *post mortem*.

Just a quick reminder: the pH value expresses the negative value of the decimal logarithm of the molar concentration of hydrogen ions. The concentration of hydrogen ions is a measure of the acidity of solutions. In acidic solutions, the concentration of hydrogen ions H^+ is greater than 10^{-7}; the pH value is therefore less than 7.0 (Musil et al. 1976).

The pH value of rested muscles is 7.1 – 7.3 (Ferguson and Gerrard 2014) and decreases to a final 5.4 – 5.8 in *post mortem* muscles.

Metabolism, during which energy is released in the muscles after slaughter, causes a fall in pH values due to the accumulation of H^+ ions, and also leads to the production of metabolic heat. *Rigor mortis* (stiffness of death) sets in due to the loss of energy reserves. The temperature of the muscles decreases as a result of the interruption of the blood supply and, in particular, the effect of external refrigeration temperatures. The range of the fall in pH values and the temperature of the muscles during *rigor mortis* are probably the two most important *post mortem* factors that influence the quality of meat with regard to its colour, water-holding capacity and tenderness (Kim et al. 2014).

The activity of muscle fibres during the life of an individual requires a constant supply of energy. The immediate source of energy for the muscle is adenosine triphosphate (ATP). In *post mortem* muscles, muscle fibres attempt to maintain homeostasis and the level of ATP (England et al. 2015).

Anaerobic metabolism is triggered after slaughter and ensures the supply of ATP for cellular functions (Ferguson and Gerrard 2014). Hydrolysis of ATP provides the energy (31 $kJ \cdot mol^{-1}$) required for muscle contractions:

$$ATP + H_2O \leftrightarrow ADP + P_i + H^+$$

In muscle cells, the most immediate tool to maintain the ATP level is creatine phosphate (PCr). Creatine phosphate contains a phosphoric group which is easily transmitted to ADP by means of the creatine kinase enzyme (CK):

$$PCr + ADP + H^+ \leftrightarrow creatine + ATP$$

The PCr supply is depleted very quickly in moving muscles as well as *post mortem* (Ferguson and Gerrard 2014). This increases the level of ADP. In a situation of increased muscle activity, if the consumption of ATP is greater than its production, the adenylate kinase enzyme (AK) generates the formation of ATP from ADP:

$$ADP + ADP \leftrightarrow ATP + AMP$$

Release of adenosine monophosphate (AMP) is an important regulator of energy metabolism in muscles. The final reaction of the entire energy system is catalysed by the AMP deaminase:

$$AMP + H^+ \leftrightarrow IMP + NH_4^+$$

The effect of AMP deaminase is to lower the level of nucleotides (i.e. ATP, ADP, AMP), as IMP (inosine monophosphate) is the final metabolite and is no longer able to re-convert to ATP (England et al. 2015). Complete conversion of nucleotides to IMP is associated with the end of *post mortem* glycolysis. If the activity of AMP deaminase in muscle fibres decreases, then the presence of nucleotides could lead to deepened glycolysis and lower final values of pH (the pH_u) in the muscles (meat). This dependence was demonstrated in the work by England et al. (2015). These authors inhibited the activity of AMP deaminase by pentostatin (purine analogue) and thereby reduced the conversion of AMP to IMP. This resulted in deepened glycolysis, increased catabolism of glycogen and a higher level of lactic acid. The pH_u value decreased to 5.42 ± 0.01 compared to 5.59 ± 0.02 in the control group. The tests were conducted with porcine *m. longissimus*. A lower activity of AMP deaminase was detected in the muscles of pigs with a mutation of the Rendement Napole RN^- gene, accompanied by a lower value of pH_u (England et al. 2015).

The reduction of the PCr content and, thereby, of ATP takes place together with increased levels of H^+ and lactic acid (Ferguson and Gerrard 2014). The fall of pH in the muscles is the result of the accumulation of H^+ ions during ATP hydrolysis. The formation of lactate is often considered part of glycolysis. However, lactate is the end product of anaerobic metabolism and this may be one of the least understood biochemical processes in meat science.

The formation of lactate during anaerobic metabolism serves two purposes. Firstly, this reaction regenerates NAD^+, the coenzyme required during glycolytic reactions catalysed by the glyceraldehyde phosphate dehydrogenase enzyme (Ferguson and Gerard 2014). The NAD^+ coenzyme is used for electron transfer, and not merely in glycolysis (Musil et al. 1976). If there is no NADH oxidation, glycolysis ends prematurely or continues at an extremely slow pace.

Secondly, if glycolysis continues without the formation of lactate, the concentration of H^+ hydrogen ions would increase with great intensity and exceed the buffering capacity of the muscles. This would cause a faster decrease to the pH value in the cytoplasm which would reduce the strength of muscle contractions, leading to exhaustion (Ferguson and Gerrard 2014). The formation of lactate thereby basically prolongs glycolysis by slowing down the increase in the concentration of hydrogen ions in the cytosol. Given the value of the dissociation constant, lactic acid is always dissociated and exists in the muscle tissue more as a salt (lactate) than as an acid.

The accumulation of lactate is a good measure of the extent and rate of glycolysis. It is, however, tricky to use lactate directly to determine the decrease in muscle pH. The above enzymatic reactions are controlled by the level of ADP in the cell. If there is an excess of ATP, no glycolysis is needed. *Post mortem,* glycolysis is affected by the loss of ATP (Ferguson and Gerrard 2014).

The mechanism has been described as to how the values of pH fall in *post mortem* muscles. Now – what can the pH value actually influence in the muscles (meat)? Hughes et al. (2014a) indicate that the speed and intensity (degree) of the fall in pH values influence:

- The denaturation of meat protein
- The space (distance) between myofibrils within the muscle fibres
- The contraction of muscle cells (fibres)

All these factors influence the basic traits of meat, i.e. colour, tenderness and water-holding capacity.

4. The integrity of the internal structure of muscle fibres

Proteolytic processes start at the very beginning in *post mortem* muscles, induced by the action of endogenous proteases. The products of these hydrolytic processes influence the traits of meat, especially tenderness, but also water-holding capacity. Calpains, in particular μ-calpain (Anderson et al. 2012), are considered the main group of proteolytic enzymes in muscle cells, responsible for the *post mortem* degradation of proteins. Proteolysis of key proteins in the muscle fibre, including desmin, vinculin and talin, minimises the decrease of the water-binding capacity of meat caused by the lateral *post mortem* contraction of myofibrils (Bee et al. 2007). The intensity of the hydrolysis of proteins is connected with the pH value in the muscles. When analysing pork, increased autolysis of μ-calpain was detected in the group with the lower pH value shortly after slaughter. Conversely, greater degradation of desmin and talin was observed in the group with a higher pH of the meat (Bee et al. 2007). This means that *post mortem* proteolysis does not only determine meat tenderness, but also impacts on the water-holding capacity of meat.

The influence of muscle structure on the perception of meat colour

Light impacting meat will be partly reflected and partly absorbed or scattered. The meat colour, as we perceive it visually, is always the result of three attributes (Hughes et al. 2014a). Of primary importance, however, is the reflection of light from the meat surface back into the human eye. Instrumental methods involving the use of spectrophotometers or colorimeters are employed for the evaluation of the colour, and the light reflected from the sample analysed is evaluated by colour parameters (a – redness, b – yellowness) and lightness (L). Colour parameters are strongly bound to myoglobin; the lightness is related to the structure of the meat. Together they form an overall impression of the colour of the meat.

In the scattering of light, its diversion and scattering take place after collision with particles of the medium the light has impacted on. In the case of meat (muscle), the medium is the connective tissue, muscle fibres and any liquid that is part of these structures. In *post mortem* muscles, the liquid in muscle fibres relocates due to the fall in pH values. Myofibrils begin to contract as a result. Up to 85% of the water in the muscles is held by capillary forces in myofibrils. Longitudinal and transverse contraction of myofibrils induces pressure, the action of which causes water to move into the extra-myofibrillar space. These phenomena have an effect on the scattering of light and the perception of meat colour.

The incident light can be either absorbed by pigments (myoglobin) in the surface layer of the meat or scattered by structural elements of the muscle fibre. The deeper the light is transmitted into the structure of the meat, the greater its absorption and the less the light is scattered (Hughes et al. 2014b). The result is a darker appearance of the meat. This process is dependent on the pH value. At a higher pH, muscle fibres exhibit a greater diameter and less scattering of incident light. Conversely, in muscles (meat) with a lower pH value, more intense contraction of both myofibrils and entire muscle fibres takes place. Consequently, the light is scattered to a greater extent after its impact and the meat appears lighter.

The value of lightness also increases and the meat becomes lighter during aging of the meat *post mortem*. Degradation of internal structures of the muscle fibre occurs. Degradation of M and Z lines in the sarcomere appears after 6 days of aging. Cracks in the I-bands are evident after 12 days. Protein degradation is also evidenced by the fact that almost double the amount of free amino acids were found in beef meat (*m. longissimus*) after 28-day *post mortem* aging as compared to non-aged beef. Proteolysis therefore increases light scattering and reflection. The result is an increased value of lightness (Hughes et al. 2014a).

The influence on meat tenderness

Meat tenderness is influenced by:

- The amount and solubility of intramuscular connective tissue
- The extent of contraction of sarcomeres in the *rigor mortis* stage
- *Post mortem* proteolysis of myofibrillar and cytoskeletal proteins
- The content of intramuscular fat (Warner et al. 2010)

There is an inverse dependence between sarcomere length and meat tenderness: greater muscle shortening leads to lower tenderness, i.e. greater toughness of the meat. The reason for this may be the less intense proteolysis inside muscle fibres, probably due to the reduction of the I-band which is therefore less exposed to the action of endogenous proteases (Hughes et al. 2014a).

The smallest sarcomere shortening appears in bovine muscles (meat) at temperatures from 14 to 19 °C; the minimal meat toughness also occurs at the same time (Hopkins et al. 2014). These temperatures are, however, unsuitable for the storage of meat from the viewpoint of the growth of bacteria and the shortened shelf life of the meat.

The influence on the water-holding capacity of meat

The water molecule is bipolar and is therefore attracted to particles with an electric charge such as proteins. The pH value decreases during the process of conversion of skeletal muscles into meat. As soon as it reaches the isoelectric point (p*I*) for the main proteins of the muscle cell, in particular myosin (p*I* = 5.4), the number of positive and negative charges in the molecule is the same. These charges are then attracted to each other. This reduces the amount of water held by these proteins (Huff-Lonergan and Lonergan 2005). The interior space of myofibrils is reduced at the same time.

The water in the meat is largely present as free (bulk) water (Tornberg 2013). It can be relatively easily released from the structures of meat in which this free water is held (the space between thin and thick filaments). The ability of meat to hold water or – conversely – the water loss from the meat from the viewpoint of the structure of the muscle fibre is influenced by the following factors:

- The extent of longitudinal and transverse contraction of myofibrils in the *rigor mortis* stage, and the size of the space between thin (actin) and thick (myosin) filaments
- The permeability of cell membranes (sarcolemmas) to water
- The development of channels in the structures of muscle fibres which allow water loss and the size of the extracellular space
- The *post mortem* degradation of proteins forming the cytoskeleton

When the muscles reach the stage of *rigor mortis*, the diameter of the muscle fibres may be reduced by 14 – 16%. Subsequently, the extracellular space in the muscle increases (Hughes et al. 2014b). The space between the thin and thick filaments in the sarcomere is by no means constant, but varies depending on the pH value, the sarcomere length, the ionic strength of the environment, the osmotic pressure, and on whether the muscle is in a state of contraction or relaxation. There can be as much as a threefold change in the volume of this space which quite clearly affects the ability of the meat to hold water (Hughes et al. 2014a). During contraction, part of the volume of free water is forced out, thereby becoming extra-myofibrillar, though still remaining intracellular. If the cell membrane (sarcolemma) becomes sufficiently permeable, this water may be released into the extracellular space.

Myofibrils are interconnected and anchored by protein structures to the cell membrane in the muscle fibres (cells) of living animals. These structures constitute the cytoskeleton. When it is intact, it transmits the myofibrillar contraction in the *post mortem* state to the whole muscle fibre, tending to force water out into the extracellular space. If cytoskeletal degradation occurs during *post mortem* proteolysis, then the contraction of myofibrils does not transmit to the entire cell and the water remains in the intracellular space. This explains the increase in the water-holding capacity of aged meat (Hughes et al. 2014a).

Degradation of the proteins of cell membranes (sarcolemmas) such as, for example, integrin, also occurs in *post mortem* proteolysis (Bee et al. 2007). This increases the volume of channels that cause the loss of water, i.e. meat juice.

Conclusions

Meat quality is determined by a set of properties, the most important of which are colour, the water-holding capacity of meat, and the tenderness or juice content of the meat. These essential characteristics are influenced by many factors, such as the chemical composition of the meat (muscle), the proportions of individual types of muscle fibres in the muscle, the physicochemical properties of the muscle, and the integrity of *post mortem* myofibrillar proteins. Meat properties are highly significantly influenced by the pH value. The rate of fall and the final value of pH can influence the properties of proteins in the muscle fibre. This is then reflected in the colour of meat and its water-holding capacity; the meat tenderness is also altered.

It is necessary to respect the above in breeding practice in pig farming (the proportion of different types of muscle fibres in modern breeds), and especially in the pre-slaughter treatment of slaughter animals. Severe stress factors, such as transport to the slaughterhouse, the unloading of animals at the slaughterhouse and transfer to the point of stunning, can affect the energy metabolism of muscles just before slaughter with a potential impact on meat quality resulting from *post mortem* pH values.

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