Conditioning of meat: the key to consumer acceptance

Conditioning of meat, otherwise known as ageing or ripening of meat, is defined as the holding of unprocessed meat at a temperature above freezing point in the absence of microbial spoilage.

During the period of holding at a temperature between 0-3°C, several changes occur in meat, as a result of which the meat becomes soft and pliable.

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During the process of ageing, atmospheric oxidation proceeds slowly in the dark and bacterial action is retarded to a large extent. Proteolytic enzymes, namely proteases within the muscle fibres, remain active and fragment myofibrils in natural course. Cathepsin and autolytic enzymes play a major role in the ageing of meat.

A combination of these alterations bring about desirable change in the sensory attributes (such as flavour and juiciness) of meat, especially an increase in tenderness. Ageing is important in beef and carabeef, while pork and lamb do not require ageing since they are slaughtered when they are young and inherently tender.

Additionally, the presence of unsaturated fats found in pork-fat oxidises during the ageing process and develops rancidity and off flavours. During the conditioning or ageing process there is tenderisation of meat which involves degradation of myofibrillar and cytoskeletal proteins (troponin, desmin, nebulin, vinculin, dystrophin and titin) in the muscle.

The tenderisation process involves changes in the muscle structure due to degradation of proteins by the breakage at the junction of the I-band and Z-disk, Z- to Z-line attachments are disrupted by the degradation of intermediate filaments, and finally the degradation of Z- and M-line attachments to the sarcolemma.

Eventually, the ultra structural changes that occur within muscle during the ageing process are assumed to be the result of enzymatic degradation of myofibrillar and cytoskeletal proteins. During muscle contraction, thick and thin filaments slide over each other and decrease the length of sarcomere, resulting in the decrease of the length of fibrils, fibres and the whole muscle.

If these muscles remain in a contracted state during the development of rigor, it causes a massive overlap of actin and myosin filaments leading to severe meat toughness. It is also necessary to avoid rapid chilling of meat post slaughter since cold shortening occurs in the meat, resulting in toughness.

Ageing of carcases with little or no fat covering is not recommended by meat specialists since these carcases lose moisture rapidly and discolouration of the meat surface occurs. There are several factors affecting tenderness of fresh meat. Pre-slaughter factors include breed, age of animal, exercise and nutritional profile prior to slaughter, and post slaughter factors include changes in meat during the initial 24 hours, which is very much crucial.

Types of conditioning

For conditioning of meat, cooling of the dressed carcase for 1-2 days at -0.5-3°C and quarters and sides for 10-12 days at 2-3°C is desirable.

In case of retail cuts, the carcase is held for 24 hours at 4.5-7°C. In commercial practice of ageing, the carcase should be held for 2-6 weeks. There are two methods of post-mortem ageing being used commercially – dry-ageing and wet-ageing.

Dry ageing is the traditional process where the entire carcase or wholesale cuts (without protective covering) are placed in a refrigerated room for 21-28 days.

The temperature is maintained between 0-1°C/32-34°F and relative humidity of 86% with an air velocity of 0.5m/s.

During this time, the enzymes in the meat break down the muscle and connective tissue and the meat develops tenderness with a distinct flavour. The relative humidity should be optimum since it helps in retarding microbial spoilage and moisture loss. High temperatures can accelerate the ageing process but the microbial spoilage increases with increased temperatures.

Air velocity is crucial since it helps in moisture removal from refrigerated areas. Insufficient air velocity allows excessive moisture to condense on the product, resulting in off-flavours as well as spoilage.

Too high air velocity causes excessive surface drying and an inedible outer crust is formed which must be trimmed off and discarded.

The carefully controlled environment, the time involved and the trim losses, along with weight loss, makes dry ageing a costlier process.

Wet ageing is carried out in vacuum bags where the refrigerated temperature is between 0-1°C/32-34°F. The process takes about 7-10 days for tenderness and flavours to develop. In wet ageing, humidity and air velocity become superfluous.

The ageing process continues when a primal or sub-primal cut is in vacuum packaging. The vacuum packaging does not allow loss of moisture, so the meat may absorb more moisture, which results in increased juiciness and tenderness of the meat.

Since most meat is vacuum packed, wet ageing is the predominant method of post-mortem ageing of meat. The relative advantages include low cost and longer shelf life of the meat.

The meat from dry ageing has a distinct roasted, beefy flavour, while the wet aged meat has a more bloody and metallic flavour.

Factors affecting the ageing process

- **Ageing rate and time**

Differential rate of ageing means some carcases/cuts tenderise fast, while others tenderise slowly. Muscles that are moderately high to high in connective tissue do not tenderise appreciably since the connective tissue does not fragment sufficiently during ageing. Increasing the ageing time beyond 18 hours results in little benefit to enhance palatability and cause flavour changes.

- **Different muscle groups**

The tenderloin is the most tender muscle group and requires little post-mortem ageing, while the eye of the round is the least tender muscle and requires moreageing.

Mechanism of conditioning

Ageing is a process in which major changes takes place in muscle fibres but meat proteins are also affected by the action of proteolytic enzymes present in meat.

Calpains, cathepsins and proteasome systems have been studied widely to establish their roles in the conditioning of meat. Increased tenderness of conditioned meat may be increase in ionic strength that solubilises the myofibrillar protein particularly those of thick filament.

These factors are physico-chemical in nature and of endopeptidasic behaviour include the osmotic pressure, calcium ions, and the oxidative processes.

The suggested endopeptidases acting individually, or in association, are cathepsin B, cathepsin D, cathepsin L, cathepsin H, m-calpain, μ-calpain, interstitial collagenase, 20 S proteasome and unknown endopeptidases.

Most of the mechanisms proposed for ageing of meat are based on changes occurring at the level of cytoskeletal proteins and structures of the sarcomere (Fig. 1).

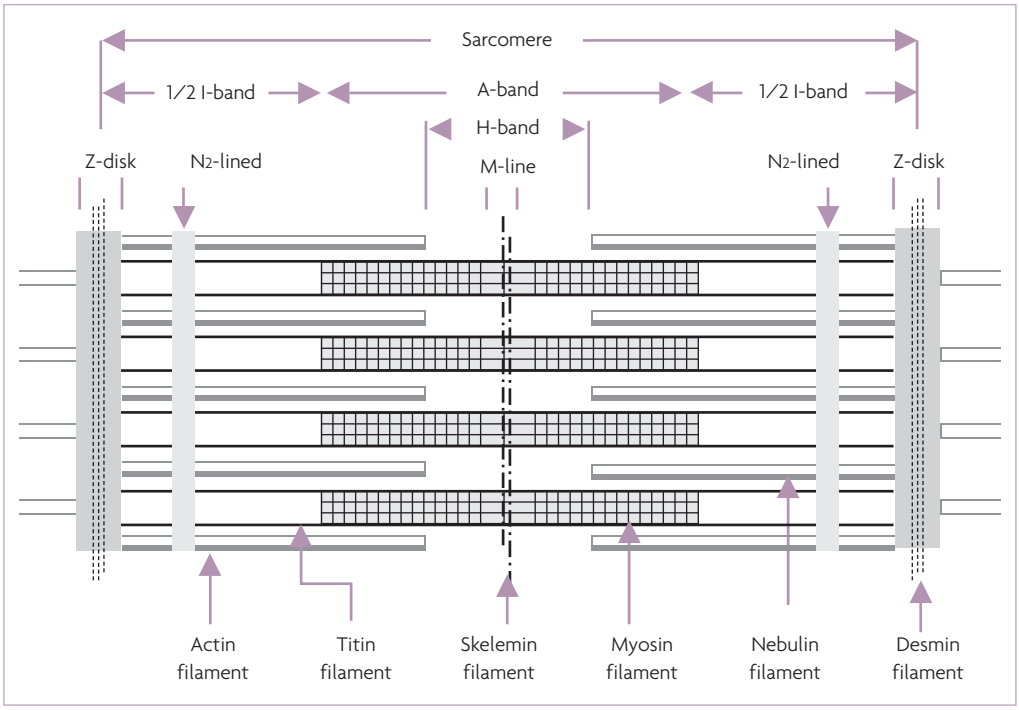

Fig. 1. Diagrammatic representation of cytoskeletal network of the sarcomere, showing its protein filament systems and structures (Prates, 2002).

● Cathepsins

Cathepsins occur in the lysosomes of sarcoplasm. They are released in post-mortem conditions and exhibit maximum activity in slightly acidic condition i.e low ultimate pH and high temperatures and bring about the degradation of myosin and actin. They also degrade the Troponin-T, cross links of collagen and mucopolysaccharides of ground substance.

Cathepsins B and D occur in lysosomes as well as parts of the sarcoplasmic reticulum.

Over 15 lysosomal cathepsins have been identified. Of them eight have been identified in skeletal muscles. Etherington et al. (1990) showed that cathepsins are also basically liable for myofibrillar protein degradation, ensuing meat tenderisation.

This suggestion was in light of the fast arrival of cathepsins from lysosomes at low pH, which causes the progressive myofibrillar weakening during post-rigor mortis. Then again, later after death, PM changes associate most intimately with changes due to lysosomal enzymes.

The interruption of the myofibrils at the N2-line level which has been distinguished as a key factor for meat tenderisation might be attributed to the activity of lysosomal proteins, especially cathepsins B and L.

Myofibrils are widely degraded in vitro by cathepsins B and L and the principle locales assaulted by these proteins appear to be situated close to the N2-lines and at the periphery of the A-band.

Cathepsin H is both an endopeptidase and exopeptidase and is reported to act on myosin. Cathepsin B degrades actin and myosin to a smaller extent than cathepsin D, which fragments both actin and myosin into small peptides. Cathepsin L acts on actin, myosin, α actinin, troponin-I, and troponin-T. Cathepsin has a minimal role in post mortem proteolysis.

● Calpains

In red meat, calpains are more important in the ageing mechanism. This was discovered almost a decade after cathepsins. Calpains have been extensively researched for their role in muscle proteolysis. Calpains are calcium activated proteases located in the cytosol. It slowly disrupts the Z line by releasing α actinin, a protein that holds the thin filament into Z line.

Calpains are activated at neutral to alkaline pH in the presence of calcium, hence originally referred to as Calcium Activated Sarcoplasmic Factor (CASF).

They occur in two forms,

α-calpains (calpains-I).

These are activated by low concentration of calcium ions (50-100∝M) at pH 6.3 after about six hours of slaughter which starts the ageing process.

μ-calpains (calpains-II)

These are activated at high millimolar concentration of calcium (1.0-2.0mM) and pH 6.5-8.0 which causes further tenderisation of meat.

Combined m- and μ calpain activity may be responsible for almost 85% of post-mortem meat ageing.

Calpains are located in the region of Z line which degrades Z line proteins as well as promoting breakdown of other proteins such as tropomyosin and titin. The activity of calpains can be enhanced by infusing carcase post-mortem with 0.3M calcium chloride.

Conversely, infusion of 10mM (millimolar) EDTA sequesters the calcium and calpain activity is reduced. Calpains are inhibited by calpastatin. High calpastatin activity reduces the extent of proteolysis in muscles.

● Proteasomes

For subsequent breakdown of myofibrillar proteins after calpains are released from sarcomere, the multicatalytic proteosome (MCP) or proteasomes degrades polypeptides that have been ubiquitinated and secondly degrades by size limitation i.e only peptides that can pass through narrow 10-13 Å opening to central core of barrel are degraded into 6-12 amino acid fragments in a single pass. They have optimal activity at pH 7.0-8.0.

Intrinsic muscle factors affecting post mortem conditioning

● Oxidative processes

In addition to a decline in pH and an increase in ionic strength, there is a rise in the formation of reactive oxygen species and an increase in protein oxidation within post-mortem muscle. All things considered, the reactive species of free radicals and the nitric-oxide, have been recommended as determinants of muscle tenderisation after death.

A synergistic activity between free radical species and endogenous endopeptidases has additionally been proposed as liable for meat maturing. The production of H_2O_2 is higher during meat conditioning (4°C) than in skeletal muscle incubated at 37°C (1 nmol/min/g). Myosin is the myofibrillar protein most prone to oxidation and, less significantly, troponin-T.

These oxidised proteins may go through polymerisation or potentially fracture, which can add to the weakening of the by and large myofibrillar structure during meat maturing. Davies and Goldberg (1987) showed that oxidised proteins have an increased susceptibility to proteolysis, a significant change which could be connected to meat tenderness.

Reactive oxygen species assault specifically the side chain of amino acid residues and form carbonyl derivatives, to a deficiency of catalytic action and to an increased susceptance to proteolysis.

Supplementation of vitamin E and irradiation to produce a range of oxidation levels in longissimus muscle of beef, researchers have found that increased oxidation of muscle proteins during early post-mortem (<24 hours) negatively impacted meat tenderness.

Since known contrasts in the pace of meat tenderisation between various species can not be clarified exclusively in terms of contrasts in muscle proteolytic activity, more consideration ought to be centered around other potential elements intervening the tenderisation interaction, like free radical species.

Nitric oxide (NO), a gaseous intercellular messenger, has as of late been thought of, by Cook et al. (1998), as a factor of changes in meat maturing.

NO can mediate its effects by free radicals and calcium ions changes, which in turn affect proteolytic enzymes. The mechanism of NO is not well studied.

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Non enzymatic factors affecting ageing

● **Calcium ions theory of meat tenderisation**

The sarcoplasmic calcium ions concentration increases from 0.1-0.8 μM to about 0.2mM during rigor mortis due to the disappearance of calcium ions accumulating potential of sarcoplasmic reticulum and mitochondria membranes.

The calcium hypothesis of meat tenderisation proposes that tenderisation of PM muscle is a non-enzymatic interaction reliant on the increase in sarcoplasmic calcium particles level to 0.1mM.

This calcium ion concentration appears to debilitate the constructions of myofibrils, desmine transitional fibres and likely the endomysium and perimysium, subsequently bringing about tenderisation of meat. The background of this hypothesis are four phenomena, carried out in vitro by 0.1mM calcium particles, which influence the primary integrity of myofibrils: degrading of Z-disks, degrading of rigor mortis linkages between actin and myosin, fragmentation of titin fibres and fracture of nebulin fibres.

These responses are explicit to 0.1mM calcium particles irrespective of proteolysis. The weakening component of Z-disks during maturing of meat is by all accounts due to the binding of calcium ions to phospholipids from Z-disks, bringing about the primary degradation of Z-disks. The translocation of paratropomyosin, a novel myofibrillarprotein, from A- and I-bands junction to the thin filaments can mediate restoration of rigor-shortened sarcomere during conditioning of meat. Titin (about 3,000 kDa) is non-enzymatically split into titin 2 and a 1,200 kDa fragment, during conditioning of meat at 4°C. Similar splitting mechanism takes place when isolated myofibrils are treated with a solution of 0.1mM calcium ions.

● **Osmotic pressure**

During rigor mortis initiation, acidification of muscle is joined by simultaneous increase in osmotic pressure from 300 up to 500-600 mOsmol, which compares to ionic qualities of 0.24-0.30M contingent upon the muscle type.

During rigor mortis, the intracellular osmotic pressure increases nearly twofold and has interrelation with pH (r = 0.97).

It was recommended that pH drop during rigor mortis accounted for a significant value of this change likely through alterations of proteins as well as structures to which ions (primarily Na+, K+, Ca2+ and Mg2+) are ordinarily bound and such ionic strengths are sufficiently high to loosen the myofibrillar structure. This will also make it further susceptible to proteolysis. Myofibrils with such high estimations (0.3M) of ionic strength are responsible for solubilisation of various structural proteins (for example protein C, protein M, actin, troponin T, tropomyosin also, α-actinin.

Simultaneously, a few super underlying changes in myofibrils are seen under electron microscopy (for example loss of thick filament). Along these lines, a potential synergistic activity of the post mortem ascend in muscle osmotic pressure along with the endogenous endopeptidases could be considered typical. From the studies it has been deduced that increased osmotic pressure, along with proteolytic enzymes, has a physico-chemical effect on myofibrillar proteins that could be linked with enhancement in meat tenderness. Approximately 50% of tenderisation occurs in the first 24 hours after slaughter after which the rate of tenderisation becomes exponential.

Changes in meat during ageing

During the conversion of muscle to meat, protease enzymes break down specific proteins in muscle fibres. This breaking of protein strands called myofibrils by natural enzymes results in improved tenderness of meat. The changes can be in terms of protein denaturation, proteolysis and flavour enhancement.

● **Protein denaturation**

Denaturation refers to the rearrangement of chemical bonds present in amino acids of protein polypeptide chains without hydrolysis. The myofibrillar and sarcoplasmic protein denature to a varying degree during PM ageing.

The detachment of actin filaments at Z line result in fragmentation of myofibrils which enhances the tenderness although the muscle proteins manifest some loss of water holding capacity. Elastin does not undergo denaturation.

● **Proteolysis**

During ageing sarcoplasmic reticulum loses the capacity to retain calcium ions and their release initiates activity of calpains which degrades desmins, connectin, M-line protein, troponin and tropomyosin causing tenderisation of meat. Cathepsins also degrade certain proteins and cross links of non-helical telopeptides of collagen and mucopolysaccharides of ground substance thereby improving water holding capacity.

● **Flavour enhancement**

Enhancement of flavour occurs due to production of inosinic acid and hypoxanthine (maximum contribution to flavour) from the breakdown of ATP to mono-nucleotide AMP and IMP. There is also production of flavour compounds such as ammonia, acetone, diacetyl, acetaldehyde and hydrogen sulphide by yeast particularly and by moulds during long term preservation.

Conclusion

Post mortem conditioning or ageing is an essential step in improving tenderness and flavour which results in alteration in the physico-chemical, structural and sensorial characteristics of meat. The quality of meat prior to ageing is crucial. The chemical changes occurring in meat affect the organoleptic characteristics of meat such as appearance, colour, texture, and aroma. Post-mortem ageing of carcases is a natural process which improves tenderness of cuts under refrigerated conditions. Natural enzymes break down specific muscle strands, which results in enhanced tenderness and wider consumer acceptance. Optimum conditioning is very much desirable since too much conditioning results in weight loss, surface spoilage and increased cost of refrigeration storage. ■

References are available from the authors on request