Technological advancements in meat tenderization-A review

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Abstract
Tenderness is one of the most important quality parameters of meat in consumer perceptions. Tenderness of meat depends on various pre-and post-slaughter factors. In the recent past, much of the advancements were occurred in the methods employed for tenderization of meat. These newer methods of meat tenderization resulted in better efficiency and high quality meat products. In this review, tenderness of meat and their mechanism, methods of tenderization and their evaluation techniques have been discussed briefly.

Keywords: Meat, tenderness, ageing, electrical stimulation, tenderization, enzymes.

Introduction
Meat tenderness is one of the most important eating quality parameters (Gonzalez et al., 2001; Nowak, 2011). The other quality parameters being juiciness, flavor, color and fat content. The factors responsible for meat tenderness are the length of sarcomere (Hostetler et al., 1972), the intramuscular connective tissue (Greaser and Fritz, 1995) and proteolytic effect of the muscle (Whipple et al., 1990; Shakelford et al., 1991). After the death of an animal, proteolysis of protein occurs due to endogenous enzymes which have been found to be responsible for tenderization of meat (Ouali, 1990; Koohmaraie, 1992; Koohmaraie, 1994; Penny, 1980; Dransfield, 1992; Asghar and Bhatti, 1987; Mane et al., 2014). According to recent studies, Caspase is a proteolytic system which may be considered to be highly responsible for proteolysis (Nowak, 2011).

Tenderness plays a very important role in deciding the quality of meat by consumers (Koohmaraie, 1994; Koohmaraie, 1996; Boleman et al., 1995; Mendiratta et al., 2010) and is considered to be a critical component of processed meat. It should be ensured that meat reaches an optimum level of tenderness before consumption. In order to tenderize meat, it is aged to about five days in chillers before dispatching into the market for consumption which incurs additional cost to the industry. There are various methods available for meat tenderization. One such method is the use of an electric current which causes fast depletion of energy by triggering muscle contractions. This stage at which all of the muscle energy is depleted from muscles (rigor mortis) can be accelerated from 12-24 hours post-mortem to as little as 2 hours. The muscles reach the rigor mortis stage sooner while they are still warm indicating the tenderization is accelerated at a significant rate.

The red meat industry has undergone significant changes in the past few years. Mechanization has resulted in high-efficiency and high volume cattle slaughter-dressing facilities. Cold shortening has been reduced by the use of temperature conditioning and electrical stimulation. Some newer methods of meat tenderization have been employed which resulted in better efficiency and high quality meat products (Breidenstein and Carpenter, 1983).

Advancements in the methods employed for tenderization of meat

Electrical stimulation (ES)
Electrical stimulation of pre-rigor carcasses has been studied as a method of processing to improve meat quality. Electrical stimulation improves tenderness and enhances lean colour and marbling of beef (Cross, 1979). Solomon et al. (1986) suggested that electrical stimulation of carcasses of young bulls might eliminate toughness and dark-colored lean. Stiffler et al. (1999) studied the effect of electric current on shearing force required to cut the beef (656 beef carcasses) and its sensory properties. The results showed that the shear force was reduced by 23% and the ratings by sensory panel improved by 26%. Electrical stimulation reduces muscle pH below 6.0 and prevents the occurrence of cold shortening. It may release lysosomal enzymes, which degrade proteins and allow more rapid tenderization by changing the
structure of muscle bands through physical disturbance. This technology requires the use of high-voltage electrical current (300V to 700V), operator safety must be ensured.

**Mechanism and set-up:** The pH of a living animal muscle is in the range of 6.8-7.2 which is decreased within 24-48 hours of the death of an animal to 5.6-5.8, which is an ultimate pH of meat. It is evidenced from many studies that meat tenderness is enhanced by the application of high or low-voltage electrical stimulation. When an electric current is passed through a carcass, a decrease in pH occurs resulting in meat tenderization (Simmon, 2006; Seideman and Cross, 1982). This process is known as Electrical Stimulation (ES). The exact mechanism of electric stimulation is still unclear. Electric stimulation induces vigorous muscular contractions and increases muscular energy expenditure which has been found to be the noticeable effect of ES. Various researches have either postulated or concluded that ES increases the rate of postmortem glycolysis (a process by which glycogen is broken down into lactic acid) which leads to a rapid decline in muscle pH. The early onset of rigor mortis before the carcass temperature reaches 15°C minimizes cold shortening. Cold shortening is a condition where muscles are exposed to temperatures below 15°C before they have gone into rigor. This causes the muscles to shorten and likely to be tough. ES causes a release of certain enzymes into muscle which break down proteins in muscle resulting in physical disruption of muscle. Collectively these mechanisms influence meat tenderness, color, flavor and shelf-life (Dransfield, 1987; Cornell and Taylor, 1986; Simmon, 2006; Geesink et al., 2001). This process and technology has benefited meat industry and consumers by providing uniform, consistent and desirable product.

One of the types of electrical meat tenderizing system is non-intrusive systems and the currently available non-intrusive technologies can only measure meat tenderness. These are near-infrared spectroscopy, ultrasound, image analysis and colorimeter (Koohmaraie et al., 2005). A more advanced non-intrusive system is an integrated measurement and tenderizing system. This system can measure pH non-intrusively and at the same time tenderize meat using ES. There is a unique system called SmartStim developed by Carne Technologies Ltd in New Zealand (SmartStim™). It provided a platform for the application of wireless pH sensor network for meat tenderizing process. The architecture of the SmartStim system is shown in Fig 1.

The system consists of three major components-SmartStim application software, stimulation electrode and pulse generator. The carcass is carried on a conveyor brought into contact with the stimulation electrode and ground electrode. The stimulation electrode is segmented. The red segments are used to apply test pulses and the green segments to apply stimulation pulses. The ground electrode provides a return path for electric currents. The carcass response to these pulses is sensed using load cells. The application software running on a PC controls the whole process of pH analysis, stimulation and data storage.

![Fig 1: SmartStim system architecture (Source: Devan, 2010)](image)

The potential commercial benefits of ES are reduced inventory for ageing meat (usually 2-5 days for frozen product), reduced product shrinkage (between 0.5-1% of product weight during storage of chilled product) and increased retail display life in supermarkets (up to 1 day for chilled export lamb) (Simmon et al., 2008). A further benefit is carcass grading so that different grades can be used for different end products such as fresh packed, sausages, vacuum packing and export quality. Fig 2 shows the processing space for creating commercial opportunities for producers and processors. It is of critical importance that there is a balance between stimulation and chilling rate which is to be achieved in a space shaded green. If processed more towards the fast rigor process in red zone would exacerbate PSE (Pale, Soft, Exudative) meat and processing in slow rigor process towards cold short would result in shrinking of muscles and toughening of meat.

SmartStim meat tenderizing system is completely wired pH analysis system which is used to analyze pH and manage meat tenderizing process. The WSN is wireless and dramatically reduces time and cost in system wiring. On the other hand, a wired connection requires a physical wire to be laid to the desired location of the central controller which incurs significant materials and labour costs. In case central controller is relocated or system is expanded, additional costs will add up. One more disadvantage with the use of wired system is long cables and can easily pick up the noise through inductive and capacitive coupling. Noise is one of the reasons for
loss of signal quality and excessiveness in sample variability hence degrades the system reliability and performance (Devan, 2010).

Safety and installation: Food Safety and Inspection Service (FSIS) in conjunction with Occupational Safety and Health Administration (OSHA) has developed safety and sanitation standards and requirements for the installation and use of ES units or devices. Electrical stimulators may be installed upon approval by the Facilities Group of Meat and Poultry Inspection (MPI) and by the Equipment Group, Facilities Group, Technical Services, Meat and Poultry Inspection, FSIS, USDA, Washington, DC 20250.

Persons who work near or operate such equipment must recognize the potential danger associated with high voltage electrical stimulators which produce lethal quantities of electricity. Because of this, FSIS requires barriers at all openings to stimulator, flashing or rotating lights and audible signals to warn plant personnel that unit is operating. “Danger-High Voltage” signs must be displayed prominently and emergency stop buttons to shut off the electrical current must be plainly labelled. The power supply must be locked in an OFF position when not in use to prevent unauthorized personnel from turning on the stimulator. Also a fail-safe system must be installed around the stimulator to prevent personnel from entering in to the area while the unit is operative. Low voltage units which are now available to packing plants offer a significant safety advantage compared to high voltage electrical stimulators (Stiffler et al., 1982).

Hydrodynamic shock wave-pressure technology-Hydrodyne process

The Hydrodyne process and equipment have been designated to tenderize meat by exposing meat cuts to an explosive charge that generates and applies a shock wave (Solomon, 1998a). This supersonic, hydrodynamic wave with a targeted pressure front of 68,948 kPa occurs in fractions of a millisecond and is applied to packaged meat suspended in water. The wave passes through objects that are an acoustical match with water (meat, which is approximately 75% water, is a close acoustical match) and ruptures proteins. The sarcomeres are ruptured indiscriminately through the myofibrillar proteins, z disks and surrounding structures (Zuckerman and Solomon, 1998). The explosive which is used to create a shock wave is a combination of nitromethane (liquid) and ammonium nitrate (solid). These components are not explosive until combined (Solomon et al., 1997). The amount of explosive and distance from explosive to surface of the packaged meat product determine the amount of force imposed on meat (Solomon et al., 1997). Solomon (1998b) reported this process performed at 3 d post-slaughter improved shear force values of Longissimus, Semimembranosus, Biceps femoris, and Semitendinosus muscles by 5.5, 6.2, 4.1, and 7.2 kg, respectively, and enhanced taste panel tenderness evaluation. Electron microscopy revealed the enhancement of tenderness was due to myofibril fragmentation (Zuckerman and Solomon, 1998).

Hydrodynamic pressure wave generated in a steel chamber is less effective as compared to disposable/plastic container for tenderizing meat. Even, the composition and configuration of the explosive containers influence the magnitude of performance of this Hydrodynamic process on meat tenderization. This process results in slightly more brown appearance in cooked patties predisposed to persistent pink colour at elevated internal temperatures. However, extremely tough meat exposed to a hydrodynamic shock wave pressure front was made significantly tender and acceptable. Regardless of the type of meat cut and level of initial toughness, hydrodynamic pressure technology has been successful at increasing the value of these meat products by improving tenderness instantaneously.

Ultrasound

Use of ultrasound has been investigated as a method to tenderize meat (Meek et al., 2000). It has been suggested that ultrasonic techniques cause lysosomal rupture and disruption of myofibrillar protein and connective tissue which result in tenderization of meat (Lyng et al., 1997). The lysosomal rupture is due to the cavitation process. There exists two types of cavitation of interest. The first one is reffered to as stable cavitation in which the bubble or cavity grows to a resonant size and oscillates due to ultrasound. Within a biological media (steaks), the bubble is suspected to produce hydrodynamic forces which affect the integrity of muscle structure. The second and more severe form of cavitation is called collapse or transient cavitation. The violent hydrodynamic forces due to a collapsing bubble or transient cavitation can cause severe damage within biological media such as meat.
specimen in this case, thereby damaging the fiber structure of the muscles (Smith et al., 1991). In a study of low frequency, high intensity ultrasound baths on beef steaks, Lyng et al. (1997) reported that the ultrasound baths were not effective in improving the tenderness of intact beef steaks. These results are inconsistent with a study in which high intensity; low frequency (26 kHz) ultrasound was determined to tenderize Semitendinosus beef muscle when sonicated for 2 and 4 minutes (Smith et al., 1991).

**High Hydrostatic Pressure**

The use of high pressure processing (HPP) to tenderize meat has a potential to revolutionize red meat industry since tenderization effects are highly variable between meat carcasses (Mane et al., 2014). By understanding biochemical mechanisms of muscle breakdown, processors can utilize HPP to “turn on” and promote endogenous enzyme systems that tenderize muscle protein. HPP can increase the activities of certain enzyme systems such as those of the calpain family resulting in an increase in tenderization. Similarly, the activity of added proteases such as papain can be enhanced by HPP (Raghubeer, 2007).

Hydrostatics is the study of characteristics of liquids at rest or the force that a liquid imposes on a submerged object (Solomon et al., 1997). MacFarlane (1973) studied the effects of hydrostatic pressure on beef and lamb. An improvement in tenderness was determined when meat was treated with hydrostatic pressure of $1.05 \times 10^7$ kg/m$^2$ at 30$^\circ$C to 35$^\circ$C for 2 min. Kennick et al. (1980) confirmed the results of previous study and determined that hydrostatic pressure accelerated meat aging and improved tenderness. Water-holding capacity (WHC) was decreased due to cellular disruption in pressurized samples (Kennick et al., 1980). Further sensory studies have not been conducted to determine consumer response to pressurized meat. Kennick et al. (1980) reported obvious visual contraction of treated muscles and MacFarlane (1973) reported a noticeable change in firmness of treated raw muscles. This technology lacks extensive development and industrial applications found in other areas.

**Proteolytic Enzymes**

The scientists agree that tenderization of meat during storage results from a proteolytic degradation of myofibrils and associated components by endogenous muscle proteases. This was followed by discovery of different proteolytic systems and their ability to mimic biochemical and structural changes affecting postmortem muscle. It has been evidenced that this process of meat tenderization is multi-enzymatic in nature and involves a large set of endogenous proteolytic enzymes acting in a synergistic manner (Foegeding et al., 1996).

Many studies suggest that tenderness is dependent on such enzymes as cathepsin and calpains. Some researchers suggest that proteosomes may be responsible for final tenderness while others suggest that caspases are responsible for the same (Bernard et al., 2007; Kemp et al., 2010). However, many studies have assigned this role to the calpains system (Neath et al., 2007; Kemp et al., 2010).

Calpain system is the major enzyme within skeletal muscle involved in meat tenderization. Calpains require calcium for activity and calcium can be added to meat to activate the system and induce more rapid and extensive tenderization. Calcium chloride (2.2% solution) is injected into meat cuts either pre- or post-rigor which results in an enhanced tenderness within 24 h postmortem changes (Mane et al., 2014). This process of tenderization is called calcium-activated tenderization. This type of meat tenderization has been shown to enhance tenderness in tough beef muscle without having an adverse effect on flavor, color or microbial count. Consumer response towards this kind of meat has been found to be positive.

Cathepsins are a group of enzymes comprising exo- and endo- peptidases and categorized into cysteine (cathepsins B, H, L, X), serine (cathepsin G) and aspartic (cathepsins D, E) peptidase families (Sentandreu et al., 2002; Kemp et al., 2010). The participation of cathepsins in tenderization is doubtful as there is no proof that during post-mortem storage of meat, cathepsins are freed from lysosomes. Moreover, cathepsins have the
ability to disintegrate myosin, actin and α-actinin during normal ageing of a muscle, a small quantity of these proteins is degraded (Koohmaraie, 1988; Whipple and Koohmaraie, 1991; Purslow et al., 2001; Koohmaraie, 2006; Koohmaraie, 1996; Geesink et al., 1994).

Enzymatic processing has an advantage over acid or alkaline hydrolysis of proteins in that it requires simple equipment and does not result in destruction or racemization of amino acids (Underkofler et al., 1957).

**Supplementation of Vitamin D₃ to tenderize meat**

It has been researched that one of the factors that turns-on the tenderization process is an elevation of calcium in muscle (Morgan, 2007). A correlation between vitamin D₃ and calcium elevation has been studied. Early studies using lactating dairy cows showed that oral administration of vitamin D (VITD) for 2 weeks prepartum increased serum calcium (Hibbs et al., 1951; Hibbs and Poundsen, 1955). Additionally, injections of vitamin D metabolites (i.e., 25-hydroxyvitamin D₃, 1, 25-dihydroxyvitamin D₃) increased serum calcium concentrations of dairy cows (Bar et al., 1985; Sachs et al., 1987). These procedures proved to be potential methods that could be used to elevate muscle calcium and in turn activate postmortem tenderization process.

One of the most consistent findings between all of the trials investigating an impact of VITD supplementation on meat tenderness is a noticeable spike in serum calcium that peaks during second day of supplementation (Fig 4). Pooled results suggest that supplementing beef cattle diets with at least 0.5 million IU of VITD per animal each day caused a 30% to 35% increase in blood calcium concentration. Maximal calcium concentration was maintained for an entire time of supplementation. Animals not fed with supplemental VITD showed no change in calcium concentration throughout the entire pre-harvest dietary period.

The first evidence that associated the involvement of calcium with process of meat tenderization during postmortem aging was noted over 30 years ago (Davey and Gilbert, 1969). They indicated that weakening and disappearance of muscle structure during postmortem aging was inhibited by ethylene diamine tetra-acetic acid (EDTA). They also speculated that EDTA might exert its effect by chelating calcium ions. It appears that VITD supplementation accelerates an aging process by shifting calcium to inner areas within muscle cells. In normal muscle, calcium is primarily housed in compartmentalized structures. If VITD can solicit calcium from these areas and into a closer proximity to muscle cells, calcium mediated enzymes can tenderize meat more easily and readily during postmortem aging. Initial results demonstrated that VITD supplementation primarily improve beef tenderness ratings by reducing the percentage of tough steaks, bringing them back into a more acceptable tender category (Swanek et al., 1999; Kargas et al., 2001; Montgomery et al., 2000; Montgomery et al., 2002). However, more recent findings involving VITD supplementation and its impact on cooked beef tenderness conflict these findings and certainly raise questions about the commercial application of VITD (Scanga et al., 2001).

![Fig 4: Influence of vitamin D supplementation on blood calcium concentrations](Image 330x478 to 527x584)

A study was conducted in University of Limpopo Experimental farm at Syferkuil. Experiment was conducted during winter period with the temperatures ranging between 16°C and 25°C. It was concluded from the results that vitamin D₃ supplementation improved tenderness and flavor of aged Venda cock meat. This offers a solution to the problem of hardiness of indigenous chickens. However, Vitamin D₃ did not improve tenderness and flavor of unaged Venda cock meat (Mabelebele et al., 2012).

**Pre-rigor injections of ionic compounds**

Infusion refers to an introduction of a solution via vein and perfusion should refer to the introduction of a solution via an artery (Polidori and Francesco, 2003). Infusion or perfusion of compounds to change the rate of glycolysis, rate and state of contraction and rate of proteolysis appear to be feasible methods of manipulating postmortem tenderization process in meat (Lee et al., 2000; Koohmaraie et al., 1990; Morgan et al., 1991; Wheeler et al., 1991). It was reported in mid 1950s that infusion of bovine carcasses with salt solutions caused a considerable improvement in tenderness (Geesink et al., 1994). Salts generally influence the
Postmortem proteolysis and identification of protein markers for tenderness using proteomics approaches

Tenderness plays very important role in determining consumer acceptability of meat. Recent studies, role of proteomics in postmortem proteolysis and its relation to meat. Proteomics has also been used by several groups to identify potential protein markers for tenderness. However, applying this new knowledge to improve tenderness is a significant challenge for meat scientists. Fortunately, recent advances in technology and reduction in costs mean that today’s potential sites are routinely genotyped and data is being used to improve stock by genomic and marker-assisted selection. The success of genetic breeding programs is dependent upon access to high-resolution phenotype data. If our efforts to detect potential protein markers for tenderness are successful and they have a sufficient genetic contribution then it should be possible to associate these with genetic markers and improve breed and meat tenderness through selective breeding (Veiseth-Kent and Hollung, 2011).

Evaluation of tenderness using newer methods

Electronic evaluation (instrument grading) of carcasses to determine quality, yield grade and tenderness is a technology goal of numerous research projects. One such instrument is the BeefCam equipment. A study was conducted in which the system was used for evaluation of 296 steer and heifer carcasses (Patterson et al., 2000). Of those certified to produce tender beef, 98.6% produced tender steaks as based on the Warner-Bratzler shear force data and consumer sensory panel ratings.

Conclusion

The final tenderness of meat is determined by the rate and extent of postmortem proteolysis of key myofibrillar proteins in muscle. The much faster the proteolysis of myofibrillar proteins occurs; the much faster will be the tenderization. There has been lot of technological advancements in the past few years in the process of tenderization resulting in better quality meat and improved palatability. But yet there are some limitations in these methods which can be overcome by some other methods developed or proposed in the near future. The use of electrohydraulic shock waves has proved to be a promising technology for tenderization. Much research needs to be done to find some other alternative methods for meat tenderization with better efficiency than the existing methods.

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