Emerging trends and technologies adopted for tenderization of Meat: A review

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Abstract
Meat tenderness appears as top rated issue to be solved concerning meat sensorial quality, which requires enhanced knowledge and further work to understand the processes involved. Meat tenderness plays an important role, where entire pieces of meat are cooked, fried or barbecued. In these cases some types of meat, in particular beef, have to undergo a certain ripening or ageing period before cooking and consumption in order to achieve the necessary tenderness. In the fabrication of many processed meat products the toughness or tenderness of the meat used is of minor importance. Many meat products are composed of comminuted meat, a process where even previously tough meat is made palatable. Further processing of larger pieces of meat also results in good chewing quality as these products are cured and fermented or cured and cooked, which makes them tender. 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, emerging trends and technologies in meat tenderization have been discussed briefly.
Keywords: Meat, technologies, tenderization, proteolytic enzymes, Ultrasound, cavitations, Hydro-dyne process

Introduction

Meat tenderness is one of the most important quality parameters of meat in consumer perceptions. It is one of the most important eating quality parameters. (Nowak, 2011). Tenderness is considered the most important palatability characteristic of meat. Therefore a critical component of processing meat is to ensure that meat reaches the optimum level of tenderness before consumption. Meat is 'aged' up to five days in chillers to improve tenderness before being released to the market for consumption, which constitutes significant cost to the industry. Tenderization can be accelerated by electrical stimulation, whereby currents are used to trigger muscle contractions and accelerate the depletion of muscle energy. Rigor mortis, the point at which all muscle energy is depleted, can be accelerated from 12-24 hours post mortem to as little as 2 hours. Reaching rigor mortis sooner while the muscles are still warm after slaughter means that the tenderization process is significantly accelerated. Tenderness is actually a quality of meat gauging how easily it is chewed or cut. Tenderness is a desirable quality, as tender meat is softer, easier to chew, and generally more palatable than harder meat. Conversely, tender pieces of meat generally acquire higher price than harder ones. The tenderness depends on a number of factors including the meat grain, the amount of connective tissue, and the amount of fat. Tenderness can be increased by a number of processing techniques, generally referred to as 9o or tenderization. The factors responsible for meat tenderness are the length of sarcomere, the intramuscular connective tissue and proteolytic effect of the muscle. 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 (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 (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.

Emerging trends and technologies adopted for tenderization of Meat

There are various methods available for meat tenderization. Some newer methods of meat tenderization have been employed which resulted in better efficiency and high quality meat products (Breidenstein and Carpenter, 1983). various newer methods employed in meat tenderization are discussed briefly in this manuscript.

Meat tenderization by proteolytic enzymes after osmotic dehydration

The treatment of proteolytic enzymes is one of the popular methods for meat tenderization. In this case, it is very important how to introduce the enzymes into the meat cut. This method involves the dipping the meat cut in a solution containing proteolytic enzymes after contact-osmotic dehydration. After the dehydration of each piece of meat from culled cow for 18 h by contact-dehydration sheet, each sample was dipped for 3 h in a solution containing papain or proteinases from Aspergillus traditionally used for soysauce production in Japan. It was stored at 3∼4°C for 24, 48 and 168 h, and subjected to texture measurement, sensory evaluations, biochemical analysis and histological observations. The penetration efficiency of the enzyme solution (of around 80%) after the contact-osmotic dehydration seemed to be sufficient. A marked decrease in hardness by texture measurements was observed in the meats treated with proteolytic enzymes and higher sensory scores for tenderness were observed in the meats treated with enzymes as compared with the untreated meat. The papain-treated meat received the highest score in tenderness, but the scores given to juiciness and taste were lower than that of the control. The rapid increases of the fragmentation of myofibrils from the enzyme-treated meat were observed at first 24 h of storage as compared with that of the control. Remarkable degradation of myosin molecule in the myofibrils from the enzyme-treated meats was observed on SDS-PAGE profiles. Considerable degradation of myofibrilar structure especially due to proteolytic removal of Z-lines, was observed among the myofibrils from enzyme-treated meats by electronmicroscopy. The remarkable deformation and disruption of honeycomb-like structure of endomysium were also observed in the meats treated with enzymes. (Gerelt *et al.*, 2000).

Plant enzymes (such as papain, bromelain, and ficin) have been extensively investigated as meat tenderizers. New plant proteases (actinidin and zingibain) and microbial enzyme preparations have been of recent interest due to controlled meat tenderization and other advantages. Successful use of these enzymes in fresh meat requires their enzymatic kinetics and characteristics to be determined, together with an understanding of the impact of the surrounding environmental conditions of the meat (pH, temperature) on enzyme function. This enables the optimal conditions for tenderizing fresh meat to be established, and the elimination or reduction of any negative impacts on other quality attributes.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 multienzymatic in nature and involves a large set of endogenous proteolytic enzymes acting in a synergistic manner. Many studies suggest that tenderness is dependent on such enzymes as cathepsin and calpains. Calpain system is the major enzyme within skeletal muscle invoved 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 postrigor which results in an enhanced tenderness within 24 h postmortem changes. 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 (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, 2005).

Meat tenderization by electrical stimulation (ES)

At postmortem, one of the major interventions adopted in the meat industry for enhancing meat quality traits is carcass electrical stimulation (ES), a practice whereby an electric current is transmitted through the carcass of animals that are freshly slaughtered and eviscerated. In the course of postmortem glycolysis, the muscle undergoes rigor development marked by series of histological, physical and biochemical processes. Therefore, modification of one or more of the processes could eventually alter meat quality traits. The need to curb variability in meat quality traits and to enhance sensory properties of meat necessitate the application of ES. However, it must be realized that the beneficial effects of ES can only be achieved if there is sufficient muscle glycogen before an animal is exsanguinated. (Dutson et al. 1982). 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. The potential commercial benefits of ES are reduced inventory for ageing meat (usually 2 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).

Meat tenderization by Ultrasound

Ultrasound is one of the new clean technologies applied to meat. In science and technology of meat, is mainly studied for its ability to improve meat tenderness by cavitation mechanisms. Some acoustic parameters such as frequency, intensity and exposure time influence the treatment of meat tenderization (Meek et al.,2000). Initial studies determined that the use of high frequencies did not show effects on the texture, due to not cause cavitation. The intensity in which the ultrasound reaches the meat matrix is also important, and when applied below 10W cm^{-2} or much above this value does not realize the effect. The exposure time is dependent on the frequency and the used intensity directly influences the softness. Meat quality characteristics, such as weight loss after cooking, drop in pH, color and microbiology were also analyzed by several authors, with conflicting data on the effect of ultrasound on these parameters. The particularities of each muscle hinder comparisons of results, stimulating new researches. The use of ultrasound technology to improve meat tenderness shows itself as a promising technology with the potential to be exploited (Alves *et al.*, 1991)

The use of ultrasound technology to improve meat tenderness shows itself as a promising technology with the potential to be exploited.mIt has been suggested that ultrasonic techniques cause lysosomal rupture and disruption of myofibrillar protein and connective tissue which result in tenderization of meat. 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 cavitataion 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 (Solomon MB *et al.*, 1997).

Hydrodyne process

The Hydrodyne process uses a small amount of explosive to generate a shock wave in water. The shock wave passes through (in fractions of a millisecond) objects in the water that are an acoustic match with water. Four beef muscles (longissimus, semimembranosus, biceps femoris, and semitendinosus) exposed to either 50, 75, or 100 g of explosives were significantly tenderized compared with controls. As much as a 72% reduction in shear force was observed for the longissimus muscle using 100 g of explosives. Reductions in shear force with magnitudes of 30 to 59% improvements were observed for the other three muscle types. Results suggest that tenderizing beef with the Hydrodyne process presents a potentially novel opportunity in the way the meat industry can tenderize meat (Smith *et al.,* 1991).

Hydrodynamic pressure wave generated in a steel chamber is less effective as campared 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 (Solomon *et al.,* 1997).

Meat tenderization and High Hydrostatic Pressure (HHP)

Hydrostatics is the study of characteristics of liquids at rest or the force that a liquid imposes on a submerged object. High hydrostatic pressure (HHP) treatment can influence meat protein conformation and induce protein denaturation, aggregation, or gelation. The means whereby HHP treatment exerts effects on meat protein structure change are due to the rupture of noncovalent interactions within protein molecules, and to the subsequent re-formation of intra- and inter-molecular bonds within or among protein molecules. Depending upon the meat protein system, the pressure, the temperature, and the duration of the pressure treatment, meat can be either tenderized or toughened. Muscle texture variation induced by heat treatment is due to breakage of hydrogen bonds, whereas changes from high pressure treatment are due to the rupture of hydrophobic and electrostatic interactions. Pressure treatment has little effect on the toughness of connective tissue. Juiciness, springiness, and chewiness are increased upon HHP treatment. Prerigor HHP treatment tenderizes meat, whereas tenderizing effects of postrigor HHP treatment are only measureable if pressure and heat treatment are combined (Sun XD *et al*., 2010).

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).

Raghubeer (2007) 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 × 107 kg/m2 at 30°C to 35°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.

Meat tenderization by Vitamin D

The first evidence that associated the involvement of calcium with the 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 tetraacetiz acid (EDTA). They also speculated that EDTA might exert its effect by chelating calcium ions. It appears that VITD supplementation accelerates the 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 more readily tenderize meat during postmortem aging. Initial results demonstrated that VITD supplementation primarily improves beef tenderness ratings by reducing the percentage of “tough” steaks, bringing them back into a more acceptable “tender” category (Swanek et al., 1999; Karges et al., 2001; Montgomery et al., 2000 and 2002). However, more recent findings (Elam et al., 2002; Scanga et al., 2001) involving VITD supplementation and its impact on cooked beef tenderness conflict these findings and certainly raise questions about the commercial application of VITD.

Vitamin D supplementation improved tenderness of meat by elevating the muscle calcium. Elevation of muscle calcium concentration as a result of VITD supplementation seems to be a practice that promotes meat tenderness. It appears that supplementing beef cattle diets with 0.5 million IU/day for the week immediately prior to harvest can improve beef tenderness ratings while not creating any safety concerns associated with VITD residues in meat products. However, since there does not appear to be a consistent response to VITD supplementation and its impact on cooked beef tenderness, commercial application at this time is premature. It must be understood that VITD supplementation is just one of the tools which can be used to insure a satisfactory beef eating experience. Pre- and post-harvest factors which promote beef palatability still must be in place to capture the enjoyable eating experience associated with grain-fed U.S. beef. (Montgomery *et al*., 2002).

Infusion of ionic compounds for tenderization of meat

Pre-rigor ionic compound injection to change the rate of glycolysis, rate and state of contraction, and rate of proteolysis appears to be a feasible method of postmortem meat tenderization. Infusion refers to an introduction of solution via vein and perfusion should refer to the introduction of a solution via an artery. Infusion or perfusion of compounds to change the rate of glycolysis, rate and state of contraction and rate of proteolysis be feasible methods of manipulating postmortem tenderization process in meat. Infusion of bovine carcasses with salt solutions caused a considerable improvement in tenderness. Salts generally influence the functional properties of meat products and are believed to affect contraction and shortening, protein-protein interactions, protein solubility, proteolytic enzyme activity and lattice swelling (Polidori and Francesco, 2003). Use of sodium chloride (NaCl) and phosphate has been found to show a positive result on meat tenderness (Beekman et al). Salt (NaCl) improves meat tenderness by either dislocation of actomyosin or solubilization of proteins from myofilaments. Injection of NaCl or sodium pyrophosphate (NaPPi) into hot boned muscles reduces cold-induced toughening. The authors suggested that meat tenderization could be due to the ionic strength of NaCl and NaPPi solutions. Because of their buffering capacity at neutral pH (pH 6.5 to 9.0), polyphosphates including pyrophosphate, triphosphate and metaphosphate are employed for improving the meat tenderization. They raise the meat pH and assist in solubilization of myosin and increased uptake and retention of water (Offer and Knight, 1988).

Role of proteomics in meat tenderization

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; Tawheed A at eil., 2014).

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