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Application of ultrasound on meat tenderization: A review

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Abstract

Recently, there has been a lot of interest in utilizing ultrasound to tenderize meat. Since it can disturb the microstructure of the muscle and activate the enzymes that soften meat, this approach may improve the tenderness of the meat. A summary of the main conclusions from a number of studies looking into how ultrasound affects meat tenderization is given in this review. The findings suggest that ultrasound has the potential to transform the meat processing sector and considerably increase the tenderness of meat. To get consistent and acceptable results for various meat varieties and cuts, maximizing ultrasonic parameters such as frequency, intensity, and exposure time is essential. The encouraging outcomes imply that ultrasound is a useful technique for raising consumer satisfaction and meat quality.

Keywords: *Meat, meat tenderization, aging, ultrasound*

1. INTRODUCTION

The expected increase in demand for premium meat, especially red meat, highlights consumers' growing focus on the dining experience when choosing their preferred meat products. This underscores the importance consumers place on choosing high-quality meat that can deliver an enhanced and enjoyable culinary experience, emphasizing its significance in their decision-making process (Thorslund et al., 2016). Palatability, encompassing the taste and sensory attributes of meat, greatly influences consumers' choices in selecting meat products (Dong et al., 2022). Quality attributes of meat are of utmost importance to consumers, encompassing visual aspects such as color and marbling, as well as sensory attributes including tenderness, juiciness, and flavor. These factors collectively contribute to the overall palatability and desirability of meat products (Kim et al., 2018). Tenderness, which refers to the perceived ease of meat breakdown during chewing, has been found to have a significant impact on consumer purchasing decisions, particularly when it comes to repeat buying behavior (Lepetit & Culioli, 1994; Miller et al, 2001; Morton et al, 2019). If consumers are unable to distinguish between different levels of tenderness, then any attempts to enhance the tenderness would hold little significance (Miller et al., 2001). However, if consumers can indeed perceive variations in tenderness, it becomes crucial to measure and determine the value that tenderness holds in the marketplace (Boleman et al, 1997). The meat sector has devised numerous methods to enhance the consistency of premium cuts and elevate the tenderness of less desirable muscle tissues.

Meat's level of tenderness is affected by multiple factors. These factors include the age and breed of the animal (Klont et al, 1998), as well as the specific cut of meat. Additionally, the presence of connective tissue and the degree of marbling within the meat play crucial roles (Veiseth et al, 2004). Muscle characteristics are influenced by various factors, including animal species, age, nutritional status, sex, and muscle type (Bao et al., 2021). These factors collectively contribute to the unique attributes of muscles, showcasing the intricate interplay between biological factors and individual muscle properties. The maximum level of toughness in meat is influenced by the length of the sarcomeres, which, in turn, Is dictated by various circumstances such as temperature, pH, and the level of restraint when the muscle enters the rigor phase (Marsh & Leet, 1966). The enhancement of tenderness that occurs during the aging process is due to the degradation of essential structural proteins within the myofibrils, facilitated by internal proteases. This tenderization effect can vary across species and muscles (Kemp et al, 2010). While there is consensus regarding the significance of enzymes in the aging process of meat, there remains ongoing debate concerning the specific role of individual proteases. To be considered a potential candidate, an enzyme must be present within the muscle, have the ability to interact with the myofibrils, remain active after the animal's death, and be capable of reproducing the pattern of proteolysis observed in vivo when tested in vitro (Koohmaraie & Geesink, 2006).

Further research on post-mortem interventions aimed at enhancing meat tenderization should be prioritized and advanced (Dong et al., 2022). Within the realm of emerging technologies, ultrasound stands out as a particularly promising and environmentally friendly alternative to conventional food processing methods (Jadhav et al., 2021). Ultrasound involves the use of sound waves with frequencies above 20 kHz (Caraveo-Suarez et al., 2023). It offers a novel approach to control, improve, and expedite various food processing procedures, all while ensuring that the quality of food products remains uncompromised (Li et al., 2019).

The goal of this review is to offer a thorough examination of the application of ultrasonic technology to meat tenderization. The purpose of the review is to provide light on the effectiveness and possible uses of ultrasound in enhancing meat tenderness by analyzing the body of existing research in this field. This study is significant because it provides information on the state of the knowledge about ultrasound as a new method of meat tenderization to researchers, meat processors, and industry professionals. The food sector as a whole stands to gain from the development of more effective and long-lasting techniques for enhancing meat tenderness, which can be achieved through an understanding of the benefits and limitations of ultrasound in this particular context.

2. THE CONCEPT OF MEAT TENDERIZATION

The process of meat tenderization is multifaceted and involves various interconnected mechanisms (Madhusankha & Thilakarathna, 2021). After the animal is slaughtered, the breakdown of myofibrillar proteins has a direct impact on determining the tenderness of the meat. There are several mechanisms involved in the tenderization of meat, all of which work towards achieving the desired tenderness. These processes include the degradation of collagen, both regarding the amount and the type, which leads to a decrease in the size of muscle fiber bundles. Additionally, changes in the length of sarcomeres throughout the rigor mortis phase, as well as chemical and structural alterations that occur during the aging process, are other different kinds of mechanisms that contribute to tenderization (Renand et al, 2001; Rhee et al, 2004; Veiseth et al, 2004). The excessive contraction of sarcomeres and the hydrolysis of proteins associated with myofibrils during the rigid phase necessitate the involvement of enzymes for successful tenderization (Dong et al, 2022).

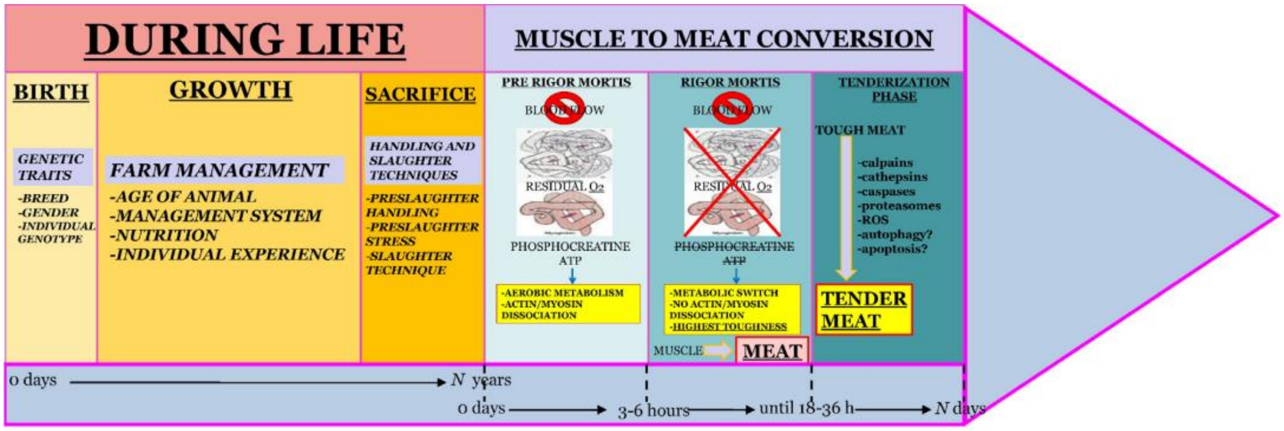

Figure 1. A timeline illustrating the progression of meat tenderness development (Lana & Zolla, 2016).

2.1. Toughness of meat

After slaughter, animal muscles undergo rigor mortis, which involves a series of physiological, biophysical, and biochemical changes. Meat texture is determined by two main components. The first component relates to muscle fibers, which are the building blocks of striated muscle. The striated appearance is due to the structure of myofibrils, which are cylindrical and composed of repeated sarcomeres separated by Z discs. Changes in the contractile apparatus of the muscle, specifically the sarcomeres, during rigor mortis affect meat texture. Processing and post-mortem handling, such as efficient chilling systems and specific temperature and pH control, can induce muscle shortening and result in tougher meat. However, the actions of endogenous proteases can partially resolve the toughness caused by shortening. Methods that activate, enhance, or prolong the action of these proteases, such as pH and temperature control, calcium induction, and aging, can improve the texture of myofibrillar proteins (C. M. Kemp et al., 2010). The second component of meat texture is attributed to the connective tissue, often referred to as background toughness (Lepetit, 2008). The contribution of connective tissue to meat toughness depends on the structure and amount of collagens and elastin present. Post-mortem processing and handling practices have limited impact on this component of meat toughness, but cooking style and temperature can partially improve it (H. Chang et al., 2011).

2.2. The Significance and Consumers' Perceptions of the Toughness and Tenderness of Meat

Based on the Meat Standard Australia (MSA) grading system, which has collected consumer observations for two decades, meat palatability is determined by three main characteristics: tenderness, flavor, and juiciness. A survey conducted by O'Quinn et al., (2018) found that 69% of consumers rejected meat when its tenderness was deemed unacceptable, while only 10% rejected it when the tenderness was acceptable. Among the three traits, tenderness had the greatest impact on overall acceptability, accounting for over 50% in the mid-1990s but recently dropping to approximately 40% ((Huffman et al., 1996; Chail et al., 2017). The recent application of various methods for tenderization has greatly reduced the influence of tenderness on overall acceptability. Despite this improvement, the impact percentage of tenderness remains substantial, and there is a strong demand in the modern meat processing industry for protocols that enhance tenderness while also improving flavor and juiciness (Madhusankha &

Thilakarathna, 2021). Tenderness plays a crucial role in determining the eating quality of meat, making tender meats more likely to satisfy customers and lead to repeat purchases. This repeated purchasing behavior ensures stability in the meat industry, as it motivates manufacturers to invest in large-scale production and develop new products (Banović et al., 2009; Madhusankha & Thilakarathna, 2021).

2.3. Meat Tenderization Mechanism

Meat tenderization is a multifaceted process involving various interconnected sub-mechanisms. These mechanisms include the degradation of collagen in terms of both quantity and type, the reduction in the diameter of muscle fiber bundles, changes in sarcomere length during rigor mortis and chemical and structural alterations that occur during aging (Madhusankha & Thilakarathna, 2021). It's important to note that tenderization is an ongoing process that begins from the birth of the animal and continues through to the consumption stage (Rhee et al., 2004). The primary mechanisms related to meat tenderization involve protein degradation and protein oxidation. The tenderness of meat is influenced by protease enzymes that specifically target the protein components within muscle fibers. The structure and connectivity of skeletal muscle tissue, along with the activity of these proteolytic enzymes, play a crucial role in the development of tenderness. Additionally, the role of endogenous enzymatic activity, particularly the activation of actomyosin dissociation through the provision of heat, has also been emphasized (Macbride & Parrish Jr, 1977; D. Wang et al., 2013). Different treatments can lead to the dissociation of individual protein types.

The degradation of meat cuts with high collagen content is influenced by the cooking method and the endpoint temperature. Light et al., (1985) explain that collagen converts to gelatin at around 80°C, while collagen shrinkage occurs at 60-70°C. The combination of collagen shrinkage and collagen-to-gelatin conversion contributes to meat tenderization. However, excessively high temperatures aimed at achieving tenderness can result in moisture loss and the hardening of myofibrillar proteins, which collectively reduce meat tenderness (Barbanti & Pasquini, 2005).

2.4. Factors Contributing to Meat Tenderization

The primary constituents of meat are myofibrils, which consist mainly of actin, myosin, and other accessory proteins, along with various types of collagen and elastin in the connective tissue (Lana & Zolla, 2016). The chemical structure and arrangement of these proteins contribute to the development of a firm texture in meat. The process of tenderization involves the degradation of structural meat proteins with the aim of reducing meat toughness and achieving a more desirable texture.

Genetics has been shown to have a significant influence on the tenderness of ruminant meat. Beef tenderness is a crucial quality trait, and one of the key factors that affects the total number of muscle fibers, fiber cross-section area, and muscle fiber type within a species is the breed (Lefaucheur, 2010). Monsón et al., (2005) highlight that breed has a substantial impact on beef tenderness and juiciness. Consequently, there is a strong interest in genetic selection to enhance meat tenderness (Hanzelková et al., 2011). Researchers have identified certain cattle breeds such as Pinzgauer, South Devon, Jersey, and Piedmontese, whose meat is comparatively more tender compared to other breeds (Koohmaraie et al., 1995).

In a study comparing meat quality between male and female Simmental cattle, where the animals were fed the same diet and slaughtered at the same age, the researchers found that the meat tenderness of female cattle was superior to that of males (Petričević et al., 2015). A similar trend was observed in a study by Andrade et al., (2021), which reported that the meat from female buffaloes was more tender compared to that of males. These findings suggest a tendency for female animals to exhibit greater tenderness in their meat compared to males in these particular breeds.

The rate and extent of pH decline in muscle during the early post-mortem phase can have a significant impact on muscle tenderization. As rigor develops, pH decline occurs and can influence tenderness by affecting muscle shortening, especially in cases of cold shortening. However, pH decline can also impact tenderness through other mechanisms (Melody et al., 2004). It has been observed that beef with either a high or low pH at 3 hours post-mortem results in a less tender product compared to beef with an intermediate pH. This suggests that maintaining an optimal pH range during early post-mortem stages is crucial for achieving desirable tenderness in beef.

Modern meat is often criticized for not being juicy enough, which is often attributed to low levels of intramuscular fat. Intramuscular adipose tissue, also known as inter-fascicular fat, is a unique fat depot. It is the last fat tissue to be deposited and the first to be used by the animal as an energy source. The selection for leaner carcasses results in lower levels of intramuscular fat. However, the amount of marbling greatly affects the flavor of the meat (Thompson, 2004), and a minimum of 2.0-2.5% intramuscular fat is necessary to achieve desirable eating quality. The perception of tenderness and juiciness in humans seems to be interconnected, and juicy meat is often perceived as more tender compared to a similar sample with less juiciness. According to a study conducted by Killinger et al., (2004), it has been observed that higher levels of marbling in meat contribute to increased tenderness, as determined by shear force measurements. This is attributed to the fact that higher marbling accumulation results in the weakening of connective tissue rigidity, leading to a more tender meat texture.

Increasing the duration of time on feed generally leads to greater fat deposition, which in turn improves meat tenderness. This improvement is primarily attributed to the effect of increased marbling score and the protection of the carcass during cooling (Špehar et al., 2008). Several studies, such as the one conducted by Sami et al., (2004), have reported positive outcomes of longer time on feed on beef tenderness, marbling, and sensory characteristics. It is worth noting that animals finished on concentrate feed tend to reach the desired slaughter weight at an earlier stage compared to those finished on pasture. As a result, concentrate-fed animals are typically slightly more tender due to being slaughtered at a younger age. The influence of growth rate on meat tenderness appears to be mainly associated with changes in muscle protein turnover. If a higher growth rate corresponds to increased rates of muscle protein synthesis and degradation, one can expect an improvement in meat tenderness, and vice versa (Špehar et al., 2008).

2.5. Process of Converting Muscle into Meat

Immediately after the death of the animal, a series of processes occur to convert the muscles into meat. This begins with the pre-rigor mortis phase, which starts after the animal's death and involves the cessation of blood flow and vital functions. However, residual oxygen remains, allowing for aerobic respiration through hemoglobin and myoglobin, which transport oxygen to the cells. During this phase, muscle tissues maintain a certain level of metabolism, and the presence of ATP and phosphocreatine prevents the onset of rigor mortis. This short period typically lasts for 3 to 6 hours before the muscles enter the rigor mortis phase (Madhusankha & Thilakarathna, 2021).

During rigor mortis, ATP is rapidly consumed through glycolysis. When ATP levels become insufficient for metabolism, the rigor mortis phase diminishes. At this point, the muscle transitions into a state of maximum toughness, which is then mitigated by the post-rigor mortis and tenderization phase. In this later stage, meat qualities and tenderness develop over time. Proteolytic enzymes naturally present in the muscle become activated and start to tenderize the muscle tissues. It is also possible to introduce proteolytic enzymes externally into the meat matrix during this phase (Madhusankha & Thilakarathna, 2021).

2.6. Calpains

The calpains are the group of proteases that best fulfill the mentioned criteria (Goll et al, 2003). Initially identified in skeletal muscle tissue, calpains have since been recognized as abundant proteases found in animal cells throughout the body (Dayton et al, 1976). The distinctive feature of calpains is their dependency on calcium for activation. In the human genome, researchers have identified fourteen genes associated with calpain (Ono et al, 2016), with calpain 1, 2, and 3 being particularly linked to the process of tenderization. Calpain 1 and 2 share similar structures, comprising an 80 kDa large subunit that contains the active site, along with a 28 kDa small subunit (Morton et al, 2019). Calpain 1 and calpain 2 differ in their calcium requirements for half-maximal activity. Calpain 1, also known as m-calpain, requires approximately 50 mM of calcium, while calpain 2, also known as μ-calpain, necessitates around 500 mM of calcium. These names were assigned based on their respective calcium sensitivities. However, these calcium concentrations are significantly higher than the physiological levels found in the body, which are typically around 0.1 mM. Both calpains undergo an initial autolysis step, which reduces their calcium requirement. Autolyzed calpain 1, for instance, exhibits a half-maximal activity at approximately 3 mM calcium concentration (Goll et al., 2003). Calpains are enzymes with intricate specificity, and they frequently alter the function of their target molecules through precise cleavage. A majority of the substrates that calpains act upon are proteins found in the cytoskeleton. Their actions play a crucial role in modulating cellular structure, particularly during processes like the formation and growth of muscle fibers. In essence, calpains bring about significant changes in cell organization and function by selectively cleaving specific proteins within the cytoskeleton (Sorimachi & Ono, 2012).

The hypothesis regarding calpains suggests that the aging process of meat starts with a decline in the levels of ATP after the animal's death. This decline in ATP affects the cell's ability to regulate low levels of calcium in the sarcoplasm. This happens due to the compromised functionality of ATP-dependent pumps in the sarcoplasmic reticulum and mitochondria (Hopkins & Thompson, 2001). As a result, calcium levels increase, leading to the activation of calpain 1 enzyme. Calpain 1 then targets specific cytoskeletal proteins that uphold the integrity of the myofibril structure. In essence, the calpain hypothesis explains how the activation of calpain 1 due to elevated calcium levels affects the integrity of cytoskeletal proteins involved in maintaining meat structure during the aging process. Among the proteins involved, it is believed that titin, a sizable protein, plays a crucial role by linking the M-line to the Z-disk. Another significant protein is desmin, which connects bundles of myofibrils. Vinculin, found in the costameres, is essential for attaching myofibrils to the sarcolemma, the muscle cell membrane (Taylor et al., 1995). These proteins are considered the most important in their respective functions within the muscle structure. Nebulin and troponin T, which are important constituents of the thin filaments in muscle fibers, can also be broken down by calpain. This breakdown process, known as proteolysis, results in the destabilization of myofibrils and ultimately leads to meat tenderness. Nevertheless, it is crucial to acknowledge that actin and myosin, which are the predominant proteins found in myofibrils, are not easily broken down by calpain during the post-mortem stage of meat processing (Morton et al., 2019).

The act of inhibiting the activity of μ-calpain and m-calpain by calpastatin is also dependent on the presence of calcium. This is because the binding of calpastatin to calpain requires calcium (Bhat et al., 2018). Calpastatin inhibits the activity of calpain by preventing its proteolytic activation, binding to membranes, and expression of catalytic activity. These findings were initially observed by Lonergan et al., (2010) and later verified by (Geesink et al., 2006). The presence of elevated levels of calpastatin in the hypertrophic muscles of lambs is closely related to the hydrolysis of proteins after death and the resultant tenderness in meat quality, as noted by (Cruzen et al., 2014).

According to Chang et al., (2020), calpain, an enzyme, has been found to degrade important proteins in myofibrils. However, their study suggests that the activation speeds of calpain may vary depending on the composition of other muscle fiber types. Calpain is known to degrade the filamentous structure that connects adjacent myofibrils at the level of each Z-disc, as well as weaken the binding force between the I-band and Z-line. These findings indicate that calpain has the potential to disrupt and break down myofibrils. According to Uytterhaegen et al., (1994), the organized structure of muscle fibers and the connection between adjacent muscle fibers can be disrupted by the hydrolysis activity of calprotease. When calpain interacts with myofibrils, it has the ability to degrade desmin, a protein involved in maintaining the integrity of muscle fibers. In simpler words, the combination of calpain and myofibrils can lead to the degradation of desmin, which in turn affects the organized arrangement of muscle fibers and the connections between them.

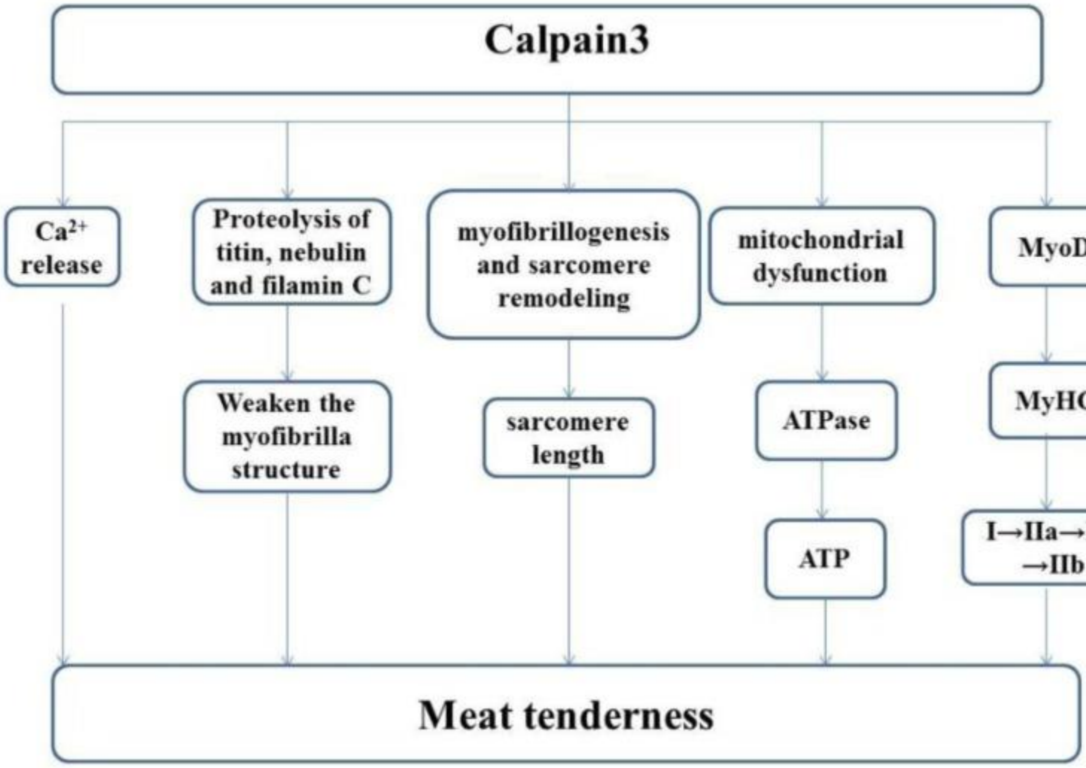

Figure 2. *Possible mechanisms by which CAPN3 contributes to meat tenderization (Lian et al., 2013).*

2.7. A Different Endogenous Protease

In muscle tissue, calpains are not the sole proteases present. Evidence suggests the presence of additional proteases also contributes to the aging process or interacts with calpain. One such group of proteases called cathepsins is responsible for breaking down cellular components within lysosomes. Notably, cathepsins exhibit activity within an acidic pH environment commonly present in meat (Sentandreu et al., 2002). Under certain conditions where the lysosomal membranes are damaged, cathepsins may have access to myofibrils. These proteases have the ability to break down actin and myosin proteins, although the extent of protein breakdown is limited during the aging process (Mikami et al., 1987). Another potential protease involved is the proteasome, which is present in significant quantities in muscle tissue. Normally, the proteasome functions through an ATP-dependent process, recognizing and degrading proteins labeled with ubiquitin (Robert et al., 1999). However, in post-rigor muscle where ATP is absent, the proteasome can remain active for up to a week after death, and its activity becomes independent of ubiquitin (Lamare et al., 2002). The proteasome has the ability to mimic certain aging characteristics, and when proteasome inhibitors are used, the proteolysis process in chilled meat is slowed down. However, it's important to note that the general pattern of proteolytic degradation in chilled meat differs from the natural aging process.

Current theories on early aging propose that it is a process involving controlled cell death, known as apoptosis (Becila et al., 2010). One key group of enzymes activated during apoptosis is caspases, which have been found to exhibit similar characteristics to post-mortem proteolysis in myofibrils (C. Kemp & Parr, 2008). It has been suggested that caspases may be influenced by serine peptidase inhibitors called SERPINs, which have been linked to meat toughness in beef (Herrera-Mendez et al., 2009). Ample evidence exists supporting the interaction between caspases and calpains, with caspases being capable of proteolyzing calpastatin, an inhibitor of calpains (K. K. Wang et al., 1998). Therefore, the current understanding of post-mortem aging centers around calpains but also Involves interactions with various other categories of proteases.

Based on proteomic studies, it has been observed that certain groups of proteins can impact the aging process. These proteins can either protect the proteases or their substrates, thus affecting the extent of proteolysis. Enzymes involved in metabolism and heat shock or chaperone proteins are among those which play a role. Additionally, there is a strong link between tenderness and proteins that defend against oxidation. Oxidation can also impact calpain, which is a protease that is particularly vulnerable to this process (Gagaoua et al., 2015; Lana & Zolla, 2015, 2016; Z. Li et al., 2017).

2.8. Exogenous Proteases and Tenderization

The meat cuts that are most useful for grilling have been shown to be the most valued. However, even with ideal aging, a small portion of these cuts may not achieve desired tenderness (Sullivan & Calkins, 2011). The other muscles generally contain higher levels of connective tissue, which cannot be broken down by the body's enzymes. Plant proteases, such as papain from papaya, bromelain from pineapple, ficin from figs, and actinidin from kiwifruit, can tender tough meat cuts (Bekhit et al., 2014). These proteases can be marinated in meat or added to it by injection or infusion. It is crucial to remember that these plant extracts frequently include a range of low-specificity proteases that can break down any type of meat protein, including connective tissue and myofibrils. Although they work well to lessen the shear force of meat, some of them—like papain—tend to over-digest the flesh, giving it a mushy texture that isn't typical of aged meat. Certain microbial enzymes and actinidin are more specialized proteases that cause a more regulated development of tenderness (Lewis & Luh, 1988; Ryder et al., 2015). These proteases can be applied through various methods, such as infusion of kiwifruit juice to lamb, and can activate calpain 2, leading to proteolysis.

3. MECHANISMS AND EFFECTS OF ULTRASOUND

Ultrasound refers to a specific type of mechanical wave characterized by its frequency range, which typically falls between 20 kHz and 1 MHz (Huang et al., 2020). When sound energy is transferred to a medium, it leads to the generation of longitudinal waves, which exhibit a continuous wave-like motion. This motion causes the particles within the medium to undergo compression and rarefaction alternately. In simpler terms, the sound waves create areas of high pressure (compression) and low pressure (rarefaction) as they propagate through the medium (Povey & Mason, 1998). Ultrasound is commonly categorized as a non-thermal process; however, it's important to note that mechanical vibrations during the propagation of ultrasound waves can generate heat through mechanical friction. This heat generation can result in a small temperature increase, typically ranging from 1 to 10°C (Zhang & Abatzoglou, 2020). The ultrasound equipment comprises three primary components: a generator, a transducer (also known as a converter), and a probe or horn (Fig 3) (Gómez-Salazar et al., 2021). Ultrasound is a form of vibrational energy generated by the transducer, which converts electrical energy into sound energy. This conversion process gives rise to a phenomenon known as cavitation (Linares & Rojas, 2022). Ultrasound is classified based on its intensity into two categories: High-Intensity Ultrasound (HIU), which refers to ultrasound with a low frequency and an intensity greater than 10W, and Low-Intensity Ultrasound (LIU), which corresponds to ultrasound with a high frequency and an intensity lower than 1W (X. Li et al., 2019). According to Domínguez et al., (2019) research, the High Intensity Ultrasound (HIU) is primarily utilized in the field of food processing, with a specific focus on meat products. The application of HIU brings about physical, chemical, and mechanical alterations in the food, resulting in improved heat and mass transfer. When applied to meat products, HIU serves multiple purposes. Firstly, it enables the measurement of the meat product's composition. Additionally, it enhances mass transfer and diffusion during the development process. HIU also gives meat products desired technical characteristics like better texture and tenderness. These effects contribute to the overall enhancement of meat products.

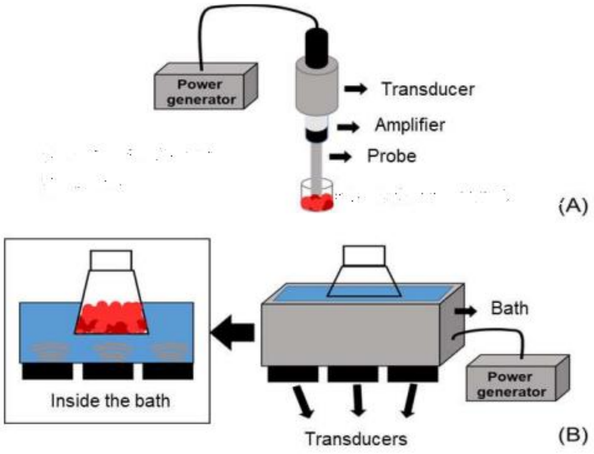

Figure 3. *Ultrasonic systems (A: Ultrasound probe), (B: Ultrasound bath) (Strieder et al., 2019)*

3.1. Ultrasonic Cavitation

Ma et al. (2021) reported that, by changing the frequency, shockwaves and ultrasound effects can be produced. When a liquid is exposed to ultrasound waves at a constant frequency, it leads to the generation of acoustic cavitation. This phenomenon entails the formation of small bubbles filled with vapor within the liquid. Subsequently, these bubbles undergo a collapse, resulting in the rupture of the liquid. This process is commonly known as cavitation (Czechowska-Biskup et al., 2005). Flint & Suslick, (1991) stated that steady acoustic cavitation occurs when small air bubbles form within a specific region of a liquid, typically facilitated by impurities present in the liquid. Conversely, transitory acoustic cavitation occurs when the fluid cannot sustain increased pressures while sound waves expand. This leads to the release of a significant amount of acoustic energy in small quantities. The energy released by the cavitation bubble collapsing causes localized high pressure and temperature changes (Fig 4). As a result, it disrupts the boundary layer and significantly reduces resistance.

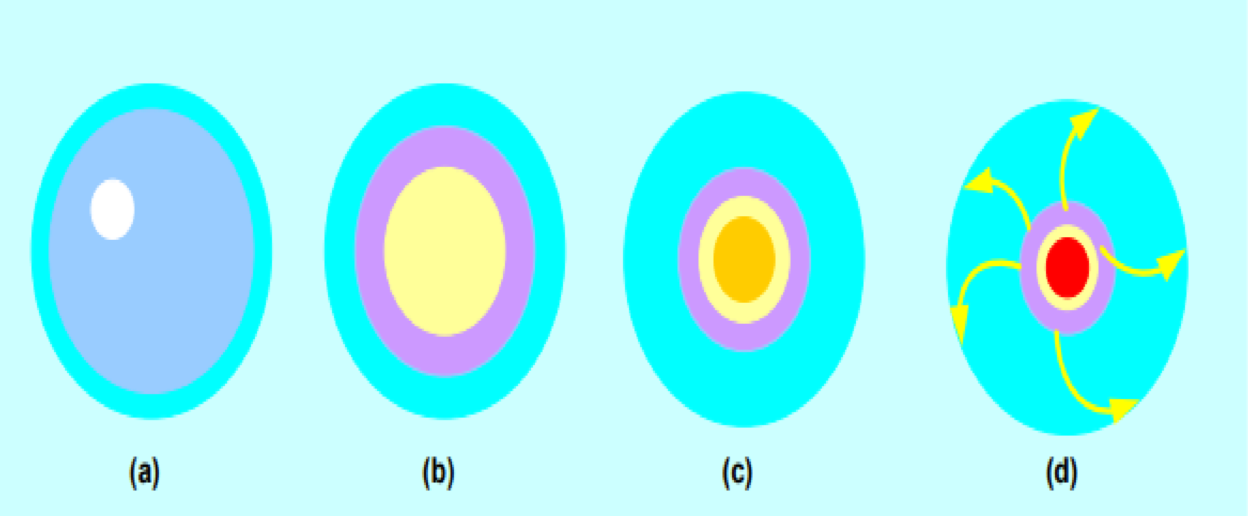

Figure 4. *In bubble cavitation, (**a**) the vapor bubbles collapse, (**b**) and (**c**) intrinsic shock waves are formed, (**d**) the temperature increases, and light is emitted (Alarcon-Rojo et al., 2019).*

3.2. Application of Ultrasound Technology on Meat Tenderization

In the 1950s, ultrasound was first employed in the meat industry to analyze the fat and muscle composition of cattle, marking the origin of its application in this field. Ultrasound can be categorized into two groups based on variations in frequency and power: i) High-frequency and low intensity (> 1 MHz, > 1 W/cm²); ii) Low frequency and high intensity (20~100 kHz, 10~1,000 W/cm²) (Alarcon-Rojo et al., 2019). The utilization of low-frequency and high-intensity ultrasound is mainly focused on meat tenderization. It is commonly accepted that the frequency, power, and duration of ultrasound treatment significantly affect the improvement of meat tenderness (Table 1).

Ultrasound technology, when used on meat, generates rapid vibrations that disrupt muscle fibers and connective tissues. This disruption effectively breaks down tough proteins, leading to improved meat tenderness. The high-frequency vibrations of ultrasound easily penetrate deep into the meat, ensuring uniform and comprehensive tenderization, even in hard-to-reach areas that traditional methods may struggle to access. Moreover, ultrasound treatment facilitates the improved diffusion of enzymes and marinades into the meat, providing additional assistance in the tenderization process. This enhanced penetration of enzymes and marinades effectively breaks down proteins, leading to more efficient meat tenderization.

The implosion of bubbles resulting in microjets can lead to physical damage, such as myofibril fracture, on the meat surface, subsequently influencing meat tenderness (Alarcon-Rojo et al., 2019). Furthermore, cavitation, induced by ultrasound, can facilitate molecular fracture and promote protein oxidation. This process triggers the formation of free radicals and accelerates chemical reactions, including the cross-linking of disulfide bonds and the aggregation of proteins, ultimately impacting protein solubility (Kang et al., 2016). On a macroscopic level, muscles undergoing protein oxidation tend to become tougher. Therefore, it is crucial to effectively control the duration and intensity of ultrasound application for meat tenderization. It is widely accepted that low-intensity ultrasound (< 10 W/cm2), particularly when applied using an ultrasound bath, has minimal impact on meat tenderness due to weak cavitation and dispersed ultrasonic energy (Alves et al., 2013).

The impact of ultrasound on meat tenderization varies depending on the postmortem time. On one hand, it can be challenging to differentiate the effects of ultrasound from the natural tenderizing processes that occur over a longer postmortem period, including the action of endogenous proteases (Lyng et al., 2007). On the other hand, when applied to pre-rigor muscles, the focus is primarily on evaluating protease activity during the onset of rigor and the subsequent aging process. Some studies have observed sarcomere stretch and Z-disk damage, but the ultimate tenderness of the meat is not significantly improved (Lyng et al., 2007). To minimize variability, it is recommended that ultrasound application on meat be performed within 24 hours after slaughter, as this is when the muscle pH reaches the desired level (pHu) for effective ultrasound tenderization (Barekat & Soltanizadeh, 2018; Jayasooriya et al., 2007).

Ultrasound has been found to activate ATPase, increase the content of sulfhydryl groups, and reduce the formation of protein aggregates. These effects result in the formation of more β-sheet structures and an optimized three-dimensional network structure of gelatin, leading to improved water-holding capacity (WHC) (K. Li et al., 2014; Saleem & Ahmad, 2016; Zou et al., 2019). While there are differing viewpoints on the exact mechanism of ultrasound in water retention, it is generally accepted that appropriate ultrasound parameters can enhance the penetration of salts or water-retaining agents, resulting in synergistic effects that improve the WHC of meat products (A. Wang et al., 2018; Zou et al., 2019).

The mechanisms by which ultrasound tenderizes meat can be summarized by considering three main aspects: (1) physical destruction of myofibrils and tissue structures, (2) increased hydrolysis of proteins during aging (including enhanced release of cathepsin) and rupture of collagen and cell membranes, and (3) physicochemical changes in proteins and improved gelling properties resulting from the effects of ultrasound. These factors collectively contribute to the tenderization process facilitated by ultrasound treatment (Shi et al., 2021).

In the study conducted by Roncalés et al., (1993), the researchers investigated the impact of ultrasonication on isolated lamb skeletal muscle fibers immersed in a phosphate buffer at pH 7. It was observed slight damage to the plasma membranes of the muscle fibers. However, more severe changes occurred when the power and treatment time were increased. Notably, after two days of aging, several peptides with a molecular weight of 30 kDa were detected, indicating potential activation of proteolysis, the breakdown of proteins by enzymes. The effects observed were more pronounced with longer treatment durations. The results of the study were interpreted as the ultrasonication process activating proteolysis.

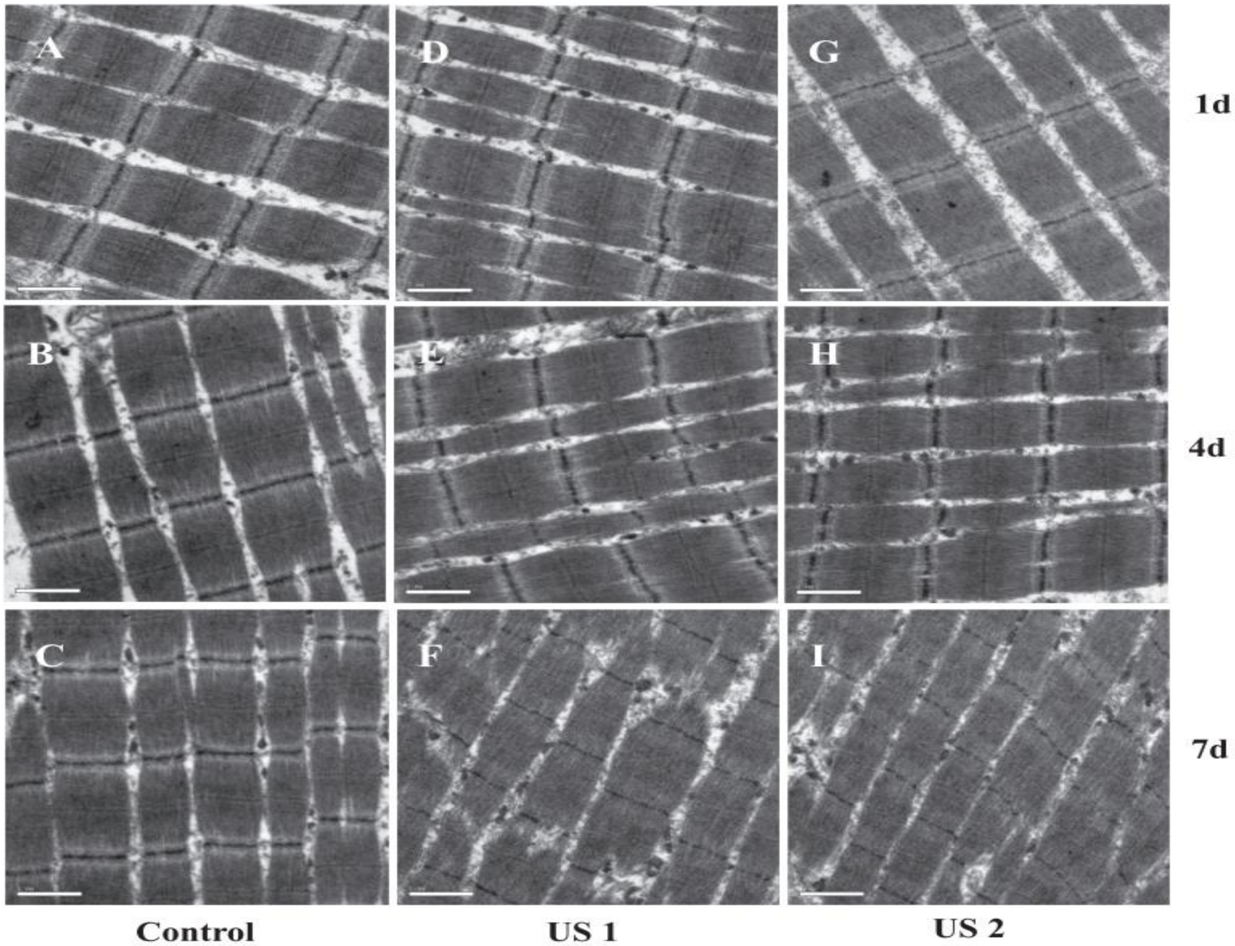

Figure 5. *The microstructure of postmortem 1, 3, and 7-day beef samples with varying ultrasound times (×2950). control: (A–C); US 1: 20 min of ultrasound (D-F); US 2: 40 min of ultrasound (G-I) (A. Wang et al., 2018).*

However, it is worth noting that during ultrasonic treatment, the molecular weight of the peptides decreased rapidly and eventually reached a limiting value.

This suggests that while ultrasonic treatment can induce proteolysis and the breakdown of biopolymers, there may be a point at which the molecular weight of the resulting peptides stabilizes or reaches a lower limit.

Él'piner, (1964) suggested that the mechanical forces generated through cavitation can degrade proteins with molecular weights higher than 20–25 kDa. Therefore, the presence of 30 kDa peptides observed after ultrasonication in the study conducted by Roncalés et al., (1993) may be attributed to the direct mechanical effects of cavitation as well as the indirect activation of proteolysis by ultrasound.

High-Intensity Ultrasound (HIU) technology has been found to enhance the quality of surimi gel containing 0.5% NaCl, as indicated by (Tang & Yongsawatdigul, 2021). This study demonstrated that the application of HIU technology improved the properties of the surimi gel (Table 1). Additionally, P. Li et al., (2021) showed that the combined use of ultrasound and low-temperature heating had a synergistic effect on inactivating key meat proteases, including calpain, cathepsin B, and total proteases. The findings from both studies highlight the positive impact of ultrasound technology, either alone or in combination with other methods, in improving the quality and characteristics of food products like surimi gel and meat (Table 1).

Table 1. *Summary of several research studies that explain how ultrasound affects the tenderness of different meats.*

Meat type	Treatment conditions	Major outcomes	Reference
Beef longissimus lumborum	20 kHz. For 10, 20, and 30 min, 100 and 300 W	The optimal conditions for achieving the highest tenderness and most proteolytic activity were found to be a 20-min treatment duration at an ultrasonic power of 100 W	(Barekat & Soltanizadeh, 2017)
Beef semitendinosus	25 W/cm2, 20 kHz, 20 or 40min	Ultrasound treatment resulted in a significant reduction in Warner-Bratzler shear force at both the 3-day and 7-day post-mortem aging periods.	(A. Wang et al., 2018)
Chicken breast	300 W, 40 kHz, 10, 20, 40, and 80 min	The analyses revealed that both enzymes and ultrasound treatment lead to the destruction of muscle fiber structure, resulting in the softening of the meat.	(Cao et al., 2021)
Threadfin bream surimi gelation at low NaCl	10 kHz, 10.01, 13.28, and 16.45 W/cm2 for 30 min	The findings indicated that the utilization of High-Intensity Ultrasound (HIU) technology can enhance the quality of surimi gel containing 0.5% NaCl.	(Tang & Yongsawatdigul, 2021)
Chicken gizzards	2.09 and 2.46 W/cm2, 500 W/20min, 500 W/30min, 600 W/20min, and 600 W/30min	Applying appropriate ultrasound treatment proved to be an effective method for enhancing the tenderness of the gizzard, with the best tenderization effect observed at 500W for a duration of 30 minutes.	(Du et al., 2021)
Pork loin	Power density was 0.14 W/g, 15 kHz, 2,200W, 0, 0.5, 1, 2, 3, 4, and 6min.	Meat can be effectively tenderized by subjecting it to ultrasonic treatment at a power of 2,200W for a duration of 6 min.	(Yeung & Huang, 2017)
Cobia	4 W/ml, 6 ± 1◦C, 60 kHz, 60, 90min.	The application of ultrasonic processing proved to be an efficient method for tenderizing cobia sashimi while maintaining its freshness.	(H. Chang & Wong, 2012)
Whelk meat	200 W 9.6 min at 45 C	Modification of muscle microstructure	(Hu et al., 2018)

Yellow-feathered chickens	40 kHz, 0.2 W/cm2 at 55 °C for 15 min	The combined use of ultrasound and low-temperature heating demonstrated a synergistic effect in enhancing the inactivation of key meat proteases, specifically calpain, cathepsin B, and total proteases.	(P. Li et al., 2021)
Dry-cured yak meat	0, 200, 300, and 400 W (ultrasonic frequency of 20 kHz)	The application of ultrasound treatment had adverse effects on the color, smell, and taste of the meat. However, it positively impacted the tenderness of the meat and improved its overall acceptability.	(Bao et al., 2022)

According to the study conducted by Barekat & Soltanizadeh, (2017) on Beef longissimus lumborum, the optimal conditions for achieving the highest tenderness and proteolytic activity were identified as a 20-minute treatment duration at an ultrasonic power of 100 W. These specific parameters were found to be effective in significantly improving the tenderness of the beef muscle and enhancing proteolytic activity, which involves the breakdown of proteins by enzymes. Therefore, the use of ultrasound treatment under these optimized conditions can be considered advantageous for enhancing the tenderness of Beef longissimus lumborum (Table 1).

3.3. Sensory Properties

A study conducted by Peña-Gonzalez et al., (2019) demonstrated that the application of ultrasound on stored meat has a positive impact on its flavor, smell, color, and texture. The results indicate that ultrasound treatment can enhance the sensory attributes of meat, making it more favorable in terms of taste, aroma, and visual appeal. This suggests that ultrasound technology has the potential to improve the overall quality and sensory experience of meat products after storage.

The study conducted by Peña-González et al., (2017), titled 'Quality and Sensory Profile of Ultrasound-Treated Beef,' revealed interesting findings regarding the odor and flavor characteristics. It was observed that after 7 and 14 days of storage, the untreated samples exhibited stronger and more intense odors and flavors, particularly raw meat odor, fresh-cooked meat odor, and fresh bovine cooked meat flavors. However, they also had a more intense pleasant boiled meat odor compared to the ultrasound-treated samples. The ultrasound-treated samples showed an increased perception of unpleasant greasy flavor, which became more pronounced after 14 days of storage. These results suggest that ultrasound treatment of beef can have both positive and negative effects on sensory attributes depending on the specific aspect being evaluated and the storage duration.

4.CONCLUSION

In conclusion, extensive research has been conducted on the application of ultrasound in meat tenderization. These studies have consistently demonstrated that ultrasound can significantly enhance meat tenderness by modifying the meat's microstructure and increasing enzymatic activity. The utilization of ultrasound technology offers a more efficient and reliable approach to tenderization compared to conventional techniques, potentially revolutionizing the meat processing industry. It is important to note that the effectiveness of ultrasound may vary depending on several factors, including exposure duration, intensity, and frequency. To optimize ultrasonic settings for specific meat varieties and cuts, further research is necessary. Exploring these areas will allow for the tailored use of ultrasound in meat processing, ensuring optimal results and customer satisfaction. The practical implications of these findings are noteworthy. By unlocking the potential of ultrasound, the industry can enhance meat quality, improve overall customer satisfaction, and meet the increasing demands for tender and flavorful meat products. Future research should focus on refining ultrasonic parameters for different meat types and cuts, as well as investigating potential synergies with other processing techniques.

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