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Prepared by: Assoc. Prof Sandra Kentish
Assoc. Prof Muthupandian Ashokkumar
Mr. Owain Critchley
THE UNIVERSITY OF MELBOURNE

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Meat and Livestock Australia Limited
Locked Bag 991
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The Application of Ultrasound to Meat and Meat Product Development

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Executive Summary

Ultrasonic processing involves the application of sound waves at a frequency beyond that of human hearing. This approach is gaining increasing acceptance in the food industry as commercial scale continuous flow ultrasonic units become available and because of its perception as a benign technology. Ultrasound causes a range of physical effects that generally promote turbulence within a system. However, it acts preferentially at surfaces and phase boundaries which means that it can play a particularly important role in enhancing rates of heat and mass transfer. Ultrasound is used routinely in the meat industry for cutting applications and as an imaging tool. A number of researchers have considered it as a mechanism for increasing meat tenderness, but this work has produced mixed results and may not be a useful application to pursue. More success has been gained in the use of ultrasound to assist with meat brining and marinating, and in the thawing of frozen meat; with a number of patents appearing in this area. Ultrasound may also be used to reduce the viscosity of liquid foods and disrupt aggregates. This approach may prove useful in modifying the properties of gelatin and blood based products and this might increase the range of value adding meat by-products. Further, the use of ultrasound may prove a useful adjunct in the extraction of serum albumin from bovine blood.

Introduction

A variety of novel technologies are emerging for the enhancement of processing efficiency, shelf-life, safety and functional properties of food products. These technologies include ultra-high pressure processing, the use of pulsed electric fields, supercritical fluid extraction, microfluidisation and ultra-violet light treatment. The use of ultrasound has been identified as a particularly promising technology for processing specific food materials[1-3]. The increased availability of efficient large scale continuous flow through ultrasonic systems over the last decade has facilitated this technology to move from the laboratory into fully operational commercial food processes throughout Europe and the USA [4]. Ultrasonic processing is establishing itself as a significant food-processing technology with the capability for large commercial scale-up and good payback on capital investment [4].

A major advantage of ultrasound to the food industry is that it is perceived as benign by the general public. Other processing techniques (microwaves, gamma radiation, pulsed electric field) can be considered cautiously by elements of the general population. However, sound waves are generally considered safe, non-toxic and environmentally friendly – this gives the use of ultrasound a major advantage over other techniques.

The use of ultrasound in food processing in general has been discussed in a number of review articles [1-6]. Mason et al. [2] have suggested that the mechanical and chemical effects generated by low frequency – high intensity ultrasound may be useful for inactivating pathogens in food products and enhancing extraction and emulsification processes. Mawson and Knoerzer [7] have provided a brief history of the applications of ultrasound in food processing in general, including examples of well-established applications such as cleaning of processing equipment[8, 9], together with newer proposed applications such as the enhancement of the extraction of food ingredients from natural products [10, 11]. Knorr et al. [6] have also reviewed the applications and potential of ultrasonics in food processing, focusing particularly on uses related to food preservation and food quality parameters. Patist and Bates [4] provide a summary of the key drivers for the deployment of ultrasonic technology into commercial production.

This review will first provide a short introduction to acoustic cavitation and the broad principles of ultrasonic processing. A detailed account is then presented of the documented use of ultrasound technology in meat processing and other potential uses not yet fully explored.

1 The Principles of Ultrasonic Processing

A sound wave is simply a longitudinal pressure wave passing through a medium. The type of sound wave is determined by its frequency (Figure 1). 'Infrasound' refers to sound waves of frequency below that detectable by the human ear. This is the zone used by whales and by submarine sonar devices. The range of human hearing is from around 20 Hz to 20 kHz. The word 'ultrasound' refers to sound waves which are at a frequency above this range. This ultrasonic spectrum can itself be divided into two zones. Power ultrasound will be the major focus of this review and refers to the frequency range from 20 kHz to around 1 MHz. Diagnostic ultrasound has a frequency in excess of 1 MHz and is used mainly for medical and industrial imaging purposes.

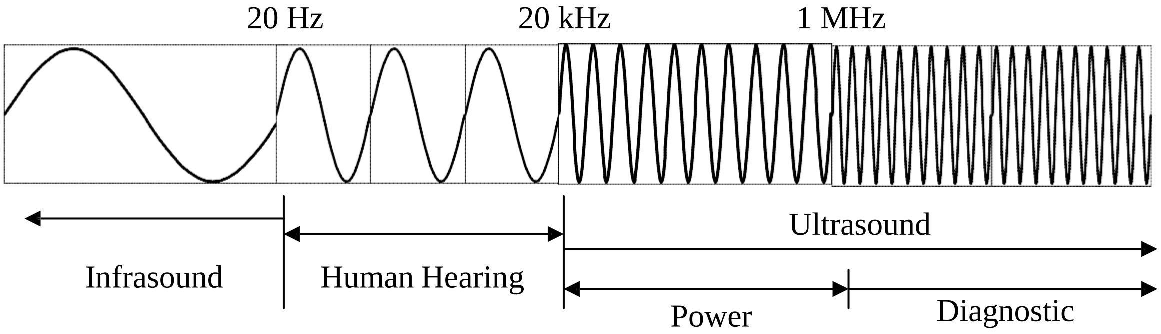

Figure 1 – The sound spectrum

1.1 Physical Effects of Ultrasound

As previously indicated ultrasound simply represents a high frequency pressure wave. As this pressure wave passes through the medium regions of high and low pressure are created. The size of these pressure variations, referred to as the amplitude of the pressure wave or the acoustic pressure, is directly proportional to the amount of energy applied to the system. As this wave passes through a viscous medium, be it air or water, it will dissipate this energy in the form of viscous flow. This is referred to as 'steady streaming'[12]. The flow pattern that results will depend upon the form of the original acoustic wave and whether the pressure wave is reflected from hard surfaces or otherwise interacts with the system boundaries. For example, Rayleigh streaming is the term used to refer to the specific flow patterns that arise from a standing wave pattern between two plane walls [12]. Higher frequency ultrasound leads to higher energy absorption and in turn generates greater acoustic streaming flow rates than lower frequencies for the same power intensity [13].

In gases such as air, which are compressible fluids, the movement of fluid in streaming patterns is always sufficient to accommodate these pressure variations. However, most liquids are inelastic and incompressible and thus cannot respond as easily in this manner.

If the changes in pressure are great enough, then the liquid can literally be ‘torn’ apart under the influence of ultrasound. Microbubbles of gas and vapor form that relieve the tensile stresses created by the pressure wave. Scientific theory would suggest that the acoustic pressure variation required for this to occur is very large, up to 30,000 atmospheres[14]. However, in practice, these microbubbles form at relatively mild acoustic pressures. It is generally believed that this is because any liquid already contains cavities of gas, or nanobubbles and that these nuclei assist in the formation of microbubbles.

The bubble formation process is known as c*avitation* and the lowest acoustic pressure at which it is observed is the cavitation threshold. It should be noted that while cavitation formally refers to the “creation” of a microbubble, most authors in the ultrasound field use this term to encompass the full range of bubble behavior once it has been formed. While the focus of this review is on acoustic cavitation, the hydrodynamic forces around pump impellers or in homogenisers can also create sufficient pressure changes as to induce cavitation.

Bubbles formed through cavitation will begin to expand and collapse under the influence of the acoustic field. The expansion/collapse cycle can be sinusoidal, mimicking that of the acoustic wave (Figures 2a,b). Alternatively, for certain bubble sizes and acoustic pressures, the bubble expansion phase is extended and is followed by a violent collapse back to a very small bubble size. This mode of bubble oscillation can persist for many hundreds of acoustic cycles, in which case it is referred to as stable, or repetitive transient cavitation (Figure 2c). Alternatively, if the acoustic amplitude is higher, the bubbles grow and collapse spectacularly within a very few acoustic cycles and the collapsed bubble then disintegrates into a mass of smaller bubbles [15, 16]. This is referred to as unstable or transient cavitation (Figure 2d). The size range at which transient cavitation occurs is often referred to as the resonance size. However, Yasui[15] shows that the bubble size range for transient cavitation is often over an order of magnitude wide and does not necessarily coincide with the linear resonance radius.

A number of other processes may also be occurring within the cavitating bubble population. If the bubbles are small, they will simply dissolve away (Figure 2a). However, the mass transfer boundary layer is thinner and the interfacial area is greater during bubble expansion than during bubble collapse. This means that more air transfers into the bubble during the expansion phase than leaks out during collapse. This causes larger bubbles to grow over a very large number of acoustic cycles in a process referred to as rectified diffusion[17, 18]. Larger bubbles may also form through coalescence of smaller bubbles[19, 20]. These coalesced bubbles may eventually be of a size where they simply float away from the sonication zone through the influence of gravity.

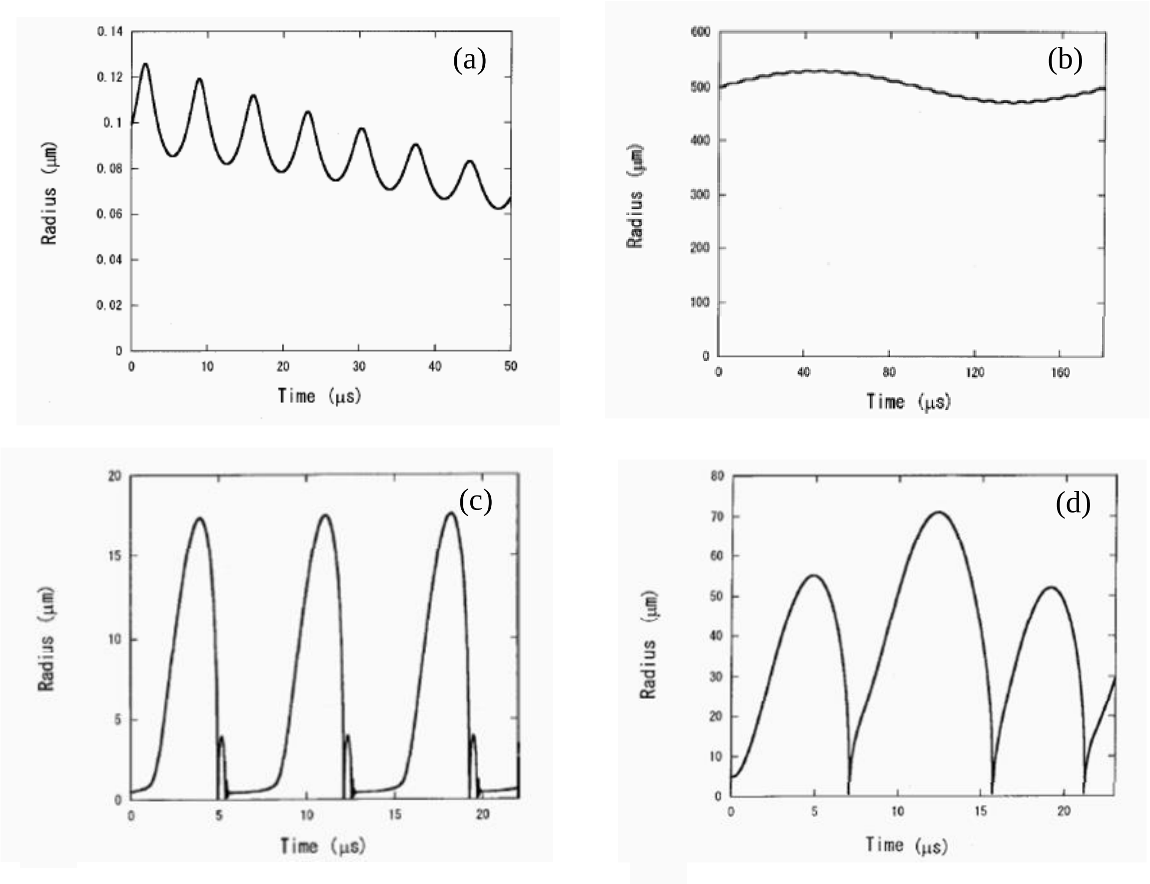

Figure 2 Simulated radius time curves at 140 kHz for (a) a dissolving bubble (initial radius 0.1 µm and acoustic pressure 5 bar) (b) a 'degas' bubble (initial radius 500 µm and acoustic pressure 5 bar) (c) a bubble in repetitive transient cavitation (initial radius 0.5 µm and acoustic pressure 2.5 bar) (d) a bubble in transient cavitation (initial radius 5µm and acoustic pressure 5 bar). This last bubble disintegrates into a mass of smaller bubbles just after the third collapse (t~22 µs). (reproduced from [15])

During stable cavitation, the oscillating bubble will generate fluctuations in velocity and pressure in the surrounding fluid (see Figure 3). This is referred to as 'cavitation microstreaming' and generates turbulence within the fluid on a microscale. More significantly, the collapse of a bubble during transient cavitation is a cataclysmic event – extremely high pressures can be generated (70 to 100 MPa)[13, 21] that result in outward propagating shockwaves. This propogation causes severe turbulence within the immediate surroundings. These dramatic 'micro' events can also easily cause polymer chains to break[22] or the cell walls of plant and animals to be destroyed[23-25]. Kenneth and Gerald[26] comment that the energy released from a single transient collapse is extremely small, but millions of bubbles collapse every second and the cumulative effect is large.

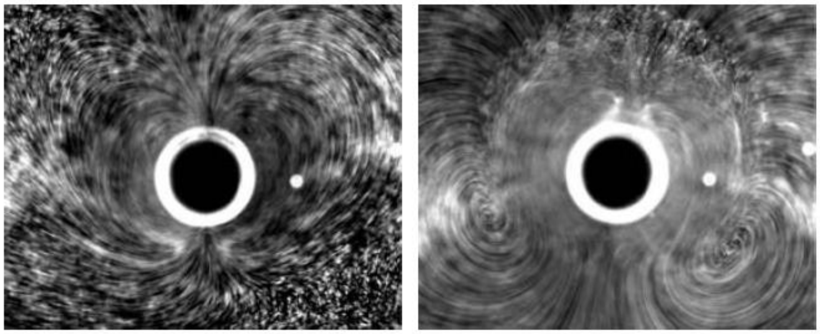

Figure 3 Cavitation microstreaming patterns around a 272 μm radius bubble excited at acoustic frequencies 9 kHz and 11kHz[34].

Of particular relevance to many food applications, is the occurrence of a transient collapse within the proximity of a solid surface. In this case, the bubble collapses asymmetrically. In doing so, a microjet of fluid or bubbles[27] can be emitted from the bubble (Figure 4). This microjet is directed towards the solid surface and this can lead to pitting and erosion. The surface action can also dislodge particles attached to the surface and break down large aggregates into smaller particles [13, 28].

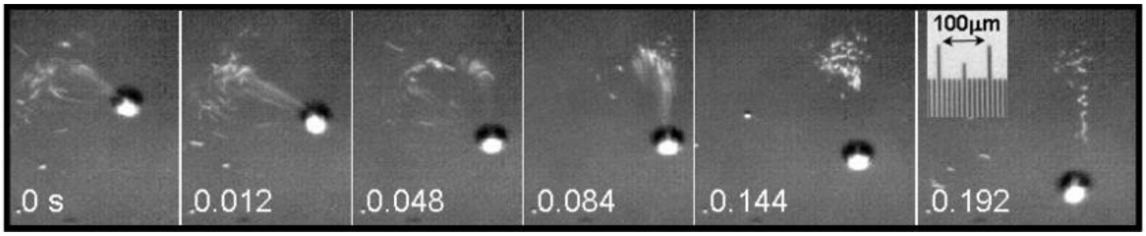

Figure 4. Selected images of the release of a fountain of microbubbles from a parent bubble in water within a confined microspace. Acoustic frequency 59.67 kHz and intensity 0.5-0.7 W/cm2. (reproduced from [10])

Acoustic standing waves can result from the reflection of sound from a solid surface or an air-liquid interface back into the solution at the same time that a wave is generated at the transducer (Figure 5). At the pressure antinode of such a standing wave pattern, the pressure fluctuates from a maximum to a minimum amplitude with time. Conversely at the pressure node the acoustic pressure is invariant and close to zero. A phenomenon referred to as Bjerknes forces causes smaller bubbles to accumulate at the antinode, while larger bubbles accumulate at the node [15, 29]. In moving to the antinodes, the cavitation bubbles travel in ribbon like structures (referred to as ‘streamers’) coalescing as they collide. In doing so, a filamentary structure, referred to as an acoustic ‘Lichtenberg’ figure is created (Figure 6)[30]. At 20 kHz, these bubbles are typically <10 micron in size, about a millimeter apart and travelling at less than 1 micron per second[31]. This bubble translation is known to dislodge particles from fouled surfaces, in cases where the surface itself acts as the pressure antinode[32].

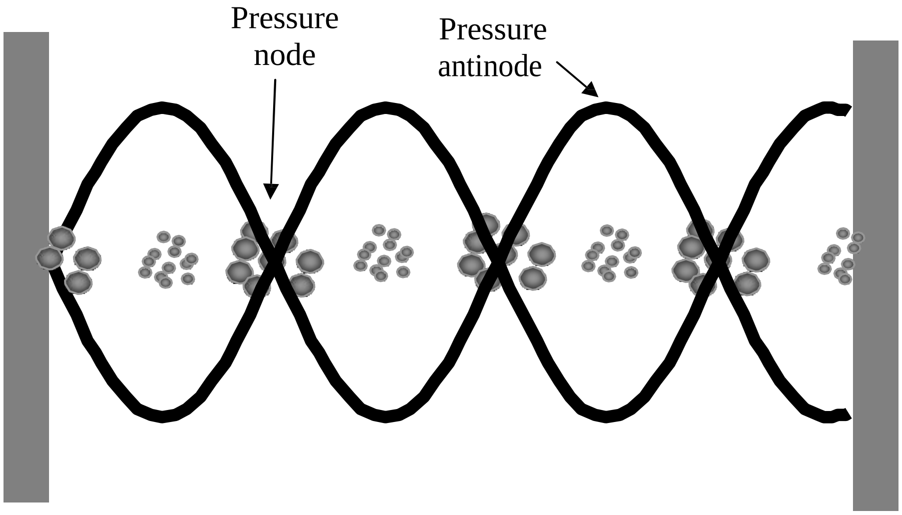

Figure 5. Effects of a standing wave pattern. Bubbles smaller than the resonance size accumulate at the pressure antinodes, larger bubbles accumulate at the nodes.

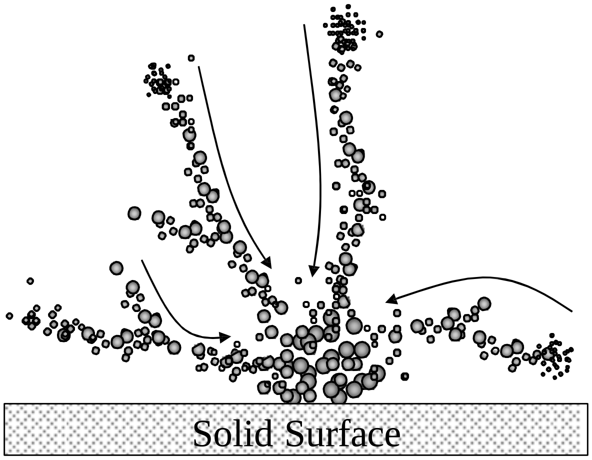

Figure 6 – the movement of bubbles towards a solid surface acting as a pressure antinode within an acoustic standing wave pattern.

Of particular interest to many of the applications discussed in this report is that many of these physical effects are strongest near to fluid/solid and fluid/fluid boundaries. Specifically, the microjetting that occurs with asymmetric bubble collapse and the acoustic streaming patterns around solid objects are strongest within a few millimeters of the surface. Similarly, the movement of bubbles towards a solid surface acting as a pressure antinode within an acoustic standing wave pattern, results in increased turbulence within this same zone. These boundary layer effects are important, because it is usually such boundary layers that offer the greatest resistance to both heat and mass transfer. By concentrating the acoustic energy dissipation in these areas, ultrasound is extremely effective in improving heat and mass transfer kinetics, often proving to be more effective than other less site specific options such as high shear mixers or microfluidic devices. Surface effects are also important in emulsification, where interfacial turbulence is associated with droplet formation [33] and in nebulisation, where acoustic streaming effects cause a 'fountain structure' to form at the air/water interface from which microdroplets are ejected[34].

Increasing the external pressure increases the cavitation threshold within an ultrasonic field and thus fewer bubbles form. However, increasing the external pressure also increases the collapse pressure of cavitation bubbles [35-37]. This means that the collapse of the bubbles when cavitation occurs becomes stronger and more violent. This use of 'overpressure' is a common feature of many commercial sonoprocessing applications. Conversely, increasing the external temperature increases the water vapor pressure inside the cavitating bubbles. This water vapor 'cushions' the bubble collapse and the collapse event is subdued. Hence, ultrasound is less effective at temperatures significantly above ambient levels.

Finally, regardless of the mechanism for dissipation of acoustic energy, be it steady streaming, microstreaming, transient cavitation or microjetting the energy is ultimately converted to heat. This means that all applications of ultrasound will result in an increase in temperature unless cooling is simultaneously applied. In most circumstances, the temperature increase is relatively mild, of the order of a few degrees Celsius, but it is dangerous to ignore this effect in system design. Experimentally, the change in temperature can be used to determine the fraction of the electrical energy originally applied to the transducer that is converted to acoustic energy and ultimately to heat. This is referred to as the calorimetric determination of acoustic energy and it is simply determined from an energy balance[38, 39]:

$$Q = mCp\Delta T \qquad \textbf{(1)}$$

where Q is the energy input in Watts, m the sample mass, Cp the heat capacity of the sample and ΔT the change in temperature.

In conclusion, the physical effects of ultrasound can be generally summarised as increased turbulence throughout the medium but with the strongest effects in the vicinity of system boundaries and interfaces. At lower frequencies (20 – 100 kHz), this increased turbulence results primarily from transient cavitation, that is, the catastrophic collapse of microbubbles throughout the fluid. At higher frequencies (>1 MHz), cavitation and the associated chemical effects is less likely and acoustic streaming effects are more dominant. Thus for example, ultrasound in the MHz range, is used in the electronics industry to clean sensitive components such as silicon wafers and disk drive parts without risking the erosive damage that might occur in the cavitational frequency range.

1.2 Chemical Effects

The violent collapse events that occur during transient cavitation also generates enormous temperatures at a localized level (>5000K). These high temperatures and the violent pressure changes occurring simultaneously can cause a number of chemical changes to occur within both the vapor phase inside the cavitation bubble and in the immediate fluid surrounding it.

Primary radicals are formed as a direct result of the high temperatures inside a collapsing bubble. If water vapor is present, H and OH primary radicals are generated and these can recombine to form molecular products as shown in Equations 2-5.

$$H_2O \quad))))) \quad H + OH \qquad \textbf{(2)}$$

$$H + H \longrightarrow H_2 \qquad \textbf{(3)}$$

$$OH + OH \longrightarrow H_2O_2 \qquad \textbf{(4)}$$

$$H + OH \longrightarrow H_2O \qquad \textbf{(5)}$$

When a single bubble is considered, the amount of radicals generated is high when the temperature inside the collapsing bubble is at a maximum. This temperature can be increased by increasing the sonication power, increasing the external pressure or decreasing the external (solution) temperature as described above. Changing from an air saturated medium to one saturated with a monoatomic (inert) gas such as argon is also effective. The heavier inert gases have a lower thermal conductivity and hence are less efficient at transferring heat away from the bubble to the surrounding fluid.

In a multibubble field, the total number of primary radicals generated is not only controlled by the bubble temperature, but also by the number of active bubbles generated. In fact, it has been shown that the number of bubbles generated is the dominant factor in controlling the radical yield. The amount of heat generated within the bubble depends upon the size of the cavitation bubble. A 20 kHz bubble grows to a maximum size of 60-100 μm and hence generates relatively large amount of heat. Thus, the amount of primary radicals generated per bubble is higher at 20 kHz compared to that generated at higher frequencies. However, for a given liquid volume and acoustic power, greater number of bubbles are generated at higher frequencies that dominates over the radical production per bubble (see Figure 7).

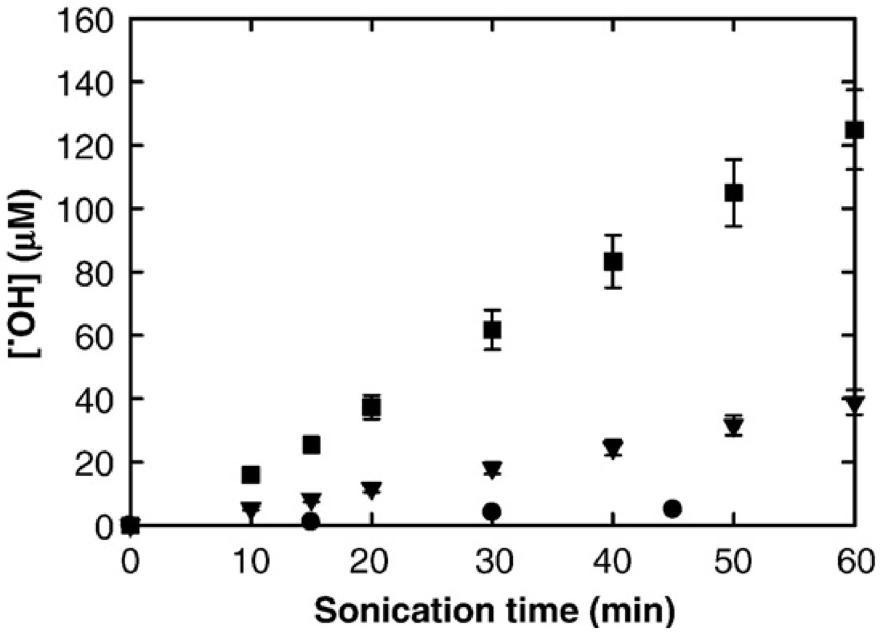

Figure 7 – OH radical yield generated in water upon sonication at different ultrasonic frequencies (0.90W.cm^{-2}). More radicals form per bubble at low frequencies, but many more bubbles are generated at the higher frequencies. Data shown are means±standard deviation of 3 experiments. (■ 358 kHz, ▼ 1062 kHz, ● 20 kHz) (reprinted from [40].

In air-saturated solutions, other reactions involving oxygen and nitrogen occur. In particular this results in the formation of NO_2 which forms nitric acid in solution. It is for this reason that sonication of air-saturated solutions often leads to a decrease in the pH.

The high temperatures within the bubble can also result in a range of pyrolysis reactions if organic solutes are present. Further, the primary radicals generated during the bubble collapse can be consumed in a range of secondary reactions that may occur within the bulk fluid and some distance from the bubble itself. For example, the OH radicals generated within the bubbles have been used to oxidize organic pollutants[41, 42] and the H atoms have been used to reduce metal ions to generate metal nanoparticles[43, 44]. Of particular importance in food applications, Suslick[45] has proposed that the superoxide species(HO_2) formed from primary radicals may induce disulfide cross-linkage between proteins. These effects have been used to generate both gas and liquid filled protein microspheres that have applications for drug delivery. Similarly, Ashokkumar et al.[40] propose that hydroxyl radicals generated during sonication can be used to enhance the degree of hydroxylation in food materials and hence increase the antioxidant activity of foods.

It should be noted that the generation of OH radicals may affect the quality of some foods, by reducing the antioxidant capacity[40]. Intense sonication is also known to generate off flavours. Reiner et al.[46] showed that extended sonication of milk generated a range of volatile organic compounds that might be responsible for a ‘rubbery’ aroma. They related these compounds to both pyrolysis reactions inside the cavitating bubbles and to free radical-induced lipid oxidation resulting from the decomposition of unsaturated fatty acid hydroperoxides. In these applications it may be important to minimize sonochemical reactions by either utilizing a low frequency where free radical formation is very low (20 kHz) or by the addition of a free radical scavenger such as ascorbic acid[40].

The formation of the free radicals is usually accompanied by the emission of light. This is known as sonoluminscence and it serves as a very useful indicator of transient cavitation in laboratory experiments (Figure 8).

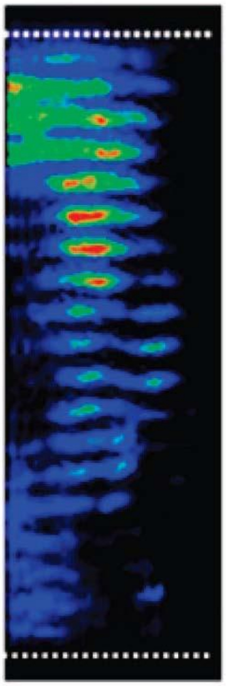

Figure 8 – A sonoluminescence image of a 10mM solution of sodium dodecyl sulfate undergoing sonication at 168 KHz and 1.1 W/cm^2 captured by an intensified CCD camera. The white dotted lines above and below the sonoluminescence structure denotes the liquid surface and the transducer position, respectively. The center axis of the vessel is located on the left side of the images The image clearly shows the pattern generated by standing waves within the sonication cell. (reproduced from [35])

2 Commercial Applications of Ultrasound in the Meat Industry

There are two major applications of ultrasound that have reached their commercial potential in the meat industry. The first of these is the use of ultrasonic cutting devices. These machines utilize the high frequency acoustic vibrations (20 – 35 kHz) to provide a smooth and clean cut for frozen, fresh and cooked meat products[47-50]. Ultrasonic cutting is a novel approach that is now used in a number of commercial operations. The energy demand for this approach is comparable to conventional methods [51] but the cut is generally sharper, particularly for soft or crumbly food such as cream cakes or muesli bars and the downtime for cleaning is reduced.

The second major commercial application is the use of ultrasound for non-destructive testing, particularly of live animals. The attenuation in velocity as a high frequency (> 1 MHz) acoustic wave is passed through a sample provides accurate information on the elastic properties of a material [53]. Further, it is capable of analysing systems that are optically opaque[52]. The use of ultrasonic imaging for predicting fat and muscle content in live cattle has been around since the early 1950s[53]. Today, ultrasound technology is routinely used by the beef industry for: evaluating seed stock[54], identifying dates to slaughter cattle[55], predicting quality, palatability, tenderness and cut-ability in carcasses [56-59]. Ultrasonic techniques have been used in the beef industry to quantitatively determine carcass value and predict heritable muscling and quality attributes[60-67]. The ultrasonic velocity in fish tissues, chicken and raw meat mixtures can be related to its composition using semi-empirical equations [68].

Ultrasound has also been commercialized as a method of sealing plastic food packaging – in a process also referred to as ultrasonic welding, the temperature induced by the cavitation processes described above is sufficient to seal the plastic [35, 79].

3 Applications considered at a Laboratory Scale

3.1 Meat Tenderisation

Post-mortem ageing of meat is the conventional practice used to tenderise meat. This has been associated with a break-down of high molecular mass proteins such as myofibrils during storage. Any means to accelerate ageing in meat would be highly regarded as value addition.

The principle behind ultrasound induced meat tenderisation is the weakening of the muscle fibers by the shock waves produced in acoustic cavitation[69]. Low frequency ultrasound induced meat tenderisation might have significant advantages over other methods mainly because it is a pure physical form of structural alteration that requires no chemical or thermal treatment. This approach has been studied extensively at the laboratory scale. However, results to date in this area have been mixed. The use of relatively low intensity ultrasonic baths (0.3-1.5 $W.cm^{-2}$) [70, 71] on meat of thickness

2.5cm has proved ineffective. For example, Pohlman et al.[71] used a 20 kHz ultrasonic bath to sonicate vacuum packaged steak at 1.55 Wcm^{-2} for 24 min. Results did not indicate any increase in meat tenderness. Similarly, the use of a high intensity sonication horns (62 $W.cm^{-2}$) for a short treatment time (15s) on beef of similar thickness both pre and post rigour has also proved inadequate[72]. Got et al.[73] worked at a higher frequency (2.6 MHz) but still observed only minor changes in meat ultrastructure when applied pre-rigor and no change in the final mechanical resistance of the myofibres.

These results reflect the high acoustic impedence of the muscle fibres. The acoustic energy is unable to pentrate sufficiently to achieve the desired effect. The use of higher levels of acoustic energy or thinner cuts of meat is more successful[69, 74-76], but this approach can also be problematic. If the energy is applied unevenly, then the release of this energy as heat may cause elements of the meat to 'cook' with myofibrular denaturation occurring, in a similar manner to the generation of hot spots in microwave heating[72, 73, 76]. For example, sonication applied to the surface of muscle samples with a 20 kHz horn at 62 Wcm^{-2} for 15 s caused increases in the surface temperatures by 12-20˚C and 3-4˚C for core temperatures, but still did not produce significant results in meat tenderisation[72]. The excessive use of energy would also make many of these applications uneconomic at scale up.

Roncales et al.[77] have approached this issue from a different perspective. They discuss the use of ultrasound to increase the activity of native proteases within the meat postmortem. Specifically, destabilization of lysozomes and vesicles might allow cathepsins to be released from their compartments as well as activating calpains by increasing the free calcium concentration. They showed that sonication of lamb liver cubes (5g) at 40W with a horn sonicator for up to 7 minutes was able to destabilize lysosomes and thus release their enzymes into the cytoplasm. Further sonication inactivated the enzymes. Similar sonication of small lamb muscle fibres (2cm x 0.5 mm) appeared to have an effect on muscle structure. The exposure to sonication used in this instance was prolonged relative to other studies and the sample size was small – this probably explains the more positive results. However, the energy input at scale up would be large i.e 8KW per kg of meat for at least one minute. In a more recent work, Jayasooriya *et al.* (2007) show that the application of ultrasound causes an increase in the pH of the muscle (Figure 9). This pH continued to change during the storage period. They speculate that these pH changes might reduce cathepsin activity but increase calpain activity and the net effect could be an increase in beef tenderness. However, they were unable to prove this hypothesis.

A patent search revealed no current patent applications for ultrasound assisted meat tenderisation indicating that research may not be prospective in this area.

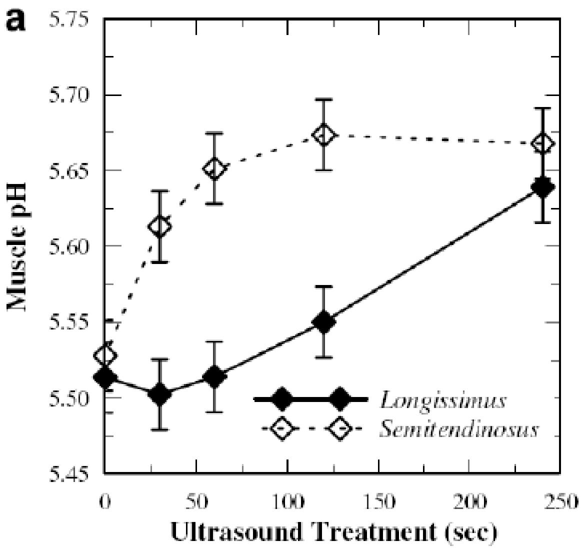

Figure 9 - The effect of ultrasonic treatment on the pH of bovine Semitendinosus and Longissimus muscles (error bars represent standard error)

3.2 Extraction and Meat Brining

It is well known that ultrasound can assist with the extraction of bioactives from food products both through cell disruption and by enhancing mass transfer in the boundary layer surrounding the solid matrix. In this respect, ultrasound may prove useful as an adjunct to collagen and gelatin extraction from bones and skin. Olsen et al. [78] evaluated power ultrasound as a processing aid in gelatin extraction from previously frozen and dehydrated fish skins. The use of ultrasound as an adjunct to extraction increased the extraction yield by 11.1%. The resulting gel strength decreased 7%. Gelation temperature was also lower in ultrasound-extracted gelatin (4.2°C). The authors argued that the changes in gel strength and gelation temperatures were related to a reduction in the molecular weight distribution of the polypeptide coils in gelatins (see Section 3.3 below). These authors further commented that the technology could also work to increase mammalian skin gelatin extraction. However, the scale of this operation, and the relatively high acoustic impedance of the bones and skin might make ultrasound uneconomic.

Alternatively, these same principles can be applied to improve the penetration of brining liquids and marinades into meat products. A number of patents have been filed in this area over the last few years[79-81]. Carcel *et al.* [82] showed that above a critical ultrasonic intensity the uptake of brine solution into meat was proportional to the applied ultrasonic intensity. At the highest level studied the total brine uptake was significantly higher than the initial water content of the meat. Siro *et al.* [83]considered the effect of ultrasound on salt uptake, water holding and water binding capacities of pork loin samples and related these parameters to texture characteristics. Samples were treated in 4% brine solution in a static mode, using vacuum tumbling and using ultrasound at 20 kHz. Results showed that ultrasonic treatment and tumbling caused favourable microstructural and textural changes and improved water binding capacity relative to the static mode. Sonication improved the diffusion of salt through the matrix with the salt diffusion coefficient displaying an exponential relationship with respect to ultrasonic intensity (Figure 10). However, optimization of the sonic power level and irradiation time was important – denaturation of protein occurred at higher power levels and/or longer times.

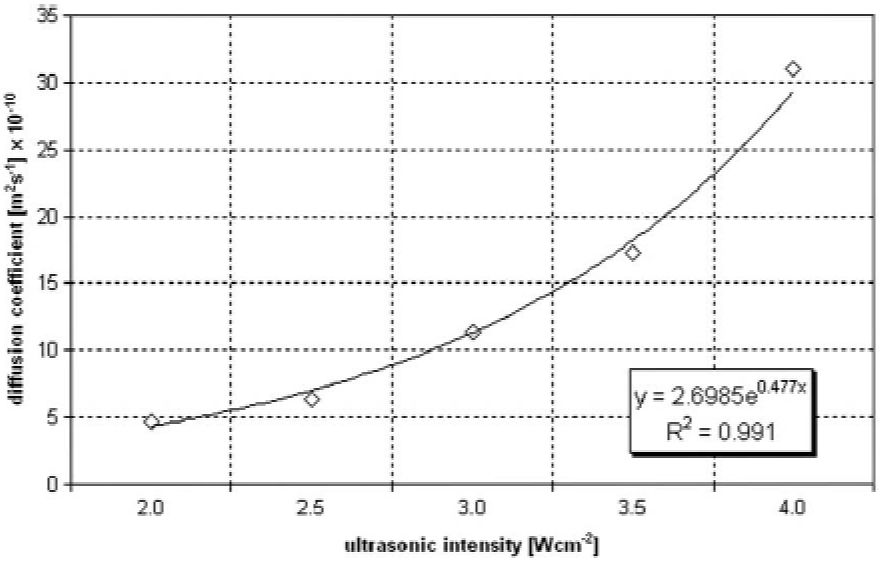

Figure 10 Correlation between ultrasonic intensity and calculated diffusion coefficients of salt in pork loin samples[83]

A patent assigned to Cargill[80] uses ultrasound on a gelatin-containing brine solution prior to injection. The sonication reduces the viscosity of the solution by disrupting the protein networks (see Section 3.3 below). The reduced viscosity solution is more suitable for brine injection.

3.3 Physical Property Modification

Ultrasound can be used effectively to reduce the viscosity of food systems by disrupting aggregates and reducing the interaction between neighboring structures. For example, both Iida et al. [84] and Zuo et al. [85]have used cavitation induced shear forces to modify the viscosity of starch solutions.

A number of workers have shown that ultrasound is similarly effective in disrupting the inter- and intra- molecular bonds in collagen and gelatin. Collagen macromolecules in solution consist of three polypeptide chains held together with hydrogen bonds. Nishihara and Doty[86] have shown that exposure of soluble calf skin collagen to 9kHz ultrasound causes a continuous decrease in intrinsic viscosity but with no change in optical rotation. This indicated that sonication causes fragmentation of the long, rodlike collagen macromolecules into shorter pieces that retained the three-stranded, helical structure.

Hodge and Schmitt[87] show that the collagen molecules break at well defined locations rather than in a random manner. Sonication alters the end groups of the collagen molecules, changing the end-to-end polymerization properties. Kanegae *et al.*[88] reported that ultrasound applied at 10 kHz frequency was able to degrade the soluble fraction of gelatin. They studied various gelatin products that were either alkali processed or acid processed and observed similar degradation kinetics of this fraction (Figure 11).

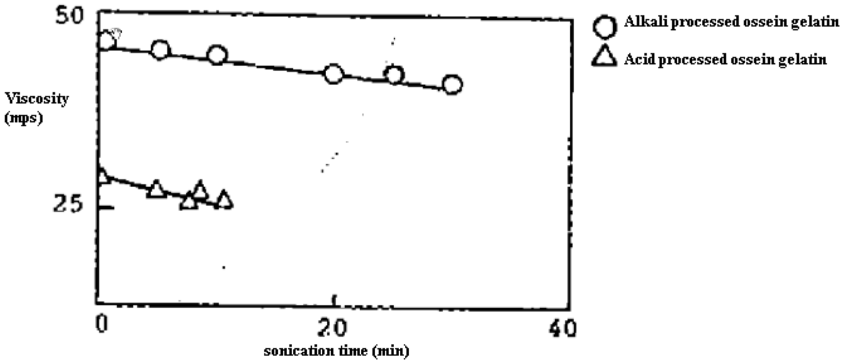

Figure 11 - Effect of sonication time on viscosity of alkali or acid processed gelatin[88]

A major study has been recently undertaken by Gülseren *et al.*[89] into the effects of ultrasound on bovine serum albumin (BSA) functionality and structure. Properties of BSA molecules were analysed using a fixed ultrasound intensity of 20 W.cm^{-2} and varying treatment times. Results showed a decrease in the surface tension of BSA solutions with sonication time, reflecting a change in the structure of the protein. Surface charge properties were also analysed and it was found that, upon ultrasonic treatment, the magnitude of charge increased. This increased surface charge indicates a larger presence of charged residues at the surface of molecule further illustrating a breakdown of the BSA structure. The temperature required for denaturation of the BSA molecules remained unchanged, however the enthalpy required for denaturation decreased with sonication time. The free sulfhydryl content also increased, consistent with an unfolding of the protein structure. Particle sizes increased at longer sonication times (>40 min) suggesting that small aggregates may have been formed. However, it was believed that the formation of aggregates was not due to the formation of covalent bonds (intermolecular disulfide bridges) between protein molecules but through electrostatic or hydrophobic interactions.

Stathopulos et al. [90] show that sustained sonication at 20 kHz of a range of structurally diverse proteins, including BSA results in the formation of aggregates that have properties similar to those of amyloids. Sonication of proteins with a substantial helical structure in the native state (such as BSA), caused an increase in β-structure with a concomitant decrease in α-helical structure.

Conversely, our own recent work has shown that sonication over shorter time frames can disrupt protein aggregates in whey protein solutions, significantly reducing their size [91]. This size reduction is retained even when further heat treatment is applied.

Teng *et al.*[92] have shown that ultrasound (25 kHz, 250 W) can increase the size of protein molecules. The researchers reported an increase in lysozyme particle size when ultrasound was applied (Figure 10). However, the authors indicated that this was only a temporary change caused by shear forces as the native size of the protein molecules could be recovered by turning off the ultrasound unit.

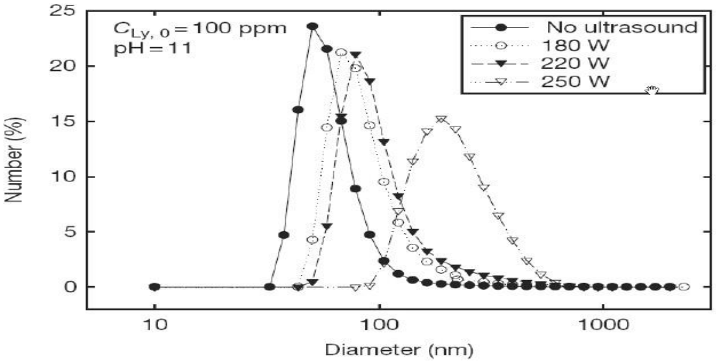

Figure 10 Size distribution of Lysozyme as affected by ultrasound applied at 180, 220 and 250W[92]

A novel application of sonication induced protein denaturation in the meat industry is in the production of skinless sausages and hot dogs [93, 94]. Ultrasonic energy is applied to a tubular surface through which the sausage meat is passed. The ultrasound causes a skin to form with greater processing efficiency than a thermal process.

Ultrasound is also used to prepare proteinaceous microspheres of both bovine and human serum albumin[95]; these are widely used as ultrasound contrast agents in medical imaging applications(e.g., Albunex and Optison). Suslick and coworkers [96, 97] suggest that the microspheres form through crosslinking of free sulfhydryl groups in the albumin protein, whereas Gedanken and coworkers[98, 99] could generate microspheres using macromolecules that do not contain free sulfhydryl groups.

3.4 Microbial and enzymatic effects

There has been considerable interest in the potential use of high power / high intensity ultrasound, often in conjunction with mild heating (thermosonication) for the inactivation of microbes and enzymes associated with spoilage, safety and quality deterioration in a range of food systems [6, 100]. The advantages of ultrasound over heat pasteurization include: the minimizing of flavour loss, greater homogeneity and significant energy savings[2, 100]. Thermosonic (heat plus sonication), manosonic (pressure plus sonication), and manothermosonic (heat plus pressure plus sonication) treatments are claimed to be better methods than sonication alone to inactivate microbes, as they are more energy – efficient and effective in killing microorganisms[5].

Of particular interest to the meat industry is the use of sonication for surface sterilisation. As discussed in Section 2.2, sonication is most efficient at surfaces, but will not penetrate the meat surface to a significant effect due to acoustic impedance. Lillard [101] showed that *Salmonellae* attached to broiler skin were reduced upon sonication in peptone at 20 kHz for 30 min. Further examples of inactivation of microbial contamination in meat also include a patent by Cargill[102], which discloses both surface sonication and the use of a sonication on a heated slurry of meat material. A patent by Medagri consists a combination of ozonated water and ultrasound for sterilisation[81] while Sukegawa considers chlorine dioxide and ultrasound[103].

3.5 Freezing and Thawing

Ultrasound may prove effective for the thawing of frozen meat. Contrary to microwaves, sound waves are more highly attenuated in frozen meat than in unfrozen tissue[104]. This means that little sound energy will be absorbed within the thawed portion of the meat with most of the ultrasound energy being absorbed at the frozen/thawed boundary. Ultrasound is thus well suited to producing stable rapid thawing. Miles et al. [104] investigate this approach using direct contact between the meat and an ultrasonic transducer. At lower frequencies (< 430 kHz), cavitation occurred resulting in surface over-heating and poor ultrasonic penetration. At higher frequencies (>740 kHz), surface over-heating again became a due to the increase in attenuation with frequency. It was only within a narrow frequency band in the region of 500 kHz that satisfactory results were achieved. Toru files a similar patent with a preferred operating range of 300 – 500 kHz[105].

A range of other workers have generated patents for the application of ultrasound to enhance the thawing of meat in warm water[106-108]. In this case, most of the thawing energy comes from the warm water with the acoustic vibration merely assisting heat transfer into the material. For example, Barat and Grau[109] patent a process for simultaneous thawing and brining as above. Kissam et al.[110] argue that low frequency, audible sound is useful for thawing as this approaches the resonance frequency of the ice crystals, causing them to vibrate. Experiments at 1.5 kHz resulted in thawing of fish in 71% less time than in warm water alone. However, the use of audible sound may be unacceptable in a commercial environment.

It has been observed in traditional methods of freezing that a uniform size of crystals is difficult to obtain due to several factors including a lack of uniform nucleation, fluctuations in temperatures and pressures, ineffective cooling due to surface encrustation of cooling coils and non-uniform crystal growth due to uneven mixing. Acoustic cavitation can promote nucleation in a phenomenon referred to as sonocrystallisation[111]. Further, the microstreaming and general turbulence associated with this process accelerates the heat and mass transfer associated with the freezing process. Finally, the shear forces present can break crystals as they form and hence result in a frozen product with much smaller crystal sizes. Ultrasound can thus accelerate the freezing process and lead to better product quality [111]. By controlling the sonication conditions, more uniform sized crystals can be produced. This approach could possibly be applied to selected meat products. However, the increased attenuation in frozen meant, relative to unfrozen tissue; would tend to mitigate against this approach.

3.6 Emulsification

The preparation of very fine emulsions is of increasing interest to the beverage and food ingredients industry, as this can permit novel oil-soluble ingredients to be added to water-based products with negligible impact to solution clarity and stability. Low frequency ultrasound has been effectively used for the preparation of such food emulsions[40, 112]. A particular advantage of the ultrasonic approach is the ease of equipment cleaning relative to traditional homogenisers or the newer microfluidic devices. This assists in the maintenance of an aseptic environment. One application of relevance to the meat industry is the use of ultrasonic emulsification in the production of tallow biodiesel[113-116]. The reaction of the triglycerides with methanol in biodiesel production is generally limited by the mass transfer rate at the oil-methanol interface. Ultrasound can assist in the generation of an ultrafine emulsion that increases the surface area available for such mass transfer to occur.

4 Conclusions and Directions for Further Work

The results of a large number of research studies and a smaller but increasing number of successful commercial applications suggest that ultrasonics can be considered as an emerging technology of significant potential in many aspects of meat processing. The physical effects generated under cavitational and non-cavitational conditions are the main cause of the efficiency enhancements or functional property modifications observed in most cases.

The use of directly applied ultrasound for meat tenderization has been extensively studied. However, the high acoustic impedence of meat means that the commercial potential of this approach is probably limited. The use of ultrasound to enhance meat brining and marinating and for the surface sterilization of meat products would appear to be more realistic opportunities. Similarly, there is evidence that meat thawing may be enhanced by the use of ultrasound, with this field producing a number of patents already. The use of ultrasound to enhance gelatin extraction would appear feasible, although the large scale and high acoustic impedance of skins and bone might make this approach less economically attractive.

Ultrasound has proved capable of substantially altering the physical properties of proteins such as gelatin and serum albumin. By ‘tuning’ the ultrasonic application, the molecular weight of these systems can be reduced, aggregates formed or aggregates disrupted. It may be possible to use this approach to increase the range of value-adding gelatin or BSA by-products, through the generation of structures such as microspheres.

One method of BSA extraction from blood is through the selective denaturation of this protein using heat. It may also be possible to replace or reduce the heat input with ultrasound. Further, it may be possible to use ultrasound to de-agglomerate the denatured protein once it has precipitated.

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