

Ultrasound technology for food fermentation applications

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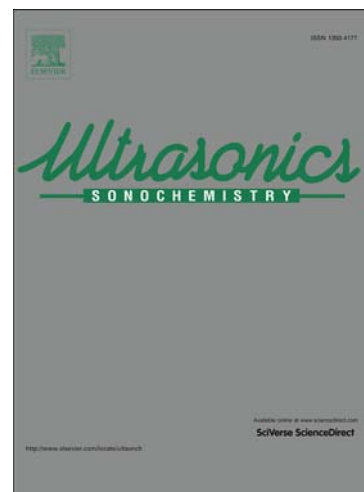
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Abstract

Fermentation processes involve the participation of enzymes and organic catalysts, generated by range of microorganisms, to produce chemical transformations. Ultrasound can be used in such processes to either monitor the progress of fermentation or to influence its progress. High frequency ultrasound (> 2MHz) has been extensively reported as a tool for the measurement of the changes in chemical composition during fermentation providing real time information on reaction progress. Low frequency ultrasound (20 – 50 kHz) can influence the course of fermentation by improving mass transfer and cell permeability leading to improved process efficiency and production rates. It can also be used to eliminate micro-organisms which might otherwise hinder the process. This review summarises key applications of high and low frequency ultrasound in food fermentation applications.

Keywords: Wine, dairy, process analytical technique, sonoporation, high frequency ultrasound, low frequency ultrasound

Research highlights

- (i) High frequency ultrasound for monitoring of fermentation process
- (ii) Low frequency ultrasound can enhance fermentation rates
- (iii) Ultrasound can accelerate microbial growth rates

1. Introduction

The lethal effects of ultrasound on microorganisms have been known for almost a century but the use of ultrasound to promote or control their activity is much more recent. In 1929 Harvey and Loomis reported that ultrasound at a frequency of 375 kHz was able to kill luminous bacteria in water at 19°C [1]. However at this early stage of the development of ultrasound, equipment was expensive and not routinely available. The authors suggested in the final paragraph of the paper that this method of killing bacteria was unlikely to be of any commercial importance because of the expense of the process. This prediction proved false because of the great interest of industry in the use of ultrasound for cleaning which led to rapid developments in ultrasound technology with a consequent reduction in the cost of equipment [2]. It was the development of ultrasonic cleaning (and welding) that led to a greater availability of laboratory scale ultrasonic equipment and subsequently the development of sonochemistry [3].

Presently power ultrasound generally at 20 or 40 kHz with an energy sufficient to produce acoustic cavitation has been developed as a standard technique in microbiology for the disruption of living cells to release their contents. Indeed the killing of bacteria and algae cells using sonication is so successful that it has been studied as a possible method for water disinfection either alone or in conjunction with other advanced oxidation processes [4].

One of the most exciting new areas of research in the field of ultrasonic effects on living cells is work using lower intensity ultrasound at a sub-lethal level. In 1992 Sinisterra reviewed the application of ultrasound to biotechnology in which he identified several processes in which cells or enzymes are activated by ultrasonic waves [5]. It was suggested that low intensity ultrasonic waves could modify cellular metabolism or improve the mass transfer of reagents and products through the boundary layer or through the cellular wall and membrane. In the case of enzymes, the increase in the mass transfer rate of the reagents to the active site seems to be the most important factor. Ten years later Christi reviewed the uses of ultrasound to enhance microbial productivity in sonobioreactors [6].

It is possible to modify the acoustic energy entering a cell suspension and hence reduce the effects of cavitation by altering either the duration of exposure, the acoustic power or the frequency of the ultrasound. Low intensity effects are

predominantly the result of microstreaming and acoustic streaming. Under such conditions, where little or no cavitation damage will occur, the beneficial effects are:

- improvements in microbial reactions (e.g. fermentation)
- activation of enzymes in enzyme modulated reactions
- increased transfer of materials across the cell membrane e.g. gene transfer (sonoporation)

Ultrasound is generally defined as sound with a frequency above that to which the human ear can respond. It can be divided into three categories based upon the frequency range involved: (i) power ultrasound (20 – 100 kHz); (ii) high frequency or extended range for sonochemistry (20 kHz – 2 MHz) and (iii) diagnostic ultrasound (>1 MHz) as shown in Figure 1. From an application perspective, ultrasound can also be broadly divided into low intensity (<1 W/cm²) and high intensity (10 – 1,000 W/cm²) sonication. Ultrasound is employed in various industrial sectors including chemical, bioprocessing, food processing, pharmaceutical, medical and defence [7, 8]. Within the food industry, high frequency ultrasound is typically used as a non-destructive, non-invasive analytical technique for quality assurance, process monitoring and control, whereas low frequency sonication is employed for process intensification. High frequency ultrasound employs very low power levels insufficient to cause acoustic cavitation which therefore produce zero or minimal physical and chemical alterations in the material through which the wave passes. Hence it can be employed for food analysis and quality control without affecting the product. In contrast, low frequency ultrasound employs power levels high enough to generate cavitation and is capable of producing physical and chemical modifications in numerous applications.

The fermentation of food has a long history and is based upon the chemical transformation of complex organic compounds into simpler compounds by the action of enzymes, organic catalysts produced by microorganisms including yeast, moulds and bacteria [9]. The modern fermentation industry is highly competitive and innovative, and has been at the forefront in assessing the potential of new technologies to improve fermentation processes and yield better quality products. The literature suggests that novel technologies for food fermentation will assist food

processors to meet both consumer demands for higher quality and safer products, and also the industry demand for energy efficient processes [10]. The food fermentation industry requires novel techniques to improve the productivity and quality of fermented products along with the new analytical tools to study and monitor complex fermentation processes. Various novel processing and monitoring technologies including ultrasound have been investigated to enhance the productivity and process efficiency of food fermentation [6, 11].

Applications of both high and low frequency ultrasound has been investigated for many years within the fermentation industry. High frequency ultrasound is typically used as a non-destructive analytical technique for monitoring fermentation processes [12]. Whereas, low frequency ultrasound has been employed for enhancing fermentation rates [13, 14], pasteurisation [15], and other specialised processing applications including wine maturation & ageing [16], degassing or deaeration of alcoholic beverages [8, 17].

In this review we will explore the ways in which ultrasound has been employed to enhance fermentation reactions of interest to the food industry. Various current and potential applications of ultrasound as a processing or analytical technique in food fermentation applications are also discussed.

2. Application of ultrasound in fermentation

2.1. Application of high frequency ultrasound

Studies have shown that acoustic based measurement systems are non-invasive, hygienic, precise, rapid, low cost and suitable for automation [18, 19]. Online measurement can be used to monitor concentrations in solutions [13] and also food composition, structure, physical state, and molecular properties [20]. Table 1 lists examples of high frequency ultrasound employed for monitoring fermentation processes.

The traditional method of monitoring fermentation processes is by the withdrawal of samples at regular intervals in order to estimate key fermentation parameters such as microbial growth, pH, acidity, turbidity and chemical composition. Classical chemical analyses such as these are time consuming and do not allow control in real time. However the use of high frequency ultrasonic waves can provide useful

information to characterise fermentation processes in real time and can be applied to either homogenous or multi-phase systems. These methods produce no degradation or chemical alterations in fermentation media [21]. The ultrasonic velocity of an ultrasonic wave travelling through a fermentation tank can be used to infer the concentration of alcohol and sugars during a fermentation process [22]. Studies have shown that empirical relationships can be developed between ultrasonic parameters and the concentration of alcohol and soluble solids in wine [23] and the density of beer [24] during fermentation.

Several ultrasonic parameters including pulse-echo, single pass “through transmission” mode, power attenuation and time of flight have been employed for the estimation of fluid density and other parameters in various liquid, semi-liquid and multiphase systems [25, 26]. For example, Novoa-Díaz, Rodríguez-Nogales, Fernández-Fernández, Vila-Crespo, García-Álvarez, Amer, Chávez, Turó, García-Hernández and Salazar [12] reported that a change in ultrasonic velocity is strongly correlated to the concentration (0 – 8 g/L) of malic acid and lactic acid during red wine fermentation. They employed an emitter–receiver ultrasound transducer (1 MHz) and observed an increase in ultrasonic velocity at a rate of 0.3 m/s with per unit (g/L) increase in lactic acid concentration and a decrease in velocity at a rate of –0.2 m/s per unit (g/L) due to an increase in malic acid concentration.

In a study of yoghurt fermentation, Ogasawara, Mizutani, Ohbuchi and Nakamura [27] employed non-contact acoustic monitoring using a pair of acoustic transducers operating at a frequency of 3.7 MHz to determine the end point of a yogurt production process. They correlated a phase difference between input and output signals measured by an oscilloscope to a phase change from liquid (milk) to gel (yoghurt) with an inflection point around 18 h indicating the end of the yoghurt fermentation process.

In addition to the measurement of concentration of chemical compounds during fermentation processes, it is also possible to use higher frequency ultrasound (> 15 MHz) to measure the concentration of yeast cells in liquid suspensions. To do this a back scattering ultrasound technique is employed with an ultrasonic emitter/receiver wide-band focused transducer centered at 75 MHz. The technique showed improved sensitivity in the detection of yeasts at a concentration as low as 10^4 cells/mL [28].

The pulse spectrum reaching the transducer after backscattering by a yeast cell depends on the size and nature of the cell, the attenuation of the medium and the characteristics of the sound wave.

2.2. Application of low frequency ultrasound

Many uses of low frequency ultrasound in food processing have been reported and within these a number have been applied to food fermentation aimed at improving enzyme/microorganism performance, foam destruction, emulsification and improving end product quality and safety. The range of applications of low frequency ultrasound for fermentation applications are outlined in Table 2 and some of the more significant applications in the key sectors of dairy production and wine making are discussed below.

2.2.1. Dairy fermentation

Milk is often pasteurised prior to its use in various fermented dairy products. Application of low frequency ultrasound alone or in combination with external pressure (manosonication), heat (thermosonication) or both (manothermosonication) is reported to improve the safety profile of milk and can achieve the desired 5 log reduction of pathogenic microorganisms including *Listeria innocua* and *Escherichia coli* [29-31]. Low frequency ultrasound processing of milk is also reported to produce beneficial physicochemical changes in macromolecules including enzyme modification, homogenisation, pasteurisation, reduction in yogurt fermentation time [32] and improved rheological properties of yoghurt [33]. Studies have shown that this method of milk processing offers the potential to achieve pasteurisation and homogenisation effects whilst reducing yoghurt production time (up to 40%) with significant improvement in rheological properties (e.g. consistency and texture) of the final product [34]. It has also been reported that sonication of milk prior to inoculation of starter culture increases water holding capacity, viscosity and decreases loss of water (syneresis). In contrast to this sonication treatment after inoculation has been shown to have no beneficial effect on syneresis although there is a reduction in fermentation time of 30 min [32]. Improved water holding capacity could be due to ultrasound induced homogenisation which causes a change in water holding capacity of the milk proteins.

Mild manothermosonication i.e. the combined application of heat and moderate pressure (2 kg/cm^2) with ultrasound (117 μm amplitude, 20 kHz frequency) of milk prior to yoghurt preparation has also been reported to produce improved structural properties compared to conventionally processed yoghurt [33]. Improved structural properties could be attributed to ultrasound effects on the fat globule membrane which would modify the ability of fat globules to interact among themselves and also with milk protein (casein) micelles. Reduction in fat globule size as a result of sonication cannot be considered as a factor alone for improved textural properties because improved texture was not observed in yoghurt prepared from conventionally homogenised milk samples [33]. In a similar study, Riener, Noci, Cronin, Morgan and Lyng [35] showed that yoghurt prepared from thermosonicated milk (24 kHz for 10 min) with varying levels of fat (0.1, 1.5 and 3.5%) had higher viscosity and water holding capacity compared to those prepared from conventionally heat treated milk (90 °C for 10 min). Yoghurt prepared from thermosonicated milk was shown to possess a honeycomb like network exhibiting large number of pores throughout the structure with small particle size ($< 1 \mu\text{m}$) compared to conventional yoghurt which showed a dense structure (Figure 2). Thermosonication (20 kHz, 480 W/55 °C for 8 min) treatment of reconstituted whey powder has been shown to increase the viable count of dairy starter culture at the end of fermentation time with improved organoleptic properties compared to thermally processed samples [36].

Ultrasound has found applications in the stimulation of probiotics. These are living micro-organisms sometimes called “friendly” bacteria that have become popular as food supplements. One such is *Bifidobacterium* sp. which is often associated with milk product supplements. Nguyen, Lee and Zhou [37] and Nguyen, Lee and Zhou [14] demonstrated the potential of low frequency ultrasound in the stimulation of probiotics (e.g. *Bifidobacterium* sp) resulting in accelerated lactose hydrolysis and transgalactosylation of bifidobacteria in milk while reducing fermentation time by up to 30 min depending on probiotic strain. [37] observed an initial decrease in probiotic cell count at the beginning of fermentation time compared to control with no significant changes in the final counts at the end of fermentation. An increase in viability of probiotics by up to 0.49-0.57 \log_{10} cfu/mL and 0.26-0.57 \log_{10} cfu/mL have been reported for *Lactobacillus* sp. and *Bifidobacterium* sp. compared to the control in the case of fermented soy milk [38].

Streptococcus thermophiles and *Lactobacillus bulgaricus* are probiotic supplements and also starter strains used to make yogurt. In a study of the ultrasonic activation of a monoculture (*Lactobacillus acidophilus*, La-5) and mixed culture (*Streptococcus thermophilus* with *Lactobacillus delbrueckii* subsp. *Bulgaricus*, YC-380) Barukčić, Jakopović, Herceg, Karlović and Božanić [36] observed that the ultrasonic activation of La-5 inoculum did not influence the viable cells count regardless of the applied conditions compared to the untreated inoculum. However, ultrasonic activation of YC-380 at 84 W for 150 s resulted in approximately 1 log cycle higher count compared to untreated inoculum (activated at 37 °C/30 min) with a decrease in fermentation time by up to 30 min. Probiotic cells treated with ultrasound have shown recovery from injury and subsequent increase in the cell growth during fermentation depending on the microorganisms and ultrasound processing conditions. These results demonstrate that the effect of sonication is culture specific, depending on specific resistance of microorganisms towards ultrasound due to variations in cell wall thickness, composition and cell size. However to date the effect of ultrasound on probiotics is not well understood.

Several theories has been proposed in attempts to understand the effect of ultrasound on microbial cells which is strongly influenced by various factors including; microbial ecology (e.g. type of microorganism, medium type and composition), ultrasound parameters (e.g. ultrasound power and frequency), sonication time, pH and temperature [39]. In general, Gram-positive bacteria are more resistant to ultrasound compared to Gram-negative bacteria, possibly because Gram-positive bacterial cells possess a thick and more robust cell wall due to cross-linking of peptidoglycan and teichoic acid [40]. Ultrasound may have positive or negative effects on bacterial cell performance owing to the level of sonoporation. Sonoporation can be defined as the formation of transient cavities or pores on cell membrane due to sonication. Figure 3 shows various interactions of cavitating microbubbles with cell membrane. Interaction of microbubbles with cell membranes has been the subject of scientific interest in the recent past. To date, the phenomena of push and pull effect of cavitating microbubble, rupturing of cellular membrane due to jet formation and penetration of microbubbles into a cell have been reported [41-43]. A low level of sonoporation has been reported to improve mass transfer of substrates or reagents across cell membrane and removal of by-products of cellular metabolism and thus improves microbial growth. Pitt and Ross [44] proposed that

ultrasound increases the rate of transport of oxygen and nutrients required for microbial cell growth and increases the rate of transport of waste products away from the cells which allows faster microbial growth rate. However, higher degrees of irreversible sonoporation can lead to leakage of cellular content because of physical disruption and/or alternation of the cell membrane lipid bilayer causing lipid peroxidation and eventually leading to cell death.

2.2.1. Wine fermentation

The production of alcoholic drinks involves the use of specific microorganisms but will always be faced with the problem of spoilage through the intervention of unwanted bacteria or yeasts. Traditional methods employed to counteract spoilage are targeted at removing the “rogue” microorganisms and involve the use of chemical preservatives (e.g. sulphur dioxide, dimethyl dicarbonate), thermal pasteurisation or removal of spoilage microorganisms by filtration. Such interventions are sometimes accompanied by the presence of off-flavours and so alternate treatments are always of interest. One such is low frequency ultrasound sometimes referred to as High Power Ultrasound (HPU). Several wine spoilage yeasts and bacteria were treated with HPU in saline (0.9% w/v NaCl), juice and red wine to assess their susceptibility to HPU. Significant killing was seen across several yeasts and bacteria commonly associated with winemaking and wine spoilage. A study of the effects of ultrasound on various types of yeast has been reported by Luo, Schmid, Grbin and Jiranek [45]. They reported the effect of ultrasound (24 kHz) for 20 min on a range of yeasts associated with wine production (*Dekkera bruxellensis*, *Hanseniaspora uvarum*, *Pichia membranefaciens*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Zygosaccharomyces bailii*) and bacteria (*Acetobacter aceti*, *Acetobacter pasteurianus*, *Lactobacillus plantarum*, *Oenococcus oeni*, *Pediococcus sp.*). The viability of these yeast was more affected compared to the spoilage bacteria investigated. These findings suggested that HPU technology could be applied to various stages of the winemaking process to control a variety of wine spoilage organisms. However the timing and type of microorganism to be targeted had a strong influence on the efficacy of this technology. In another study, Gracin, Jambrak, Juretić, Dobrović, Barukčić, Grozdanović and Smoljanić [15] employed continuous flow through high power sonication (400 W, 24 kHz, 100 μ m amplitude) to reduce spoilage microorganisms. They observed a significant reduction in

microbial counts of *Brettanomyces* (89.1–99.7%) and lactic acid bacteria (71.8–99.3%) in wine. However, treatment with ultrasound caused negative changes in wine sensorial properties with the formation of negative oxidative smell of burns or smoke and oxidised aroma.

The effects of sonication on cell growth and the formation of ethanol in the fermentation process have also been investigated [17]. The fermentation periods were reduced by up to 50% in wine, beer, and sake using ultrasound (30 mW/cm² and 43 kHz). Using yeast extract peptone dextrose solution for yeast growth, a concentration of isoamylacetate was obtained approximately 2.5 times greater than the maximum concentration under isothermal conditions (20 °C). It was suggested that sonication accelerated the formation of ethanol and other components mainly by decreasing the concentration of dissolved CO₂. Low ultrasonic intensities in a frequency range of 18 – 30 kHz have also been shown to accelerate the growth of *S. cerevisiae* with a resulting reduction in fermentation time [46]. They observed that the ultrasonic irradiation at exponential metaphase of yeast resulted in 33.3% increase yeast growth. Batch fermentations of lactose to ethanol with *Kluyveromyces marxianus* were enhanced using 20kHz sonication (11.8 Wcm⁻² sonication intensity at the sonotrode tip) and 10% and 20% duty cycles [47]. However increasing the duty cycle to 40% had an adverse effect on the process. Jomdecha and Prateepasen [48] also studied the effects of 20 kHz pulsed ultrasound on the lag phase of yeast cells. They observed that the ultrasonic energies operating at a frequency of 20 kHz in a range of 330 and 360 W s m⁻³ could decrease lag time by up to 1 h compared to control whereas, ultrasonic energy > 850 W s m⁻³ can increase the lag time resulting in reduced growth. It was suggested that the improved yeast performance was the result of morphological changes in yeast cells induced by ultrasound.

The production of high quality wines and spirits requires a period of ageing in barrels or bottles and this process will necessarily take a long time sometimes many years. Not surprisingly therefore there is a great interest in finding technologies to accelerate this stage of production. The potential of ultrasound to accelerate wine and spirit ageing and maturation to improve quality is of great interest to industry [16, 49]. Chang and Chen [50] investigated the effect of 20 kHz ultrasound on the ageing

of rice wine and found that ultrasonic treatment for 1 week had a similar effect to ageing over 1 year in fired clay containers (conventional Asian method). This technique did not work in the case of maize wine suggesting that this methodology is not universally applicable. The aging process can be accelerated by the addition of oak chips and ultrasound has been shown to enhance the release of oak-related compounds from the chips into wine during the aging process. A significant increase in total phenolic content in model wine was reported during ultrasound treatment for 150 min at 25 kHz depending on acoustic energy density and temperature [51].

3. Conclusions

Ultrasound technology has been employed for monitoring of fermentation processes (at high frequencies) and as a processing tool (at low frequencies). Efficacy of ultrasound depends both on extrinsic and intrinsic control parameters which can be altered for range of fermentation applications. The lack of standardisation in ultrasonic operating conditions i.e. ultrasound frequencies and intensity levels makes comparisons between studies difficult. Moreover, control conditions may not be reported in detail or are reported differently. The majority of reported fermentation applications have been demonstrated under controlled laboratory conditions. Industrial adoption of this technology is limited, due to the significant challenges encountered in industrial scale-up even though some niche commercial applications are reported. The interactions between ultrasound and microorganisms, particularly at sub-lethal levels to stimulate activity, are complex and not well understood. If these underlying mechanisms of action can be established, it will allow greater understanding of ultrasound processing which would assist in process scale up and industry adoption. Due to the demonstrated benefits of using ultrasound for food fermentation applications, many academic and industrial groups are actively researching this field..

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142 Table 1. Application of high frequency ultrasound in food fermentation

Process	Technique	Ultrasonic conditions	Applications	Reference
Alcoholic fermentation	Pulse – Eco technique	Frequency: 2 MHz, measurement of sound velocity	Density and ultrasonic velocity in the ternary mixture (water–ethanol– sucrose)	[22]
Wine fermentation	Pulse – Eco technique	Frequency: 1 MHz, measurement of sound velocity	Monitoring of malolactic fermentation process	[12]
Yogurt fermentation		Frequency: 3.7 MHz, measurement of sound velocity, phase difference of acoustic wave	Monitoring of phase change from liquid to gel	[27]
Malolactic fermentation in wines	Pulse – Eco technique	Frequency: 1 MHz; measurement of sound velocity	Predict the end-point of the malolactic fermentation process; malic and lactic acid concentrations	[52]
Beer fermentation	Pulse – Eco technique	Frequency: 2 MHz; measurement of sound velocity	Ternary system water–maltose–ethanol with respect to density, speed of sound and temperature (5 – 30 °C)	[53]
Model fermentation	Pulse – Eco technique	Ultrasonic velocity and attenuation measurement Ultrasonic frequency: 2 MHz	Simultaneously determining yeast and maltose concentration.	[54]

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145 Table 2. Application of low frequency ultrasound in food fermentation

Ultrasound treatment	Product	Applications	Main effects	Reference
Dairy fermentation				
20 kHz for 1 – 10 min, before or after culture inoculation	Full-fat yoghurt	Milk homogenization and yogurt fermentation	<ul style="list-style-type: none"> • Higher water holding capacity • Higher viscosity • Lower syneresis • Reduction in fermentation time by 30 min 	[55]
Microbial culture activation at 84 W for 150 s	Sweet whey	To improve fermentation process of sweet whey	<ul style="list-style-type: none"> • Reduced fermentation time • Higher viable counts 	[36]
20 kHz for 7 – 30 min at 100 W	Probiotic fermented milk	Milk fermentation	<ul style="list-style-type: none"> • Improved fermentation rates • Accelerates lactose hydrolysis • Stimulation of <i>Bifidobacterium</i> • High level of oligosaccharides 	[14, 37]
Wine fermentation				
20 kHz ultrasound for 1 week	Rice wine	to accelerate the aging of different wines	<ul style="list-style-type: none"> • Alcohol content reduction • Acetaldehyde content decrease • Ethyl acetate content increase • Polyol concentration reduction 	[50]
26 kHz ultrasound (118 W) for 20 min	Red wine	as an alternative means to control microbial wine spoilage	<ul style="list-style-type: none"> • Cell viability of spoilage microorganisms in wine decreased. • Modification of wine flavour and aroma profile 	[45]
90 kHz ultrasound (35 W) for 1 h	Grape wine (e.g., red wine, white wine)	Ultrasonic treatment with Gas Purging as a Quick Aging Treatment for Wine	<ul style="list-style-type: none"> • Tannin concentration increase • Slight increase of visible absorption 	[56]

25 kHz ultrasound (at acoustic energy density of 6.3, 14.9 and 25.8 W/L) and temperature (15, 20, 25 °C)	Model wine used was a 12% (v/v) aqueous ethanol solution, acidulated to pH 3.5 with tartaric acid.	Ultrasound technology for enhancement of release phenolics from oak chips into the wine model	<ul style="list-style-type: none"> Total phenolic yield released was not affected by acoustic energy density significantly Total phenolic yield increased with the increase of temperature during sonication 	[51]
40 kHz ultrasonic bath system in pulse mode of 1 on and 1 h off for exposure time of 1, 2, 3, 10, 15, 23.5 and 48 h.	Model wine composed of a water-alcohol solution in ratio of 9:1 (v/v), acidulated to pH 3.5 with tartaric acid. 5 g/L dry lees were added Red wine	ultrasound-assisted yeast lysis of light lees in model wine	<ul style="list-style-type: none"> Ultrasound treatment markedly increased the release of proteins Viability of the yeast was seriously affected by ultrasound: after 20 h ultrasonic treatment 	[57]
Ultrasonic processor (400 W, 24 kHz, 100 µm amplitude) in continuous flow treatment at 30 and 40 °C	Red wine	effect of ultrasound on the reduction in number of <i>Brettanomyces</i> yeasts and lactic acid bacteria (LAB) in wine samples	<ul style="list-style-type: none"> (Reduction of <i>Brettanomyces</i> (89.1–99.7%) and lactic acid bacteria (LAB) (71.8–99.3%) impaired aroma of wine due to formation of negative oxidative smell 	[15]
50 min per week with ultrasound equipment at 50 kHz	Red wine and model medium composed of water/ethanol (90:10 ml/ml) acidulated to pH 3.5 with tartaric acid	to accelerate ageing on lees of red wines and its repercussion in sensorial parameters	<ul style="list-style-type: none"> Significant increase in the concentration of polysaccharides released into the wine after only two weeks overall depletion in the anthocyanin content Oxidative taste in sensory analysis 	[58]

100 kHz ultrasound (300W) for 5 min at 20 °C	red wine and the model wine	to investigate the formation of free radicals generated by ultrasound	<ul style="list-style-type: none">• Increase in the intensity of DMPO/1-hydroxyethyl free radical	[59]
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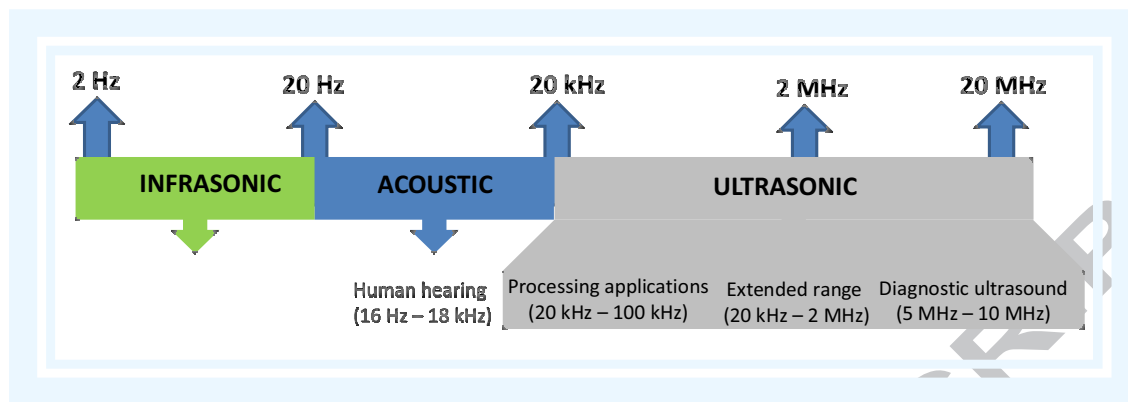


Figure 1. Ultrasound frequency range for food applications

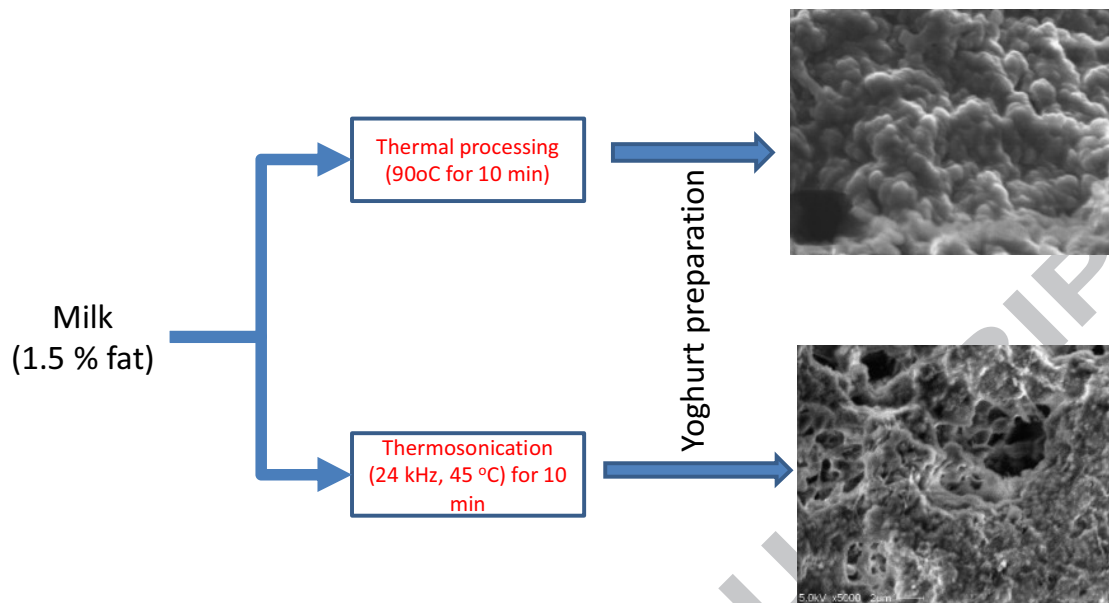


Figure 2. Microstructure of yoghurt prepared from thermosonicated and conventionally processed milk.

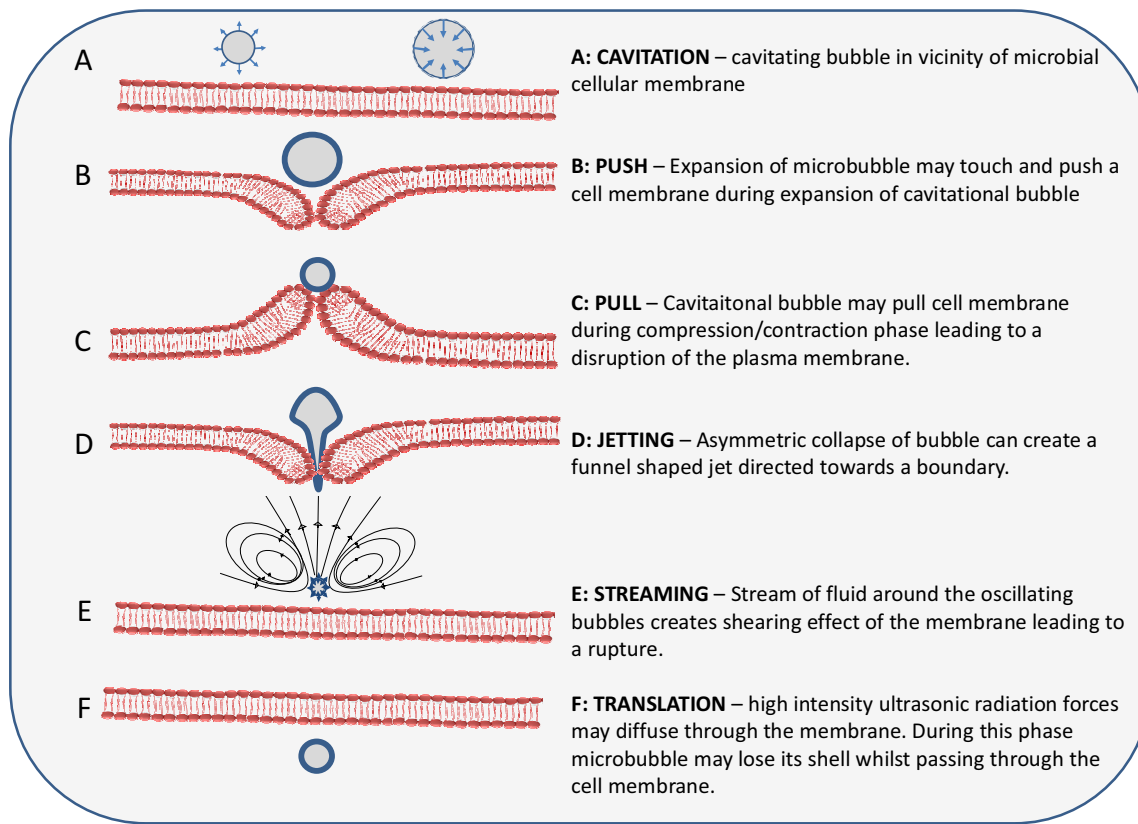


Figure 3. Mechanism of sonoporation involving various stages (adapted from [41-43]).

Research highlights

- (i) High frequency ultrasound for monitoring of fermentation process
- (ii) Low frequency ultrasound can enhance fermentation rates
- (iii) Ultrasound can accelerate microbial growth rates

ACCEPTED MANUSCRIPT