Applications of Ultrasound in Processing of Liquid Foods (A Review)

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Abstract

Ultrasonic processing of a variety of liquids, drinks and beverages has generated much interest with published literature papers increasing within this area in recent years. Benefits include enhanced emulsification with improved homogenisation and fat globule size reduction being recorded. In dairy systems increased creaming rates are observed on sonication in a process known as fractionation. Whilst fruit juices exhibit retention or enhancement of quality parameters whilst increasing levels of bioactive compounds. Sterilisation of liquids is a large feature of ultrasonic treatment with microbial activity of a range of fruit juices being monitored over time as increased stability and reduced spoilage is observed. Progress has also been made towards scale up of ultrasonic processes with several examples of batch and continuous processes being studied with reduced processing times and temperatures being quoted as a result of ultrasonic treatment. This short review covers the effect of sonication on liquids and beverages with a specific focus towards dairy and fruit juices and covers emulsification, fractionation, sterilization and some pilot scale initiatives.

Keywords: ultrasound; food; liquids; drinks; beverages; processing

1. Introduction

In recent years there has been much activity in the area of employing ultrasound to process and interact with liquid foods primarily with dairy and fruit juices. A literature search for novel research papers, written in 2015-2016 alone, resulted in over 100 papers being located thus indicating the large extent of work in this field. Several authors have also written book chapters and review articles covering the diverse areas of ultrasound use in the food industry namely that of non thermal food processing of beverages [1,2], processing in the dairy industry [3-6], processing of milk protein concentrate for food and sports drinks [7] and the implications of its use on food safety and physicochemical and nutritional properties [8]. These articles are wide ranging and examine the main effects of sonication on microbiological, nutritional and physicochemical parameters of fluid foods.

The main frequencies used for ultrasonic processing appear to be in the power ultrasound region with the range 20-25 kHz being the most popular. Ultrasound induces mechanical, chemical and biochemical effects in liquids via the production and subsequent collapse of cavitation bubbles [9]. Sound is propagated through a liquid *via* a series of compression and rarefaction waves induced in the molecules of the medium through which it passes. If the frequency of sound applied is of sufficient intensity voids within the liquid are produced which are known as cavities. These cavities often grow as a result of a process known as rectified diffusion, whereupon volatile gases already present within the bulk medium enter the cavities however are not fully expelled during the subsequent compression phase [10]. As these cavities grow they eventually become unstable and collapse releasing high temperatures and pressures on a microscopic scale resulting in extreme shear forces causing mass transfer, highly efficient mixing and homogenization in addition to high temperature 'hotspots' within the bulk liquid being processed. It is this cavitational collapse that generates the energy for various effects observed during food processing such as reduced fat globule sizes, greater homogeneity of liquids and enhanced extraction [11].

These ultrasonic effects in food liquids are monitored via the examination of the physico-chemical parameters of beverages in terms of qualities such as colour, viscosity, brix index, pH and acidity [12-14]. Benefits from the use of ultrasound include an increase in the shelf life of beverages via the reduction of contaminating microbes/bacteria and moulds [15-17] . Reduction in bacterial loading is a common feature which extends to storage monitoring over extended periods ranging from 21 to 42 days with enhanced stabilisation and quality exhibited by ultrasonically treated samples.
Additional nutritional and health benefits are being quoted by authors, such as increased levels of antioxidants and bioactive compounds [18] and increased content of flavonoids and flavonols [19] found in ultrasonically treated drinks. Whilst most encouragingly several studies have pointed to the positive responses of taste panels involving consumer groups preferring the textures, taste and flavour of ultrasonically treated beverages above those treated with conventional means [20-23].
Often ultrasound is used to extract, mix, emulsify or sterilise liquids as diverse as milk, yogurt and fruit juices. This short review is written to give an indication of the current work being undertaken and covers milk and dairy, fruit juices, sterilization and examples of pilot scale applications.

2. Milk and dairy products

Use of ultrasound as an alternative method of processing dairy products has been explored in recent years and examined in terms of physical and functional properties of the food product, food safety, shelf life and possible economic savings to the food producer [24-27]. Emulsification of milk products is of interest and several studies have examined the effect of ultrasound on the degree of emulsification and the overall homogenisation of the final drink [28,29]. Other authors employ ultrasound in order to fractionate the milk liquid and remove fat layers in order to produce lower fat content milk products [30,31]. Sonication has also been employed to disrupt casein micelles and produce milk containing smaller particle sizes. This milk has shown enhanced renneting ability with the potential of more efficient production [32]. Further details of applications can be seen in Table 1 and below.

2.1 Degree of emulsification

The stability of milk products during storage are often examined in terms of homogeneity of the milk, fat globule sizes and degree of emulsification. Often flavour compounds are also monitored in order to determine the degree of deterioration of the product [33].
Many researchers observe enhanced emulsification and reduced fat globule sizes for ultrasonically treated milk which appears to result in a more stable end product [28,29].

It is the production of cavitation bubbles which aids in the formation of more stable emulsions when liquids are treated with ultrasound. The collapse of unstable cavitation bubbles results in enhanced levels of turbulence, mixing and shear forces within the bulk liquid. Fat globules caught up in these forces are disrupted and reduced in size. The longer the sonication time the greater the droplet size reduction and the more stable the final emulsion produced [34][35]. However sonication for over 5 minutes of ultrasonic treatment is thought not to be of further benefit [36]. By combining sonication with high pressure homogenisation stable nano-emulsions were produced with much lower fat globule sizes and with much reduced energy demands for the emulsification process [37].

The functional properties of two compositions of 7% flaxseed oil/milk emulsion were examined after employing 20 kHz ultrasound, 176 W sonication for 1-8 min [28]. The sonication process improved the gelation characteristics, gel strength and elastic nature whilst decreasing the gelation time of the emulsion. This is attributed to the presence of smaller oil globules, stabilized by partially denatured whey proteins. In another study the effect of sonication on fat globules after thermosonication was monitored on a microscopic level, using a 22 mm probe (Hielscher UP400S at 400 W, 24 kHz, 120 μm amplitude) the milk samples were sonicated for 30 mins at 63ºC [38]. Fat globules appeared to be disrupted with smaller particle sizes and with a greater degree of homogenisation as compared to thermally treated milk.

The effect of ultrasound treatment on the structural, physical and emulsifying properties of three dairy proteins: sodium caseinate, whey protein isolate (WPI) and milk protein isolate (MPI) was investigated [39]. Protein solutions were sonicated for 2 min with a power intensity of ~34 Wcm^{-2}. Ultrasound treatment reduced the size and hydrodynamic volume of the proteins with no measurable reduction in their molecular weight. The authors continued this work by examining the unadsorbed and adsorbed milk protein isolate (MPI) and pea protein isolate (PPI). Under similar ultrasonic conditions it was determined that ultrasound treatment reduced the size of both the milk (MPI) and pea protein (PPI) isolates to the nanoscale (~150 nm) from micron sized aggregates (~20 µm) (see Figure 1). Emulsions prepared with these ultrasonically treated milk proteins yielded significantly smaller emulsion droplet sizes than those prepared with untreated or unadsorbed isolates. This suggests that some rearrangement of interfacial proteins occurs thus allowing for the formation of smaller emulsion droplets associated with an enhanced interfacial layer and greater electrostatic repulsive force [29].

Figure 1 goes hereabouts

Ultrasonic reduction of the particle sizes of fat globules were also noted in goat’s milk [40], skimmed milk [41], and is also thought to be the cause of increased homogenization in cream [42,43].

Ultrasound has a strong use in emulsification with 20 kHz being the most popular frequency employed, however ranges up to 2 MHz are now being explored. The power ultrasound region is still proving to be most efficient for the production of stable emulsions however the extreme temperatures and shear conditions present at this frequency can result in the generation of hydroxyl and hydrogen radicals [44] these in turn can result in lipid oxidation and deterioration of the milk product. Milk stability and lipid oxidation during storage was examined after ultrasonic treatment at frequencies (20, 400, 1000, 1600 and 2000 kHz) and specific energies (up to 409 kJ/kg) [33]. Results showed that lipid oxidation in milk can be controlled by reducing the sonication time and temperature and is to some extent dependent upon the fat content of the treated milk itself. The highest concentrations of deterioration products were seen at 1000 kHz and energies above 271 kJ/kg for raw milk and 102 kJ/kg for pasteurized skim milk which bodes well for emulsification purposes. This information could be of interest when designing larger scale up reactors for use in the diary industry to ensure milk sensory quality retention. Ultrasound appears to be a viable alternative processing technique where stable emulsions could be prepared in the absence of any emulsification enhancers thus leading to more stable additive free products.

2.2 Fractionation

An interesting application of ultrasound is its use to deliberately fractionate dairy liquids, thus forming distinct layers of fat heavy or fat low concentrations in order enhance the fat creaming rate.

Fractionation employs the premise of ultrasonic standing waves which are produced in a liquid as it is subjected to sonication resulting in the formation of nodes of low pressure and anti nodes of high pressure. Higher frequencies of ultrasound (>400 kHz) are employed to achieve fat separation/fractionation as it is thought to be a more gentle process encouraging fat movement rather than the highly disruptive/mixing force present at much lower ultrasonic frequencies. Fat globules naturally migrate to areas of high pressure (anti nodes) and become concentrated in these nodes whereupon the fat globules flocculate and coalesce resulting in an enhanced creaming rate. Using ultrasound separation rates many times faster than natural conventional creaming have been reported [30,31].

The ability of ultrasound to fractionate fat was observed in milk where ultrasonic frequencies of 0.4 MHz and 1.6 MHz were employed at 35 ºC for 5 mins. Coalescence of fat globules was confirmed by micrographs and particle size measurements [45]. The work was then scaled up to 6 Litres where various transducer frequencies and configurations were employed to enhance creaming with most efficient results being obtained with 0.4 MHz employing single and double vertical transducer configurations [46].

A process where ultrasound enhanced gravity achieved separation of milk, in order to fractionate the fat and form distinct particle size distributions, has also been examined [47]. By sonicating the milk for periods of 5-20 mins clear volume partitions were removed and the fat content and size distribution of fat droplets determined. Further stages involved 5 min sonication, with single and dual transducer configurations at 1 MHz and 2 MHz, followed by aliquot collection for particle size characterization. Ultrasound enhanced fractionation provided fat enriched fractions located at the top of the vessel of up to 13 ± 1% (w/v) with larger globules present in the particle size distributions. Semi-skim milk fractions located at the bottom of the vessel could be produced, containing proportionally smaller sized fat globules as low as 1.2 ± 0.01%

(w/v). Higher frequency ultrasound at 2 MHz was more effective in manipulating smaller sized fat and removed 59 ± 2% of the fat contained in the initial sample compared to only 47 ± 2% removed with 1 MHz after 3 ultrasound-assisted fractionation stages.

The continuous ultrasonic separation of milk fat using a 1.8 L flow reactor at volumetric flow rates was scaled up to 33 L/h by using simultaneous 1 MHz/2 MHz sonication at temperatures around 30 °C [48].

The partially skimmed, or fat enriched milk, was treated by cycling it multiple times through the reactor for further fat skimming or concentration, respectively. The visible cream layer was removed from the top of the separation vessel, and the skimmed milk retained was processed again in the following stage (see Figure 2).

Figure 2 goes hereabouts

The milk fat was separated into fat enriched and skimmed streams with final product volumes of between 0.2 and 5 L. Higher frequencies of ultrasound are well known to produce oxidative free radicals however here there is thought to be no damage to fat globules as lipid oxidation derived volatiles were also found to be below the human sensory detection level and no oxidative changes were observed.

Fractionation studies include the temperature effect of sonication on fat coalescence [30] the effect of cavitation and non cavitation on separation [49] and subsequent scale up design parameters [31]. All these studies determined that the use of ultrasound enhances the efficiency of the separation process.

2.3 Effect on milk proteins

Ultrasound is thought to enhance the destabilisation of casein micelles in milk. This can be used to advantage in the coagulation of various milk sources for example goat's milk, and reconstituted milk. Goats milk is known for its weaker coagulation abilities as compared to cows' milk however in this study the use of ultrasound at 20 kHz, 800W for up to 20 mins treatment prior to addition of rennet resulted in smaller and more uniform particle sizes in the coagulant formed [32]. This results in a firmer final product with a faster rate of coagulation observed.

Reconstituted milk was sonicated at 30 ºC using frequencies (20, 400 or 1600 kHz, energy input of 286 kJ/kg) [50,51]. It is thought that the physical and mechanical action of sonication disrupted the casein micelles sufficiently to allow proteins to be released into the milk serum which then reassembled to form aggregates of smaller sizes. This milk source was then treated with rennet and the subsequent process was shown to have enhanced properties in terms of rennet gelation rate, curding rate and curd firmness. This effect was greatest at the lowest treatment frequencies and may be used to produce a product which has different properties to that of thermally treated milk in addition to a more efficient manufacturing process.

Ultrasonically treated milk samples also showed an increase in the gel firmness, coagulum strength, final storage modulus, cohesiveness and water holding capacity [52]. These factors were stated to be due to a decrease in the soluble proteins and an increase of insoluble high molecular weight co-aggregates formed as a result of protein denaturation on sonication [52-54]. In these cases once again 20 kHz was the favoured frequency of sonication. Authors state an enhanced protein solubilisation and a delayed serum separation with no change to quality or sensory parameters of the final product.

3. Fruit Juice

Food drinks are normally processed to increase their shelf-life and thermal pasteurization is the procedure of choice due to its efficiency in preventing microbial growth in many types of beverages including fruit juices. The obvious downside of this treatment is the use of high temperatures which may lead to undesirable biochemical and nutritional variations that could affect the quality characteristics of the final product.

Consumers tend to prefer extracted juices which have a fresh taste with minimal flavour and nutrient losses. Maintaining quality via the retention, or enhancement of bioactive material, such as concentrations of antioxidants or vitamins present, would obviously be beneficial to the consumer and also to the producers themselves from a health marketing point of view.

The use of ultrasound as a non thermal processing technology has generated great interest as a viable alternative to the use of conventional thermal methods, since it appears to have minimal impact on sensorial and nutritional properties of fresh juices whilst having the ability to process liquids at much lower temperatures. Its ability to maintain juice quality whist enhancing bioactive ingredients in addition to increasing shelf life, due to the reduction of spoilage microorganisms, has proven to be of great interest to many researchers as the current wealth of publications in this area indicates. Further details on applications and experimental conditions are found below and also in Table 2.

3.1 Enhancement of fruit juice quality

Analysis of the quality of fruit juices involves monitoring of physico-chemical parameters such as pH, acidity, Brix index, colour, cloudiness and also electrical conductivity. Factors such as the total antioxidant ability are monitored via the use of common radical scavengers, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) , with total phenolics, flavonoids and flavonols also being evaluated as examples of the quality of the juice pre and post sonication. Ultrasound treatment of fruit juices at low temperatures, even as low as room temperature or below, has the potential of making significant savings to food processors and producers. As a result there are many examples of work on many different types of fruit juices all combining to provide significant evidence of the benefits of ultrasonic processing.

The sonication of grapefruit for 30, 60 and 90 min, at 28 kHz and constant temperature of 20 °C, showed that there was significant improvement in the cloud value, total antioxidant capacity, DPPH free radical scavenging activity, ascorbic acid, total phenolics, flavonoids and flavonols in all sonicated juice samples [19]. Some differences in colour values were observed but the overall quality of grapefruit juice was improved with no changes to pH, Brix or acidity levels. Employing a pulsed electric field in combination with ultrasound on grapefruit juice resulted in no significant change in pH, acidity, Brix and electrical conductivity however a significant decrease in viscosity and increase in cloud value was observed [55].

Thermosonication (ultrasound combined with thermal treatment) of fresh apple juice using an ultrasonic bath (25 kHz, 30 min, 0.06 W cm^{-3}) and probe (20 kHz, 5 and 10 min, 0.30 W cm^{-3}) at 20, 40 and 60 °C was employed for the inactivation of spoilage enzymes (polyphenolase, peroxidase and pectin methyl esterase) and microflora (total plate count, yeast and mould) [56]. The highest inactivation of enzymes was obtained with the ultrasonic probe at 60 °C for 10 min, where the microbial population was completely inactivated at 60°C. All other juice parameters such as Brix and acidity were significantly improved. Thermosonication was also employed to deactivate pectin methyl esterase in sour orange juice. The optimal processing conditions for inactivation, both in the thermal process and with thermosonication, were 21.8 min at 75°C and 9.8 min at 63°C, respectively. The results indicate that thermosonication of sour orange juice could entirely inactivate the pectin methyl esterase [57].

The effect of sonication on polyphenolic compounds, sugars, carotenoids and minerals in apple juice was also investigated [58]. Samples were sonicated for 0, 30 and 60 min at 20 °C (frequency 25 kHz and amplitude 70%, 2 W/cm^2). The concentration of polyphenolic compounds and sugars increased significantly when sonicated at 30 mins however sonication for 60 mins enhanced total carotenoids, mineral elements (Na, K and Ca) and viscosity suggesting some improvement of phytonutrients present naturally in apple juice. Examining the physico-chemical parameters of the ultrasonically treated apple determined that no significant effect of sonication was observed on juice characteristics such as Hunter colour values, cloud value, antioxidant capacity, and scavenging activity [12]. However a significant reduction in microbial population was observed suggesting improved quality and safety of the ultrasonically treated juice. In addition by using pulsed ultrasound (5s on/off, amplitude 30, 60 and 90% , ~2 Wcm^{-2}) applied via an ultrasonic probe (20 kHz, 20°C for 3min), significant increases in quality parameters were observed in all sonicated samples with consumer panels preferring the sonicated samples as compared to others during taste tests [20].

3.2 Enhancement of bioactive ingredients

One of the benefits of using ultrasound is its enhanced performance at lower temperatures as compared to other methods of processing liquids. Use of higher temperatures during processing leads to the degradation of many nutritionally beneficial compounds and can result in oxidative degradation of many others. Ultrasound has been shown to retain many bioactive ingredients both immediately after processing and also during storage.

Resveratrol-enriched grape juice was produced from 3 varieties of grape cleaned using sonication for 5 mins post harvest and then incubated for 6 h in the dark at 25 °C. This resulted in the amounts of resveratrol increasing in the final juice by factors of 1.53, 1.15 and 1.24 [59].

Bioactive and antioxidant compounds in black mulberry juice were evaluated by examining different sterilization methods namely thermal (12.81 kJ/mol), microwave (13.07 kJ/mol), and ultrasonic processing (15.99 kJ/mol) [18]. Post treatment samples were stored for 8 days at temperatures of 5, 15, and 25 °C. The antioxidant activity of thermal treated juice was shown to deplete with storage time, whilst both ultrasound and microwave treated juices showed an increase during the first 2 days which then later decreased with storage time.

The qualities of fruit and vegetable juices (orange, sweet lime, carrot and spinach juices) were examined with respect to simultaneous ultrasonic (20 kHz, 100 W, 15 min) and UV treatment (2 UVC lamps (254 nm) 8 W) and compared to conventional thermal pasteurization (80 °C for 10 min) [60]. Treated juices were analysed for total phenol content, antioxidant activity, vitamin C levels and carbohydrate content. Ultrasound treated juice retained most of its nutritional qualities whilst a scale up attempt with spinach juice was also successful in retaining nutrients for 18 days post treatment resulting in an enhanced shelf life as compared to conventional thermally treated juices. The sensory properties of the ultrasound treated juice were most acceptable to the consumer taste panel, which preferred the ultrasound treated juice in terms of taste, flavour and odour; as a result samples were rated similarly to the quality parameters of the fresh untreated juices [22].

Other studies also indicate that juices retained or enhanced their physico-chemical qualities on ultrasonic processing namely peach juice [13]; pear juice [61]; five fruit juices namely carambola (*Averrhoa carambola* L.), black jamun (*Syzygium cumuni* L.Skeels.), watermelon (*Citrullus lanatus* var lanatus), pineapple (*Ananas comosus* L. Merr) and litchi (*Litchi chinensis* Sonn.) [62]; cactus pear juice [63]; Chokanan Mango (*Mangifera indica* L.)[64,65], apple juice [66]; apple and nectar juices [67,68]; strawberry juice [69] and kiwifruit juice [70].

All authors state beneficial enhancements of antioxidants and reduction of microflora within the juices whilst either maintaining or improving the quality parameters. Only one set of authors have highlighted a negative result [71]. Here the levels of sonication required in order to inhibit the browning of fresh apple juice resulted in a reduction in the concentration of the total phenolics, flavonoids, chlorogenic acid and antioxidant activity of the juice. The authors state that ultimately enzyme browning of fresh apple juice is dependent on three parameters namely polyphenol oxidase (PPO) enzyme activity, oxygen presence, and levels of phenolic compounds. The authors suggest that despite an increase in PPO activity the reduction in enzymatic browning they observed was as a result of degassing of the apple juice on sonication, resulting in oxygen removal. Beneficial phenolic and anti-oxidant compounds in the juice were also degraded at an enhanced rate by the presence of free radicals formed in the bulk liquid as a result of ultrasonic cavitation. The combination of all these effects resulted in an unsatisfactory final product.

3.3 Non Dairy Pro-biotic Juices

Pro-biotic juices of a non dairy variety are now becoming popular with consumers who want to limit or avoid the intake of dairy. The development of juices which contain beneficial bacteria is therefore a potential area for growth in the market. Use of ultrasound to encourage the growth of pro-biotic bacteria in fruit juices has proven to be beneficial and a range of juices have been inoculated and treated by sonication often with positive results.

The use of pineapple juice as substrate for *L. casei* cultivation was investigated and it was determined that the maximum microbial viability was at 31 °C and pH 5.8 in ultrasonically treated samples. After 42 days of storage under refrigeration (4 °C), the microbial viability was 6.03 Log CFU/mL in the non-sweetened sample and 4.77 Log CFU/mL in the sweetened sample [72,72,73]. In addition high-intensity ultrasound (376 W/cm^2 and 10 min) reduced polyphenoloxidase activity by 20%; juice viscosity by 75% and enhanced both the juice colour and stabilization during 42 days of storage when compared to the non-sonicated samples [72].

Cantaloupe melon juice was examined as a non dairy probiotic juice substrate for *Lactobacillus casei* [74]. The ultrasonically treated juice was stored for 42 days at 4 °C and various parameters such as colour, acidity and levels of viable cells were evaluated. The caloric value was reduced during the storage period due to sugar consumption by the remaining microorganisms however all other parameters were retained.

Evidence exists for the beneficial use of sonication for production of non diary probiotic juices if consumer interest continues to grow the potential for enhanced novel products should also increase.

4. Sterilization

The food industry uses conventional thermal pasteurization and sterilization techniques in order to inactivate microorganisms and enzymes and to increase the shelf life of many products. While most vegetative microorganisms and some spores respond well to such techniques others prove more resistant and this can result in spoilage. Thermal processes require high levels of energy and as a result impact, sometimes unfavourably, on the nutritional content, sensory properties and quality of the final product [75]. In addition to disadvantageous effects on the products the economic costs of energy involved in these high temperature processes have a large impact on the food production process and ultimately on the manufacturer themselves. Additionally there is always the risk of food borne microbial infections associated with the consumption of inadequately sterilised/pasteurized dairy products and fruit juices.

Several studies have shown the ability of ultrasound to inactivate spoilage and pathogenic microorganisms and enzymes in dairy products [6,24,25,76-79] and fruit juices [80-83] (See Table 3 and below for further details). This is often attributed to thinning of cell wall membranes, formation of localized hotspots and production of free radicals within the bulk medium [84-86]. It is also stated that the use of ultrasound produces mechanical forces which result in the breaking and shearing of microorganism cell walls. Electron microscopic analysis of milk spoilage microbes namely *Escherichia coli*, *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* showed that sonication inflicts both internal and external damage on the microbes [87,88].

However use of ultrasound as a sole method of inactivation is not feasible with most benefits being observed with combined treatments such as thermosonication, manosonication or use of UV irradiation [89]. High intensity, low frequency (10-1000 W cm^{-2} or 20-100 kHz) ultrasound is most often quoted as being able to generate sufficient acoustic cavitation to be of benefit.

The microbial activity of a range of fruit juices over several weeks was monitored (see Figure 3). Those treated with ultrasound for 30 minutes at temperatures below 30 °C, either with or without additional UV irradiation, provided similar inactivation rates as compared to conventionally treated samples over the longest storage times [60]. Ultrasound consistently maintained microbiological safety levels for the range of fruit juices studied for up to 10 weeks post treatment however achieved at much lower treatment temperatures.

Figure 3 goes hereabouts

Thermosonication of skim milk and cream at average powers of 104 W (133 µmp-p) and 115 W (152 µmp-p) for 1 and 3 min destroyed coliforms and over 99% of the total aerobic bacteria as well as decreasing plasmin activity by almost 94%. On longer term 30 day storage the total aerobic bacteria counts of thermosonicated skim milk and cream samples were less than 20,000 cfu/mL [41].

The effect of thermosonication (35 kHz) on Ayran, an acidic milk drink, were also investigated at different temperatures (60, 70 and 80°C) and times (1, 3 and 5 min). The effects on the physicochemical, microbiological and sensorial of the drink were examined during storage [23]. According to the results, thermosonication was most effective at 70°C for 3 mins with both *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* counts decreasing as the temperature and treatment time increased. The sensory properties of the thermosonicated samples were also better than the thermally treated samples.

Two flow-through reactors for hydrodynamic and ultrasonic cavitation were employed for the simultaneous pasteurization and homogenization of fresh cow milk [4]. Hydrodynamic cavitation was employed via a loop reactor at 6 bar pressure under a CO_2 atmosphere for 30 min treatment durations which resulted in up to 88% microorganism inactivation. Employing acoustic cavitation in an ultrasonic flow reactor (Sonotube®, 370 W) resulted in a 95% microorganism inactivation within 10 minutes of treatment. The authors state that efficient homogenization was achieved and that both processes can easily be scaled-up for industrial applications.

Ultrasound was combined with either sodium benzoate or citrus extract, to inhibit *Fusarium oxysporum* in orange juice. Ultrasonic treatment at 130W, 20 kHz; amplitude 40% to 100%, pulse (2 and 10 s), and duration of the treatment (from 2 to 10 min achieved a reduction of 5 log cfu/ml for at least 14 days [90]. The inactivation of spoiling yeasts of fruit juices were initially tested towards *Saccharomyces cerevisiae* inoculated in different juices (strawberry, orange, apple, pineapple and red-fruits) [81]. Optimum conditions 130W amplitude 60 %, 4 min duration with 2 s pulse was then applied to yeasts (*Pichia membranifaciens, Wickerhamomyces anomalus, Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Candida norvegica*) [91]. Ultrasonic power and duration had the most effect in reducing levels of the organisms, with the use of intermittent ultrasonic pulse treatment having only a minimal influence on the results [81,91].

The survival and growth *of Escherichia coli* in cactus pear juice was evaluated over 5 days in terms of total soluble solids, pH, titratable acidity, [15]. Total inactivation was observed after 5 min of ultrasound treatment 20 kHz, 1500W at most amplitude levels (60%, 70%, 80% and 90%).

The microbial shelf life of yeasts *Candida parapsilosis* and *Rhodotorula glutinis* in cloudy apple juice was also examined [80]. Samples treated with ultrasound produced a reduction of aerobic mesophilic counts and psychrophilic bacteria of approximately 3 and 5 log CFU/mL respectively. Consumer taste test panels also expressed a preference for the sonicated juice [21].

Other authors investigated bacteria, yeast and spores in a range of liquid beverages for example the inactivation of *Geobacillus stearothermophilus* in skim milk powder [92], *Alicyclobacillus acidoterrestris* spores in orange juice [82]; inactivation of *Alicyclobacillus acidoterrestris* spores and *Saccharomyces cerevisiae* inoculated in natural squeezed apple juices [83]; *Escherichia coli* ATCC 35218, *Salmonella Enteritidis* MA44 and *Saccharomyces cerevisiae* KE 162 and indigenous flora in commercial (CAJ) and freshly pressed (NAJ) apple juices [93]; inactivation of *Listeria monocytogenes* and *Escherichia coli* suspended in apple and orange juices [94]; *Escherichia co*li O157: H7 and *Salmonella Enteritidis* in mango juice [95]; *Escherichia coli* K12 cells suspended in apple cider [96]; *Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Debaryomyces hansenii* in milk [97]; *Escherichia coli* ATCC 25922 and *Saccharomyces cerevisiae* in pomegranate juice [98,99]. *Saccharomyces cerevisiae* in orange juice [100]; *Listeria m*onocytogenes in nonfat, low-fat and full-cream milks [101].

All authors state an inactivation of bacteria or yeasts when employing sonication with thermosonication being the most common process employed. Authors also claim an increase in shelf life with no reduction of flavour stability or texture. The most often quoted frequencies used are in the range of 20 kHz with short duration times, often well under 5 minutes. Treatment temperatures are most commonly 50-60 °C with a few below being investigated. Nevertheless temperatures are well below those normally employed for thermal pasteurization and sterilization thus leaving open the option of potential economic savings in terms of energy use for mass producers

5. Potential for pilot scale applications

Some authors have previously reported examples of scale up work using ultrasound in food processing describing equipment used for batch and continuous processing [102], with applications ranging from homogenization, pasteurization and solid/liquid mixing [103][104]. Novel ultrasonic transducers have been specifically tailored to the requirements of a range of industrial food processes. These comprise a variety of transducers designed with the radiators adapted to different specific uses in fluids and multi-phase media [105]. Often bespoke systems are required for optimal results whereupon each process has been assessed on an individual basis and configurations can range up to 1000L volumes using a range of ultrasonic frequencies. With regard to the very most recent work several authors have attempted to reproduce results found on a laboratory scale to slightly larger proportions or attempted to identify processes which could be scaled up to some extent (see Table 4).

A novel type of ultrasound equipment employed for the extraction of virgin olive oil has been developed [106]. Two ultrasound assisted extraction processes (35 kHz, 150W, 4.25L) were compared to the traditional method. The sonication treatment was applied to olives submerged in a water bath (before crushing) and also to olive paste (after crushing). A reduction of the processing time with enhanced extract yields was observed with the whole olives exhibiting greater yields of minor compounds and greater ease of extraction as compared to the traditional method.

Olive oil with oleuropein was produced both on a laboratory and pilot plant scale [107]. Olive oil was treated with 25 kHz, 200W frequency sonication using a 30L bath type batch system. Extraction of the target phenolic compounds was enhanced within 45 minutes when sonicated as compared to those obtained using conventional methods. The final oil exhibited a higher radical scavenging capacity indicating an enrichment of these compounds within the final product.

A doughnut shaped ultrasonic reactor was employed at 20 kHz sonication frequency to enhance corn slurry saccharification [108]. Continuous flow experiments were conducted by pumping corn slurry at various flow rates (10–28 L/min) through the ultrasonic horn and the yield and particle sizes of the sugars were monitored. The sonicated samples were found to produce 2–3 times more reducing sugars than the unsonicated controls.

In another study Tween 80, or milk protein isolate (MPI), were employed as emulsifiers with continuous laboratory scale processing carried out by positioning the ultrasonic probe (20 kHz, 750W) orthogonal to the path of flow of the pre-emulsion with flow rates of 25– 250 mL/min and an amplitude of 20–40% (see Figure 4.(a)). Experiments were increased to pilot scale employing the Hielscher Ultrasonics UIP1000hd equipment which again had the probe orthogonal to the path of flow of the pre-emulsion (see Figure 4.(b)) [109].

Figure 4 goes hereabouts

In the pilot scale experiments flow rates ranged from 2700 to 5700 mL/min (163–343 L/h) with ultrasound at 20 kHz, 1000W, amplitudes of 50– 100%. Processing volume, residence time and ultrasonic amplitude, as well as emulsion formulations, emulsifier type and concentration, were studied for the effect on emulsion droplet size. Emulsions prepared with ultrasound yielded submicron droplets, ~200 nm. Efficiency was determined to be the result of smaller processing volumes required for continuous ultrasonic emulsification [109].

Emusifying agents Tween 80 and Span 80 were used to prepare stable emulsions of coconut oil in water [110]. The effect of different ultrasonic operating parameters on a 50mL–2 L scale were examined in addition to the stability and droplet sizes of the final emulsions produced. Several reactor designs were suggested with the authors stating that the power dissipated per unit volume of emulsion being of prime importance and that the final droplet size of the emulsion can be correlated with the time of sonication and power density employed.

Using milk protein concentrate and a batch mode, pilot-scale process, involving ultrasound pre-treatment, enzymolysis, membrane filtration and drying; the production of angiotensin converting enzyme (ACE), inhibitory and anti-oxidative peptides was investigated. The milk concentrate solutions, 50L containing 2% or 5% protein, were sonicated for 4 min at 800W then hydrolysed for 2h followed by ultrafiltration. Results indicated that membrane filtration can separate ACE inhibitory and anti-oxidative peptides in complex protein hydrolysates [111].

The performance of ultrasound based sterilization for processing of different fruit and vegetables was evaluated. 5L volumes of spinach juice were studied and larger scale results met microbiological and physiochemical safety limits even after 20 days storage under refrigerated conditions [60].

Several experimental parameters were optimised for processing large volumes of whey and casein-based dairy systems in pilot scale ultrasonic reactors [112]. Flow rates ranging from 200 to 6000 mL/min. were examined and a significant reduction in viscosity observed. The gelling properties and heat stability of the sonicated samples were retained even after spray drying and reconstitution. The authors state that the sonication procedure may be used to improve dairy process efficiency and improve production times.

Finally two 400 kHz transducers were employed to separate out palm oil from an ex-screw press feed. The plates were placed into direct contact with the feed and the rate of oil recovery was greatly increased by sonication. The final oil quality was also unaffected in terms of its bleachability index, vitamin E and free fatty acid content thus indicating that sonication enhances recovery whilst maintaining the quality of the final oil [113].

Recently reported work in this area appears to suggest that authors are beginning to scale up processes which appear to be very promising to a more industrial focus. This suggests that users are becoming more open to the potential of employing ultrasound in a range of food processes ranging from sterilisation and emulsification to reducing processing times and temperatures.

6. Conclusion

In conclusion the benefits of using ultrasound to process liquid foods are clear. Enhanced emulsification and sterilization can be achieved at much lower temperatures than conventional processing, thus producing a more stable product whilst preventing deterioration and retaining many beneficial bioactive ingredients.

Ultrasonically enhanced fat fractionation, casein disruption and production of dairy pro-biotic drinks offers the potential of reduced processing times, and as a result economic savings, as well as possible products which have different attributes than those produced through conventional means.

Scale up of several processes has been achieved thus opening up the possibility of more industry led ultrasonic processes with viable economic savings to the producers in terms of processing times, temperatures and value added products.

Finally consumers also appear to be satisfied with several studies showing that ultrasonically treated products appear to perform better with taste panels in terms of quality, texture and flavour of the final products themselves.

All these factors lead to a positive outcome for future use of ultrasound within the liquid food processing industry.

Tables 1 – 4 go here

7. References

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Figure 1. Protein size distributions for (a) 1% untreated MPI (solid line) and 1% ultrasound treated MPI (dashed line) and (b) 1% untreated PPI (solid line) and 1% ultrasound treated PPI (dashed line). ([33] doi:10.1016/j.colsurfa.2015.07.065) Reprint permission under a Creative Commons license

Figure 2. Schematic depiction of the stage-based ultrasonic processing protocol. Each 'stage' refers to 5 min of sonication, after which sample is collected at the very top and bottom of the separation vessel. These samples represent the extents of separation after each stage. [43]

Figure3. Microbial activity of juices treated with different methods (combinations of US and UV as well as thermal) during storage at 4 °C for 10 weeks [57].

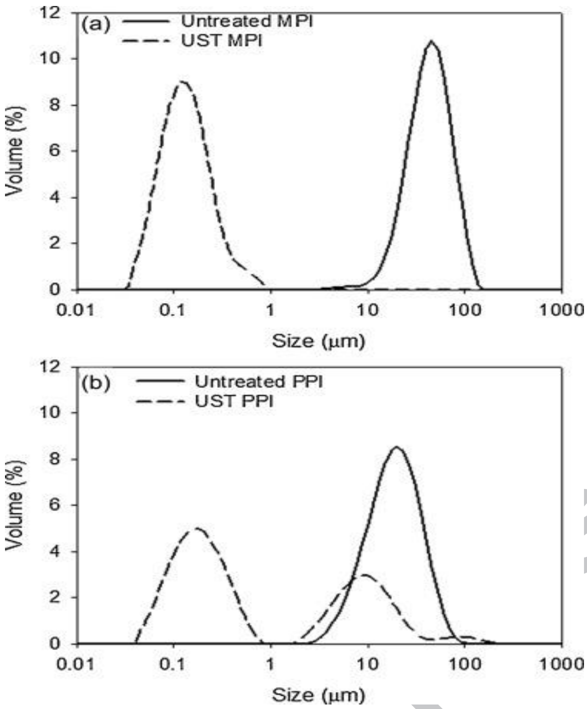
Figure 4. Schematic of continuous ultrasonic emulsification for (a) lab scale and (b) pilot scale trials. [104] doi:10.1016/j.jfoodeng.2015.05.001). Reprint permission under a Creative Commons license

Table 1 – Ultrasonic parameters and effects on milk and dairy products

Table 2 – Ultrasonic parameters and effects on fruit juices

Table 3 - Ultrasonic parameters and effects on sterilization

Table 4 – Examples of Ultrasonic Pilot Scale



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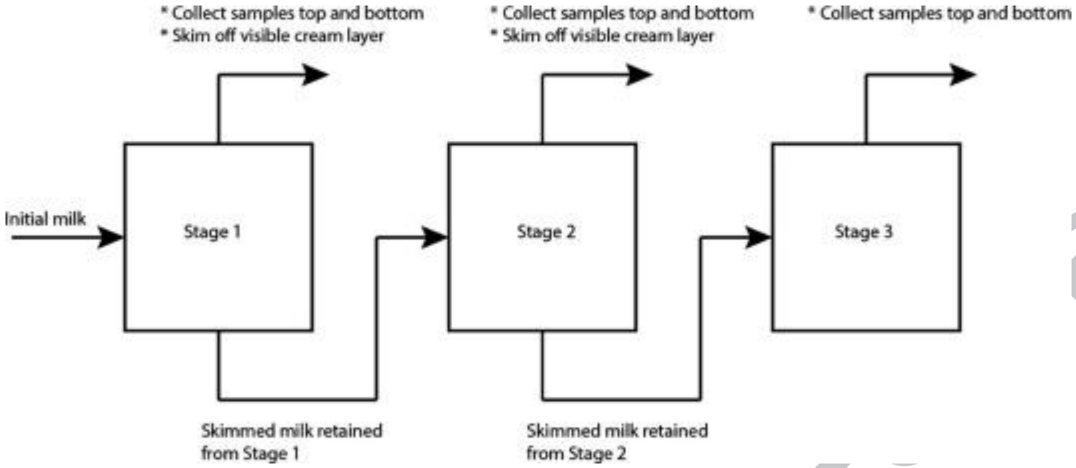

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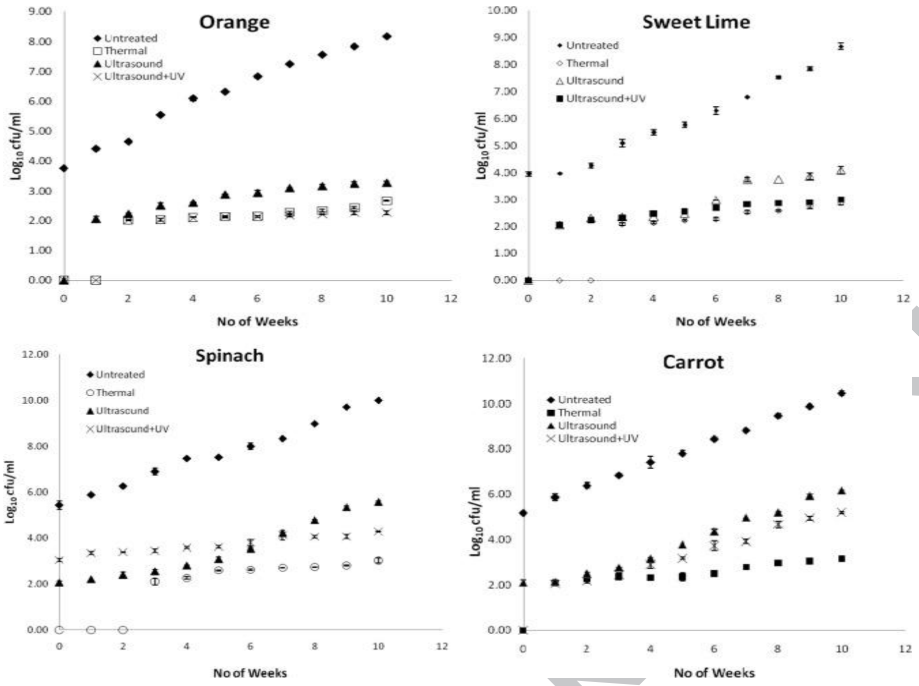

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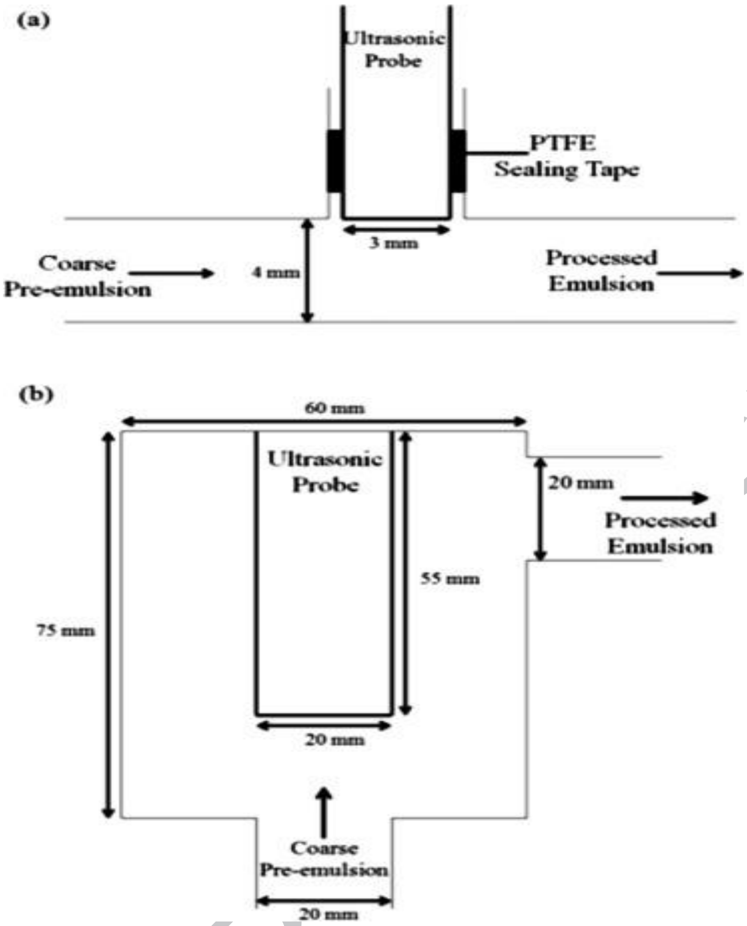

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Table 1 – Ultrasonic parameters and effects on milk and dairy products

Sample	**Experimental parameters**	**Effect of Ultrasound**	**Author**
Oil/Water	20 kHz, 400 W, amplitude 30 µm, approx 21 °C	Production of nanoemulsions with mean particle sizes down to 40 nm	[35]
Flax seed Oil/Water	20–24 kHz, 400 W	Effective production of emulsions. More than one to five minutes sonication is ineffective	[36]
Oil/Water	24 kHz , amplitude 100 µm, 20° C, up to 300 secs	More stable nano-emulsions prepared by combining high pressure and high power ultrasound	[37]
Flax seed oil emulsions in dairy systems	20 kHz, 176 W, 1-8 min UV light (13.2 W cm^{-2}) 65 °C for 30 min	Produced stable emulsions for a minimum of 9 days storage at 4 ± 2 °C	[28]
Dairy proteins	20 kHz, ~ 34 W cm^{-2}, 2 min	Reduction in the micelle size and hydrodynamic volume for sodium caseinate, whey and milk protein isolate	[39]
Citrus oil in water	20 kHz , 2-4 mins 3 to 8 g/kg volumes using 10 mL/L oil, or oil concentrations from 5 to 20 mL/L using 6 g/kg cellulose	Ultrasonic homogenization was more effective in preparing stable emulsion than shear homogenization	[114]
Milk and Pea protein isolates	20 kHz, amplitude 95% (108 µm at 100% amplitude) (~34Wcm^{-2} for 2min	Ultrasound treatment reduced the size of both MPI and PPI to the nanoscale (~150. nm) from micron sized aggregates (~20. µm).	[29]
Skim Milk and Cream	20 kHz, 115 W for 3 min, 104 W (133 µmp-p) and 115 W (152 µmp-p) for 1 and 3 mins	Decreased the fat globule size and reduced aerobic bacterial levels by 99%	[41]
Milk	(20, 400, 1000, 1600 and 2000 kHz), at temperatures (4, 20, 45 and 63 °C) for various sonication times and ultrasound energy inputs of up to 409 kJ/kg.	Volatile compounds were detected at 400 kHz and 1000 kHz frequencies and specific energies greater than 271 kJ/kg	[33]
Milk	20 kHz, 70.2 W, 152 ± 3 Jcm^{-3} per min	Solubilisation of the powders and release of individual casein micelles into solution is accelerated	[42]
Skim Milk	20, 400 or 1600 kHz energy input of 286 kJ/kg, <30 °C	Low frequency ultrasound caused greater disruption of casein micelles.	[50]
Milk	20 kHz, 600W, 50% amplitude for 0.5, 1, 2, and 5 mins	Particle size reduced from 28.45 µm to 0.13 µm after 0.5 min of sonication. Solubility increased significantly from 35.78% to 88.30% after 5 min of pre-treatment	[26]
Raw milk, retentate and cream	20 kHz, 450 W, 152 ± 3 Jcm^{-3} per min, up to 30 mins	Sonication at < 10°C indicated aggregation of fat globules	[115]

Goats Milk	30 kHz, 100 W ultrasonic power and high hydrostatic pressures of up to 600 MPa for a maximum of 9 minutes	Enhanced homogeneity of fat globules	[40]
Goats Milk	20 kHz, 800 W, 0-20 min at 38°C	Particle sizes decreased. Increase in whey protein denaturation, soluble calcium and phosphorus, gel firmness, coagulum strength, final storage modulus, cohesiveness, water holding capacity and cross-linking	[32]
Milk	1 MHz and 2 MHz	Fractionation of fat in milk to form distinct particle size distributions.	[47,48]
Milk	0.4–3 MHz, 5L reactor, 35 °C, 4 min	Creaming, aggregation of fat globules	[116]
Raw and recombined milk	400 kHz , 1.6 MHz, 4 mins, 35 °C	Enhanced Creaming, aggregation of fat globules	[45]
Whole Milk	600 kHz (583 W/L) or 1 MHz (311 W/L) temps of 5, 25, or 40 °C. up to 5 min treatment time	Greatest degree of fat separation at 25°C	[30]
Whole Milk	1 MHz, 2 MHz, 5 L , up to 20 mins	Fat separation was enhanced by reducing transducer distance	[31]
Milk	20 kHz and elevated CO_2 pressure (50 to 100 bar)	Reduction in viable numbers of aerobic and lactic acid bacteria Levels were reduced by approx 3-log fold	[117]
Milk	30 kHz, 2- 8 W, 1-3 mins	Activated the metabolic activity of lactic acid bacteria increased the rate of the fermentation process by 10%. Improved the quality	[118]
Milk	20 kHz, 150–750 W, conventional pressure treatment (10–30 MPa/5 MPa).	Homogenization reduced the fat globule size to 0.78 µm. The pH reduction rate and the duration of pH lag phase were reduced	[52,53]
Milk	20 kHz , 100, 125 and 150W	Delayed serum separation and increased viscosity.	[54]
Skim milk	20 kHz, 101 kW m^{-2} 15 min	The rennet gelation time, curd firming rate, curd firmness, and the connectivity of the rennet gel network were improved significantly in rennet gels made from milk ultrasonicated at pH 8.0 and re-adjusted back to pH 6.7 compared to those made from milk sonicated at the natural pH 6.7.	[52]
Milk	1 MHz, 348 W and 2 MHz, 280W decreasing power level to 0	Lipid oxidation volatiles were below sensory detection levels with no oxidation observed	[119]

968 **Table 2 – Ultrasonic parameters and effects on fruit juices**

Sample	**Experimental Parameters**	**Effect of Ultrasound**	**Author**
Grapefruit	28 kHz, 30, 60 and 90 min, 20 °C	Significant improvement in the cloud value, total antioxidant capacity, DPPH free radical scavenging activity, ascorbic acid, total phenolics, flavonoids and flavonols	[19,55]
Apple	25 kHz, 0.06 W cm^{-3}, 30 mins and 20 kHz, 0.30 Wcm^{-3} , 5 and 10 mins , 20, 40 and 60 °C	Inactivation of enzymes (polyphenolase, peroxidase and pectinmethylesterase) and microflora	[56]
Apple	25 kHz , 2 W/cm^2 , (70% amplitude), 0, 30 and 60 mins, 20 °C	Concentration of polyphenolic compounds and sugars increased significantly. No significant effect on juice characteristics with significant reduction in microbial population	[12] [58]
Apple	20 kHz, 20°C , 3mins, pulse durations of 5s	Significant increases in colour values, cloud value, ascorbic acid and antioxidant capacity	[20]
Grape	40 kHz sonication for 5 mins post harvest and then incubated for 6 h in the dark at 25 °C	Resveratrol increase by factors of 1.53, 1.15 and 1.24.	[59]
Mulberry	20 kHz, 650 W, 30 mins 20 °C	Preserved more bioactive compounds and antioxidant activity with ultrasonically treated samples exhibiting more total phenolics, anthocyanin and antioxidant activity.	[18]
Orange, sweet lime, carrot and spinach juices	20 kHz, 100 W, 15 min UV treatment (2 UVC lamps (254 nm) 8 W)	Ultrasound treated juice retained most of its nutritional qualities whilst a scale up attempt with spinach juice retained nutrients for 18 days post treatment resulting in an enhanced shelf life. Positive sensory evaluation panel	[22,120,121]
Sour orange	20 kHz, 80W for 21.8 min at 75°C and 9.8 min at 63°C	Inactivation of pectin methyl esterase	[57]
Pineapple	19 kHz, 376 Wcm^{-2}, 10 mins	Development of non dairy probiotic beverages for L. casei cultivation	[72,73]
Melon	19 kHz, 376 Wcm^{-2}, 10 mins	Development of non dairy probiotic beverages for L. casei cultivation	[74]
Pear	20 kHz, 750W, 10 mins at 25, 45 and 65°C	Retention of ascorbic acid and other phenolic compounds with significant reduction in enzyme activities and complete inactivation of microbes	[61]
Chokanan mango	40 kHz, 15, 30 and 60 min at 25 °C	Retention of individual phenolic compounds, significant enhancement in antioxidant activities. Positive sensory evaluation panel	[64,65]

Apple	55 to 3300W/L. 23°C (ambient) to 60°C and processing times 5 to 20 min	Partial inactivation of Polyphenoloxidase and Peroxidase	[66]
Apple and Nectar	20 kHz (amplitudes 60, 90 and 120 mm) for 3, 6 and 9 min at temperatures of 20, 40 and 60 °C	Formation of new compounds	[67,68]
Strawberry and Kiwi	40 kHz, 180W, 10 and 30 mins	Reduced yeast and mould counts	[69,70]
Cactus Pear	20 kHz, 1500W (80% amplitude), 15 and 25 min	Minimum increase in pectinmethylesterase activity (from day 14), and similar total plate counts to pasteurized juice. An increase of phenolic content was observed after 14 days of storage and an increase in antioxidant activity (ABTS, DPPH) by the end of storage	[63]

973 **Table 3 - Ultrasonic parameters and effects on sterilization**

Sample	**Experimental Parameters**	**Effect of Ultrasound**	**Author**
Inactivation of *Listeria innocua* and mesophilic bacteria in milk	24 kHz, 120 µm, 400 W, 63 °C, 30 min	5 log-reduction was obtained after 10 min when sonication is used in combination with temperature	[122]
Enterobacter aerogenes, in skimmed milk	20 kHz, 850 kHz, up to 50W for 60 mins 30 °C or less	High freq ultrasound was not able to reduce bacterial levels even on prolonged treatment	[76]
Enterobacter aerogenes, *Bacillus subtilis*, *Staphylococcus epidermidis*, *S. epidermidis*, *Staphylococcus pseudintermedius*	20 kHz, 13W, 20 mins 30 °C or less	up to 4.5-log reduction for *E. aerogenes* and *B. subtilis* however *Staphylococcus* spp. were not affected	[77]
Total aerobic mesophilic and coliform bacteria in Milk	Sonication 24 kHz, 400 W up to 15 mins 65 °C Photosonication 100% (120 µm; 240 W) 13.2 W cm^{-2}	UV light contributed to the ultrasonic lethal effect with greater log reductions observed with photosonication than with sonication alone.	[78]
Inactivation of *Pseudomonas fluorescens, Escherichia coli, Listeria monocytogenes in milk*	20 kHz, up to 15 mins	Most cell counts reduced by 100% within 10 mins of sonication with no loss of proteins observed	[87]
Electron microscopic analysis of dairy microbes	20 kHz, 750 W, at 100%/124 µm wave amplitude	Bacterial levels reduced, extensive damage observed	[88]
Inactivation of *Geobacillus stearothermophilus* in skim milk powder.	20 kHz, 45 °C, and 30 secs for cells 20 kHz, 67.5 °C, and 17.5 secs for spores	Cell reduction (4.8 log) at 19.75% total solids and spore reduction (0.45 log) 31.5% total solids	[92]
Skim Milk and Cream	20 kHz, 115 W for 3 min, 104 W (133 µmp-p) and 115 W (152 µmp-p) for 1 and 3 min	Reduced aerobic bacterial levels by 99%	[41]
Inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* in pomegranate juice..	20 kHz , amplitude levels of 50, 75 and 100% , 0, 3, 6, 9, 12 and 15 min, 25 ± 1°C	100% amplitude level for 15 min, reduced levels by 3.47 and 1.86 log cfu/mL, respectively	[98]
*Fusarium oxysporu*m in orange juice.	20 kHz, 130 W, amplitude 40% to 100%, pulse (2 and 10 secs), 2 to 10 min	The use of benzoate and citrus extract controlled *F. oxysporum* growth in orange juice and achieved a reduction of 5 log cfu/ml for at least 14 days	[90]
Saccharomyces cerevisiae, Pichia membranifaciens, Wickerhamomyces	20 kHz, 130W amplitude 20% to 60%), pulse (2 and 6 s), 2 to 6 min	Reduction of spoilage organisms	[81,91]

anomalus, Zygosaccharomyces bailii, Zygosaccharomyces rouxii, Candida norvegica in strawberry, orange, apple, pineapple and red-fruits juice			
Escherichia coli in cactus pear juice.	20 kHz, 1500W (60%, 70%, 80% and 90% amplitude), 1, 3 and 5 mins	Total inactivation was observed in both fruit juices after 5 min of ultrasound treatment at most amplitude levels, evaluated over 5 days	[15]
Streptococcus thermophilus and *Lactobacillus delbrueckii subsp. bulgaricus* in Ayran, an acidic milk drink	35 kHz, 60, 70 and 80°C, 1, 3 and 5 mins	Counts decreased as the temperature and time increased. Sensory properties of the thermosonicated samples were better than the thermally treated samples after storage	[23]
Inactivation of *Alicyclobacillus acidoterrestris* spores in orange juice	24 kHz , 460 W/cm^2, 33 W and 105 W/cm^2, 162 W	Thermosonication required at least 8 °C lower temperatures than thermal treatments to achieve the same spore inactivation. (D75°C-value of 49 min for 20.2 W/mL vs. 217 min for 0.33 W/mL). (D85°C-value decreased from 69 to 29 min)	[82]
Inactivation of *Alicyclobacillus acidoterrestris* spores and *Saccharomyces cerevisiae* in natural squeezed apple juices.	20 kHz, 600 W and 95.2µm wave amplitude; 10 or 30min at 20, 30 or 44±1°C) and pulsed light (PL) (Xenon l& 3 pulses/s; 0.1m distance; 2.4-71.6 Jcm^{-2}; initial temperature 2, 30, 44±1°C)	Combination of these technologies led up to 3.0 log cycles of spore reduction in commercial apple juice and 2.0 log cycles in natural juice; while for *S.cerevisiae*, 6.4 and 5.8 log cycles of reduction were achieved	[83,93]
Escherichia coli ATCC 35218, *Salmonella Enteritidis* MA44 and *Saccharomyces cerevisiae* KE 162 and indigenous flora in commercial (CAJ) and freshly pressed (NAJ) apple juices.	20 kHz, 600 W and 95.2µm wave amplitude; Pulsed light 0.73 Jcm^{-2}, 155 mL/min	Combined Ultrasound and pulsed light led up to 3.7-6.3 log reductions of inoculated microorganisms. Browning development during storage was prevented	[93]
The inactivation of *Listeria monocytogenes* and *Escherichia coli* suspended in apple and orange juices	35 °C, 110 µm, 200 kPa	Increase of 116 W increased the inactivation rate approximately 10-fold in both juices	[94]
Escherichia coli O157: H7 and *Salmonella*	25 kHz , 200W, 50 and 60°C for 10 and	Inactivation rate was different for both pathogens	[95]

Enteritidis in mango juice,	7 mins respectively		
Escherichia coli K12 cells suspended in apple cider,	20 kHz, 100 kPa, 59°C, 4 min	5-log reduction was achieved Quality profile of sonicated sample similar to raw apple cider	[96]
Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus and *Debaryomyces hansenii* in milk.	24 kHz, amplitude (70 and 100%) and duration (50, 100, 200 and 300 s)	Population reduction but caused milk deterioration	[97]
Escherichia coli and *Saccharomyces cerevisiae* in pomegranate juice	20 kHz amplitudes (50, 75, and 100%) and times (0, 6, 12, 18, 24, and 30 min)	More than a 5-log inactivation of *E. coli* and a 1.36-log inactivation of *S. cerevisiae*	[99] [98]
Saccharomyces cerevisiae in orange juice	20 kHz, 778.2 W ultrasonic power, and 11 min of exposure, 350 W microwave power, 35 °C	Complete inactivation	[100]
Candida parapsilosis and *Rhodotorula glutinis* in cloudy apple juice	24 kHz, 400W, amplitude 100%, 35°C, 360 secs/100 mL	Shelf life of sonicated juices around 21 days with flavour maintained	[80]
Simultaneous pasteurization and homogenization of fresh cow milk	Hydrodynamic cavitation in a loop reactor working at 6 bar pressure in a CO_2 atmosphere for 30 min. Acoustic cavitation in a ultrasonic flow reactor (Sonotube®, power 370 W)	88% microorganism inactivation using hydrodynamic cavitation and acoustic cavitation achieved microorganism abatement of 95% within 10 mins	[4]

Table 4 – Examples of Ultrasonic Pilot Scale

Sample	Experimental conditions and effect of ultrasound	Author
Liquids	Range of applications discussed covering a range of equipment	[103] [89], [105]
Olive oil	Malaxation of olives examined after prior sonication 35 kHz, 150W, 4.25 L, 19 °C using an ultrasonic bath. Prior sonication reduced malaxation time	[106]
Olive Oil	25 kHz, 60 W, 30L, 16 °C, 45 min. Final olive oil is greatly enriched in phenols whilst other beneficial compounds were not degraded	[107]
28% w/v corn slurry	Batch systems operating at a frequency of 20 kHz, 3.3kW, 20-40 secs, Continuous flow at various flow rates (10–28 L/min) through an ultrasonic reactor at constant amplitude of 12 μm. Ultrasonic system yields 2-3 times more saccharides	[108]
Emulsification of protein solutions	Batch and continuous ultrasonic emulsification processes on both lab and pilot scales 20 kHz 750W and 1000W, Emulsions prepared with ultrasound yielded submicron droplets, ~200 nm, with Tween 80 and Milk protein isolate	[109]
Coconut oil	Production of nanoemulsions of coconut oil using 3 processors namely 20 kHz, at750W, 20kHz at 1000W and 20kHz at 1500W up to 2 L volumes, Droplet size of the final emulsion can be correlated with the time of sonication and power density employed	[110]
Reconstituted whey protein in dairy	Flow through continuous sonication process at 20 kHz, 4 kW power, flow rates ranging from 200 to 6000 mL/min. significant reduction in viscosity ranging between 6% and 50%	[112]
Production of angiotensin converting enzyme inhibitory and antioxidative peptides from milk protein concentrate	Batch mode, pilot-scale process, involving ultrasound pre-treatment, enzymolysis, membrane filtration and drying 50 L batches, duration 4min at 800W Large amounts of angiotensin converting enzyme inhibitory peptides were recovered	[111]
Palm oil	Two 400 kHz transducer plates operating at 13.4 kJ/kg were used to separate out palm oil feed on a pilot plant scale. Greater amounts were recovered using sonication	[113]
Processing of different fruit and vegetable juices in terms of sterilization and microbial growth	Combination of UV, crude orange peel extract and sonication of 20 kHz, 100 W, duration,15 mins with up to 5 L processed A greater than 5 log reduction of microorganisms was achieved and sonicated juice still satisfied the microbiological and physiochemical safety limits requirements when refrigerated for 20 days	[60]

- Use of ultrasound for processing of liquid foods is reviewed
- Ultrasound enhances emulsification and sterilisation resulting in more stable products
- Ultrasound increases the rate of fat fractionation and creaming in dairy products
- Some progress has been made towards scale up
- Ultrasonically treated products appear to perform better with taste panels in terms of quality, texture and flavour