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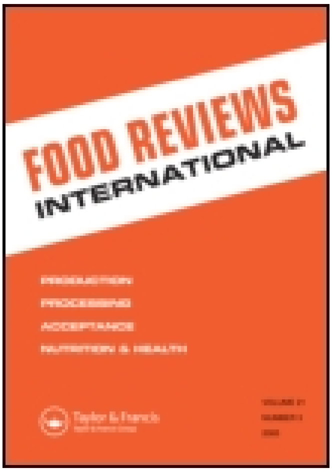

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The Use of Ultrasound and Combined Technologies in Food Preservation

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Ultrasound techniques find use in the food industry in both the analysis and modification of foods. Microbial and enzyme inactivation are other applications of ultrasound in food processing. The use of ultrasound on its own in the food industry for bacterial destruction is currently unfeasible; however, the combination of ultrasound and pressure and/or heat shows considerable promise. The future of ultrasound in the food industry for bactericidal purposes lie in thermosonication, manosonication, and manothermosonication, as they are more energy-efficient and result in the reduction of microbial and enzyme activity when compared to conventional heat treatment. The use of ultrasound and combined technologies, mechanisms, and effects of ultrasound combinations are discussed in this review.

Keywords ultrasound, thermosonication, manosonication, manothermosonication

Introduction

Today, thermal treatments are the most common processing methods for microbial and enzyme inactivation or extraction that leads to a longer shelf-life. Because of the exposure to high temperature, this method often has disadvantages for many food products. Thermal treatment can cause undesirable alterations of sensory attributes, i.e., texture, flavor, color, smell, and nutritional (vitamins, proteins) qualities. Consumers now demand minimally processed fresh-like food with high-quality sensory and nutritional attributes. For this reason, targeted non-thermal food processing and preservation methods are gaining importance. Ultrasound is probably the most versatile and simplest method and for the production of extracts and the disruption of cells. Ultrasound is efficient, safe, and reliable. The use of ultrasound within the food industry has been a subject of research for many years.(1,2,3,4,5,6)

The food industry, as well as the pharmaceutical industry, offer manifold possibilities for the use of ultrasound. Ultrasound processes are used in food manufacturing for: peeling, disintegration of cells, extracting (extract intracellular components or obtain cell-free bacterial enzyme), activation (acceleration) of an enzyme reaction in liquid foods, acceleration of a microbial fermentation, mixing, homogenizing, dispersion of a dry powder in a liquid, emulsifying of oil/fat in a liquid stream, spraying, degassing, inspection, e.g., in the beverage industry, deactivation of enzymes, microbial inactivation

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(preservation), crystallization of fats and sugars, foam breaking, meat tenderization, cleaning and surface decontamination, effluent treatment, humidifying and fogging, stimulation of living cells, and enhanced oxidation.(7,8)

Moreover ultrasound can be used for: 1) extraction of phenolic compounds from vacuolar structures by disrupting plant tissue; 2) extraction of Betacyanin (red pigments, e.g., from beets) and Betaxanthin (yellow pigments); 3) extraction of lipids and proteins from plant seeds, such as soybean (e.g., flour); 4) improvement of oil extraction from oil seeds; 5) cell membrane permeabilization of fruits, such as grapes, plums, and mango; 6) processing of fruit juices, (e.g., orange, grapefruit, mango, grape, and plum), purees, sauces (e.g. tomato, asparagus, bell pepper, and mushroom), dairy products; and 7) improve stability of dispersions, such as orange juice, i.e. reduce settling.(9)

There are two properties of sound to appreciate the possibilities. The first is the use of sound as a diagnostic tool, e.g., in nondestructive evaluation and the second is the use of sound as a source of energy, e.g., in sonochemistry.(10)

The use of ultrasound in processing results in novel and interesting methodologies, which are often complementary to classical techniques. It has been proved to be particularly useful in sterilization, extraction, freezing and filtration, providing reduced processing times and increased efficiency. Recent studies have identified a number of other areas, including the stimulation of living cells and enzymes, and improved process treatment.(4) The purpose of this article is to review some successful combinations of ultrasound with traditional food preservation technologies. In this review, the use of ultrasound and combined technologies, mechanisms and effects of ultrasound combinations will be discussed.

Theory of Ultrasound

Ultrasound is defined as sound waves with frequencies above the threshold for human hearing (>16 kHz) (see Fig. 1) and in its most basic definition, refers to pressure waves with a frequency of 20 kHz or more.(11)

Generally, ultrasound equipment uses frequencies from 20 kHz to 10 MHz (see Fig. 2). Higher-power ultrasound at lower frequencies (20 to 100 kHz), which is referred to as "power ultrasound," has the ability to cause cavitations (implosion of gas bubbles), which has uses in food processing to inactivate microorganisms. Low-intensity ultrasound provides information about physico-chemical properties, while high-intensity ultrasound is used to alter, either physically or chemically, the properties of foods, e.g., to generate emulsions, disrupt cells, promote chemical reactions, inhibit enzymes, tenderize meat, and modify crystallization processes.(12)

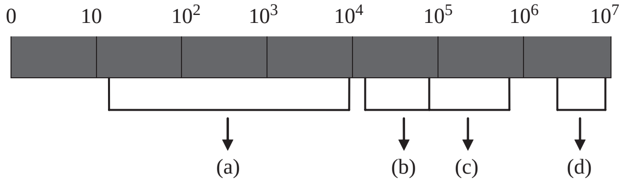

Figure 1. *Frequency ranges of sound (a) human hearing: 16 Hz-18 kHz; (b) conventional power ultrasound: 20 kHz – 100 kHz, between (b) and (c) extended for special applications: 20 kHz-1MHz; (d) diagnostic ultrasound: 5 MHz- 10 MHz.(10)

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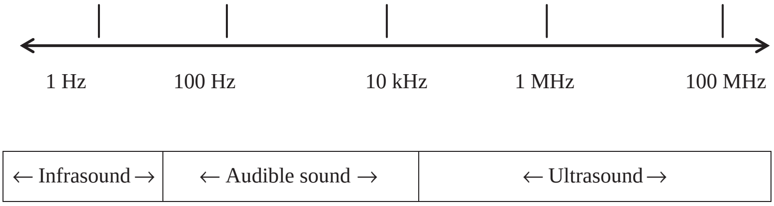

Figure 2. Acoustic spectrum.

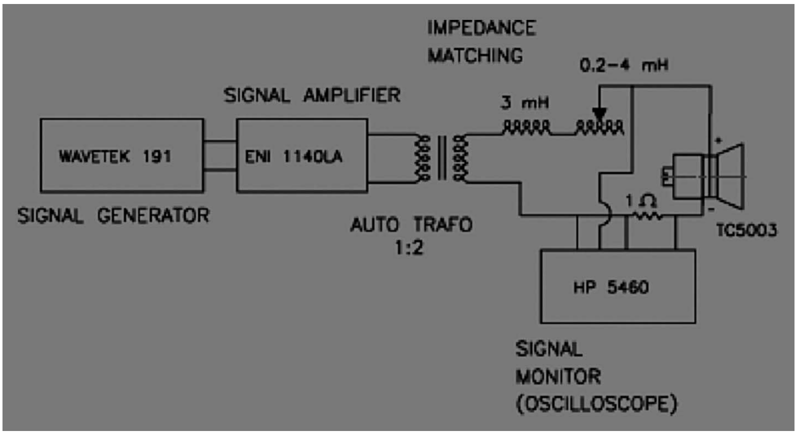

Figure 3. Components of an Ultrasound equipment.(17)

A power ultrasound will consist of three basic parts: i) generator; ii) transducer; and iii) coupler (Fig. 3).(7) Ultrasonic generators transform electrical energy into ultrasound energy (a type of mechanical energy) via a transducer. The intensity of the ultrasound treatments can be measured in terms of power.(13) Ultrasound power can be measured at different points in the ultrasound system. These measurements can be expressed as the input power to the ultrasound generator, the input power to the transducer or the transmitted ultrasound power delivered into the treated medium.

The most applicable generation of ultrasound is carried out using the electrostrictive transformer principle. The generation is based on the elastic deformation of ferroelectric materials within a high frequency electrical field and is caused by the mutual attraction of the molecules polarized in the field.(14,15) For polarization of molecules, a high-frequency alternating current is transmitted via two electrodes to the ferro-electrical material. Then, after conversion into mechanical oscillation, the sound waves are transmitted to an amplifier, to the sound radiating sonotrode, and finally to the treatment medium. Povey and Mason provide details of types of transducers that can accomplish the generation of ultrasonic waves, equipment, and their functions.(16)

Mechanisms and Effects of Ultrasound

When sound energy passes to the medium resulting in a continuous wave-type motion, longitudinal waves will be generated with the result that the motion creates alternative compression and rarefaction of the medium particles.(16) In dependence of the frequency used and the sound wave amplitude applied, a number of physical, chemical and biochemical effects can be observed which enables a variety of applications. For food processing purposes, it is important to address the generation of heat due to ultrasound applications and the related cavitation caused by a rapid change of heating to 5500°C and pressure increase to 50 Mpa.(18) The temperature and pressure indicated are generated during very short periods of time at the point were cavitation occurs with an order of temperature variation of 109°C/s.(19,20) Shock waves are generated due to cavitation, which contribute to the ultrasound effect. Formation and behavior of cavitation bubbles upon the propagation of the acoustic waves constitute the essential events, which induce the majority of the acoustic effects. The amount of energy released by cavitation depends on the kinetics of the bubble growth and collapse of the bubbles. This energy should increase with increasing surface tension at the bubble interface and decrease with increasing vapour pressure of the liquid. In particular, hydrated foods have a comparatively high surface tension, so it can be a very effective medium for cavitation.(18,21,22,23,24)

The effectiveness of ultrasound as a food processing tool has been proven in the laboratory and there are a number of examples of scale-up. In most cases the frequency used has been that which is available commercially, i.e., 20 or 40 kHz, and this has proved quite satisfactory. In such cases the variable parameters are temperature, treatment time and acoustic power.(10) The effects of ultrasound in liquid media depends on many variables, such as the characteristics of the treatment medium (viscosity, surface tension, vapour pressure, nature and concentration of the dissolved gas, and presence of solid particles), treatment parameters (pressure and temperature), ultrasound generator performance (frequency, power input), size, and geometry of the treatment vessel.(13) The influence of all these parameters on the inactivating effect of ultrasound on enzymes and microorganisms requires further study.

Ultrasound is known to disrupt biological structures and when applied with sufficient intensity has the potential to cause cell death.(25,26,27,28) The bactericidal effect of ultrasound is attributed to intracellular cavitation; that is, micromechanical shocks that disrupt cellular structural and functional components up to the point of cell lysis. Critical processing factors include the nature of the ultrasonic waves; the exposure time with the microorganisms; the type of microorganism; the volume of food to be processed; the composition of the food, and the temperature.

The lethal effect of ultrasound on some microorganisms was demonstrated first by Jacobs and Thornley (1954)(29); thus, ultrasound has been proposed as a means of sterilization of liquid foods.(29,30) The effects, however, are not severe enough for a sufficient reduction of microorganisms by ultrasound alone so most applications use combinations with other preservation methods.(31,32) Because of the complexity and sometimes protective nature of the food, the singular use of ultrasound as a preservation method is impracticable. Although ultrasound technology has a wide range of current and future applications in the food industry, including inactivation of microorganisms and enzymes, presently, most developments for food applications are non-microbial. There are not many data on inactivation of food microorganisms by ultrasound. Research activities must center on the combination of ultrasound with other preservation processes (e.g., heat and mild pressure) which appear to have the greatest potential for industrial applications.(11)

Ultrasound and Combined Technologies

Ultrasound is often more effective when combined with other antimicrobial methods; hence, different authors have attempted to use it in combination with other antimicrobial methods to increase its effect in microbial and enzyme inactivation.(33) Beneficial combinations include thermosonication (heat and ultrasound), manosonication (pressure and ultrasound), and manothermosonication (pressure, heat and ultrasound).(31)

Thermosonication

The application of ultrasound and heat has been termed thermosonication. Heat combined with ultrasound is considered to reduce process temperatures and processing times, for pasteurization or sterilization processes that achieve the same lethality values as with conventional processes.(4,34) Reduction of the temperature and/or processing time should result in improved food quality.(1,2) Ultrasound applicability was predicted for the support of conventional thermal treatments, based on the possible synergy between low frequency ultrasound and heat (see Fig. 4) for bacterial inactivation.(2,35,40)

The combined use of heat and ultrasound markedly increases the lethality of heat treatments and consequent reductions in time and/or temperature of heat processes.(36,37) Combined heat and ultrasound treatments have been reported to lower maximum processing temperatures by 25–50%. After treatment, changes in color and vitamin C were minimal.(36) Different authors have investigated combinations of heat and ultrasound to decrease the intensity of heat treatments. The heat resistance of *B. cereus*, *Bacillus licheniformis, B. stearothermophilus, and thermoduric streptococci* decreased following ultrasonication treatment at 20 kHz.(7,38,39,40,41) The effect of the combined treatment of ultrasound and heat in a continuous process on microbial destruction was demonstrated by the comparison of the integrated time–temperature intensity (F value) of each treatment.(36)

Manosonication

Although ultrasound was initially discarded for food preservation because of its weak lethal action, the simultaneous application of ultrasound with an external hydrostatic pressure of up to 600 kPa (manosonication, MS) increases substantially the lethality of the treatment.

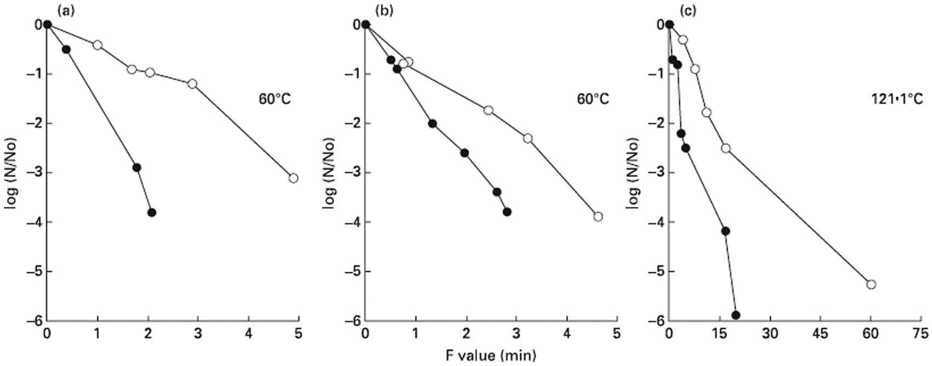

Figure 4. *Effect of combined ultrasonic and conventional heat treatment (●) in continuous flow on the survival of (a) *Escherichia coli* K 12 DH 5 α; (b) *Lactobacillus acidophilus*; and (c) *Bacillus stearothermophilus* in (○), conventional heat treatment. N/No represents the no. of colonies relative to that before treatment and F value represents the integrated time–temperature intensity in phosphate buffer (pH 7).(2)

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It has been found that MS treatments sensitize spores of *Bacillus subtilis* to lysozyme.(32) Therefore, it has been suggested that ultrasonic waves could damage the external layers of the spore, facilitating its rehydration and consequently reducing its extreme heat resistance. In contrast to the clear mechanisms of inactivation proposed for ultrasound, a much more complicated picture emerges for high hydrostatic pressure inactivation.

Manothermosonication

The concept of combination treatment has been further explored by introducing elevated static pressure in an ultrasound treatment chamber in a process called manothermosonication (MTS).(31) The lethality of ultrasound under pressure treatments is almost not modified by an increase in temperature unless lethal temperatures are reached (MTS treatments), in which case an additive lethal effect is generally attained although in some cases the total lethal effect has been found to be synergistic.(30,42,43) MTS has proved to be an efficient tool to inactivate microorganisms, especially in those conditions in which their thermotolerance is higher.(41,42,44,45,46) While in most vegetative cells the lethal effect of MTS was additive, on *Enterococcus faeciu, Bacillus subtilis, Bacillus coagulans, Bacillus cereus, Bacillus sterothermophilus, Saccharomyces cerevisiae*, and *Aeromonas hydrophila*, a synergistic effect was observed.(30,32) For example, the D value of tomato PME at 62.5°C was reduced 53-fold, from 45 min. in thermal treatments to 0.85 min. by MTS.(47) The application of ultrasound under pressure simultaneously with heat treatment results in increased microbial and enzyme inactivation. Therefore, the same inactivation level is achieved over a shorter treatment period or at lower temperature. Manothermosonication has also been used to deactivate peroxidase,(48,49) lipoxygenase,(50) lipase and protease,(51,52) and tomato or orange pectin methylesterase (PME),(53,54,55) all with an increased inactivation. Consequently, this combination could be advantageous, due to the minimization of heat-induced damage in product quality.(56)

Several mechanisms have been suggested to explain the synergistic effect of MTS on enzyme inactivation. Propagation of ultrasonic waves in a liquid medium generates bubbles (cavities) that grow up to a critical size and then collapse (cavitational collapse).(57) Ultrasound effects are mainly related to the cavitation phenomenon. As a result of intense cavitation, water molecules can be broken, generating highly reactive free radicals that can react with and modify certain molecules. Mechanical stress, generated by shock waves derived from bubble implosion or from microstreaming derived from bubble’s size oscillations, is also able to break large macromolecules or particles. This cavitational collapse creates strong shear stresses, extremely high pressures and temperatures in the so-called “hot spots,” and water sonolysis, which produces free radicals.(58) The combination of these phenomena can promote enzyme denaturation, with the relative effect depending on the structure of the protein.

There has been a renewed interest in the study of the effects of mechanical forces on enzyme stability, as it has been shown that the distribution of proteins at liquid–air interfaces strongly enhances enzyme inactivation.(59,60) Although mechanisms leading to thermal inactivation of enzymes have been extensively studied, those involved in mechanical stresses are less known. Obviously MTS treatments will depend on the effect of the pressure, temperature, and ultrasound amplitude chosen.

The mechanism of inactivation of bacterial cells by ultrasound under pressure has also been described. Most authors agree that the cavitation phenomenon is responsible for the lethal effects of ultrasound.(28,31,61) When bubbles implode under an intense ultrasonic field, very high pressures and temperatures are generated, and consequently strong mechanical forces and free radicals are formed.(19) Free radicals could therefore inactivate bacterial cells in a similar mode as that described for irradiation. However, experimental data using free radical scavengers have lead to the conclusion that the possible effect of free radicals is negligible in comparison with that of the strong mechanical effects generated by cavitation.(31,62) The effect of equivalent heat, MS and MTS treatments (99% of inactivation) on the degree of cell disruption, evaluated through phase contrast microscopy, was studied,(31) and they observed that whereas heat-treated cells maintained full cellular integrity, MS treated cells were completely broken. MTS treated cells showed a medium degree of disruption. These results confirmed that ultrasound inactivates microbial cells through envelope breakdown in an all or nothing type phenomenon.

There are however some unclear aspects that remain regarding the mechanism of action of ultrasound. In some experimental conditions, and with some bacterial species, a synergistic effect of MS and heat (MTS) has been observed. This is the case of *Enterococcus faecium*,(30) heat-shocked cells of *Listeria monocytogenes*,(42) and cells suspended in a low water activity media.(43) The reasons for the increased sensitivity of these cells to a combined MS-heat treatment are still not known. It has been suggested that moderately elevated temperatures (55–60°C) would cause a weakening effect on cell envelopes, facilitating the mechanical disruption of the cell by ultrasonic waves.(43) This weakening effect would have no relevance for thermosensitive cells, as they are killed by heat at relatively low temperatures. In addition, a synergistic effect of MS and heat has been described for bacterial spores.(32) Ultrasonic treatments cause the release of some low molecular weight polypeptides and dipicolinic acid from the spore.(63)

MTS effects have been mainly studied on enzymes and microorganisms,(47,64) but it is also possible to modify and improve textural and functional properties of tomato juice and milk proteins.(50,65) Manothermosonication has also been proposed as an alternative to heat treatments in the processing of liquid eggs.(66,67) As MTS is only suitable for treatment of liquid foods, two of the potential products to which MTS could be applied are fruit juices and milk. MTS is an efficient tool to inactivate enzymes from psychrotrophic bacteria,(51) which are responsible for some quality problems of milk and some dairy products,(68) and to inactivate thermoresistant pectin methylesterase in orange juice(54) and pectic enzymes from tomato paste.(55) Moreover, ultrasonic waves and heat combine additively to inactive pathogenic microorganisms(32) and synergistically to destroy spores.(31)

Ultrasound and Chemical Combinations

Ultrasound can also be used in combination with chemical treatments. Chemicals such as chlorine and chlorine dioxide solutions are often used to decontaminate food products or processing surfaces and it has also been demonstrated that clorine combined with ultrasound enhances the effectiveness of the treatment. This theory was demonstrated using *Salmonella* attached to the surface of broiler carcasses; bombardment with ultrasound caused the cells to become detached from the surfaces, making it easier for the chlorine to penetrate the cells and exert an antimicrobial effect.(1,69) Blume and Neis(70) researched whether the presence of soluble organic material, as well as high concentrations of suspended matter in waters and wastewaters, affects the efficiency when chlorine is used as disinfection agent. The objective was to explore the extent to which ultrasonic treatment can facilitate wastewater disinfection with chlorine in order to reduce doses of ecologically questionable chlorine and to shorten contact times. Ultrasound application provokes better chlorine dispersion in the aqueous media, which improves the fast chemical and bactericidal reaction.(70)

The bactericidal properties of chlorine dioxide (ClO_2) have been known for the last century, but it has been used in sanitation only since the 1950s.(71,72) The disinfecting power of ClO_2 is relatively constant within a pH of 6 to 10 and the presence of high levels of organic matter. The combined effects of chemical, heat, and ultrasound treatments in killing or removing *Salmonella* and *E. coli* O157:H7 on alfalfa seed has confirmed the hypothesis that combined stresses and enhanced exposure of bacterial cells to chemicals would result in higher lethality.(73) Unlike chlorine, ClO_2 does not generate toxic by-products when it encounters organic matter in solutions.(74) ClO_2 can effectively remove bacteria from food, and ultrasonication can promote the bactericidal effect in ClO_2 treatments on inoculated apples and lettuce. The decontamination efficacy of ClO_2 plus ultrasonication on apples was higher than on lettuce.(72)

Conclusion

The minimal processing concept supports the mega-trend towards health-promoting foods. With proper selection of processing methods and conditions, it is possible to preserve nutritional compounds in foods. Therefore, when developing health-promoting foods emphasis should also be placed on the preservation of beneficial compounds already existing in the raw food material as well as on the removal of harmful compounds. Often, from the legislative point of view, this might even be an easier way to develop health-promoting foodstuff than the approach in which health-promoting compounds are added to the food product. Ultrasound has been successfully used by the food industry for: the measurement of thickness of pipes, chocolate layers, fat, lean tissues in meat, canned liquids and shell eggs; detection of contaminants such as pieces of metal, glass or wood in foods; measurement of flow rates through pipes; determination of food composition; and measurement of particle size distribution in dispersed systems. However, further research is required before ultrasound becomes an alternative method of food preservation: a determination of the effect of ultrasound on microbial inactivation efficiency when used with other processing technologies (high pressure, heat or others); the identification of the mechanisms of microbial inactivation when used in combination with other technologies; the critical process factors when ultrasound is used in hurdle technology; and evaluation of the influence of food properties (such as viscosity and size of particulates) on microbial inactivation. Ultrasound also needs to be assessed and food manufacturers must decide whether the ultimate benefits outweigh the cost of converting and maintaining the processing equipment.

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