



Applications of ultrasound in food technology: Processing, preservation and extraction

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ABSTRACT

Ultrasound is well known to have a significant effect on the rate of various processes in the food industry. Using ultrasound, full reproducible food processes can now be completed in seconds or minutes with high reproducibility, reducing the processing cost, simplifying manipulation and work-up, giving higher purity of the final product, eliminating post-treatment of waste water and consuming only a fraction of the time and energy normally needed for conventional processes. Several processes such as freezing, cutting, drying, tempering, bleaching, sterilization, and extraction have been applied efficiently in the food industry. The advantages of using ultrasound for food processing, includes: more effective mixing and micro-mixing, faster energy and mass transfer, reduced thermal and concentration gradients, reduced temperature, selective extraction, reduced equipment size, faster response to process extraction control, faster start-up, increased production, and elimination of process steps. Food processes performed under the action of ultrasound are believed to be affected in part by cavitation phenomena and mass transfer enhancement. This review presents a complete picture of current knowledge on application of ultrasound in food technology including processing, preservation and extraction. It provides the necessary theoretical background and some details about ultrasound the technology, the technique, and safety precautions. We will also discuss some of the factors which make the combination of food processing and ultrasound one of the most promising research areas in the field of modern food engineering.

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1. Introduction

One of the great success stories of modern chemistry has been the evolution of a system that is increasingly more efficient at directly translating knowledge into technology and commercial products. Utilization of ultrasound in food technology for processing, preservation and extraction is such a system that has evolved to keep the wheel of development rolling. Ultrasound makes use of physical and chemical phenomena that are fundamentally different compared with those applied in conventional extraction, processing or preservation techniques. Ultrasound offers a net advantage in term of productivity, yield and selectivity, with better processing time, enhanced quality, reduced chemical and physical hazards, and is environmentally friendly.

Food products, such as fruit and vegetables, fat and oils, sugar, dairy, meat, coffee and cocoa, meal and flours, are complex mixtures of vitamins, sugars, proteins and lipids, fibres, aromas, pigments, antioxidants, and other organic and mineral compounds. Before such products can be commercialised, they have to be pro-

cessed and preserved for food ready meals and extracted for food ingredients. Different methods can be used for this purpose, e.g. frying, drying, filtering, and cooking. Nevertheless, many food ingredients and products are well known to be thermally sensitive and vulnerable to chemical, physical and microbiological changes. Losses of some compounds, low production efficiency, time- and energy-consuming procedures (prolonged heating and stirring, use of large volumes of water, ...) may be encountered using these conventional food processing methods. These shortcomings have led to the use of new sustainable "green and innovative" techniques in processing, pasteurization and extraction, which typically involve less time, water and energy, such as ultrasound-assisted processing [1,2], supercritical fluid extraction and processing [3], extrusion [4], microwave processing [5], controlled pressure drop process [6], pulse electromagnetic field [7], high pressure [8], and subcritical water extraction [9]. Food technology under extreme or non-classical conditions is currently a dynamically developing area in applied research and industry. Alternatives to conventional processing, preservation and extraction procedures may increase production efficiency and contribute to environmental preservation by reducing the use of water and solvents, elimination of wastewater, fossil energy and generation of hazardous substances.

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2. Ultrasound in food processing

In pre-historic ages, the competition of food urged the processing of food to preserve it for longer times. Nowadays, the processed foods that are flourishing in supermarkets are modern processed foods and traditional foods, but their manufacturing, processing and packaging technologies have been advanced and rationalized to an incomparable extent. The principle aims of these technologies are to reduce the processing time, save energy and improve the shelf life and quality of food products. Thermal technologies (radio frequency and microwave heating), vacuum cooling technology, high pressure processing and pulsed electric field technology are those novel technologies who have potential for producing high-quality and safe food products but current limitations related with high investment costs, full control of variables associated with the process operation, lack of regulatory approval and importantly consumer acceptance have been delaying a wider implementation of these technologies at the industrial scale. In recent years, ultrasound (US) in the food industry has been the subject of research and development. There is a great interest in ultrasound due to the fact that industries can be provided with practical and reliable ultrasound equipment. Nowadays, its emergence as green novel technology has also attracted the attention to its role in the environment sustainability. Ultrasound applications are based on three different methods:

- Direct application to the product.
- Coupling with the device.
- Submergence in an ultrasonic bath.

There are a large number of potential applications of high-intensity ultrasound in food processing of which a number are discussed below (Table 1).

2.1. Filtration

In the food industry, the separation of solids from liquids is an important procedure either for the production of solid-free liquid or to produce a solid isolated from its mother liquor. But the deposition of solid materials on the surface of filtration membrane is one of the main problems. The application of ultrasonic energy can increase the flux by breaking the concentration polarisation and cake layer at the membrane surface without affecting the intrinsic permeability of membrane (Fig. 1). The liquid jet serves as the basis for cleaning, and some other cavitation mechanisms lead to particle release from the blocked membrane [10].

Ultrasonically assisted filtration (generally referred to as acoustic filtration) has been successfully employed to enhance the filtration of industrial wastewater that is generally considered difficult to process [11–13]. Moreover, the optimized ultrasound intensity is very important to prevent the damage of filters [14]. Ultrasound can also be applied to the production of fruit extracts and drinks. In the case of juice extraction from apple pulp, conventional belt vacuum filtration achieves a reduction in moisture content from an initial value of 85% to 50%, whereas electroacoustic technology achieved 38% [15]. The use of ultrasound in combination with membrane filtration has also been investigated, with positive results [10,16]. Here ultrasonic irradiation at low power levels was employed to aid the filtration of whey solutions. The results

Table 1
Applications of ultrasound in food processing.

Applications	Conventional methods	Ultrasound principle	Advantages	Products
Cooking	Stove Fryer Water bath, ...	Uniform heat transfer	Less time Improving heat transfer and organoleptic quality	Meat Vegetables
Freezing/ crystallization	Freezer Freezing by immersion, by contact, ...	Uniform heat transfer	Less time Small crystals Improving diffusion Rapid temperature decreasing	Meat Vegetables Fruits Milk products
Drying	Atomisation Hot gas stream Freezing Pulverisation	Uniform heat transfer	Less time Improving organoleptic quality Improving heat transfer	Dehydrated products (fruits, vegetables, ...)
Pickling/marinating	Brine	Increasing mass transfer	Less time Improving organoleptic quality Product stability	Vegetables Meat Fish Cheese
Degassing	Mechanical treatment	Compression-rarefaction phenomenon	Less time Improving hygiene	Chocolate Fermented products (Beer, ...)
Filtration	Filters (membranes semi-permeable, ...)	Vibrations	Less time Improving filtration	Liquids (juices, ...)
Demoulding	Greasing moulds Teflon moulds Silicon moulds	Vibrations	Less time Reducing products losses	Cooked products (cake, ...)
Defoaming	Thermal treatment Chemical treatment Electrical treatment Mechanical treatment	Cavitation phenomenon	Less time Improving hygiene	Carbonated drinks Fermented products (Beer, ...)
Emulsification	Mechanical treatment	Cavitation phenomenon	Less time Emulsion stability	Emulsions (ketchup, mayonnaise, ...)
Oxidation	Contact with air	Cavitation phenomenon	Less time	Alcohols (wine, whisky, ...)
Cutting	Knives	Cavitation phenomenon	Less time Reducing products losses Accurate and repetitive cutting	Fragile products (cake, cheese, ...)

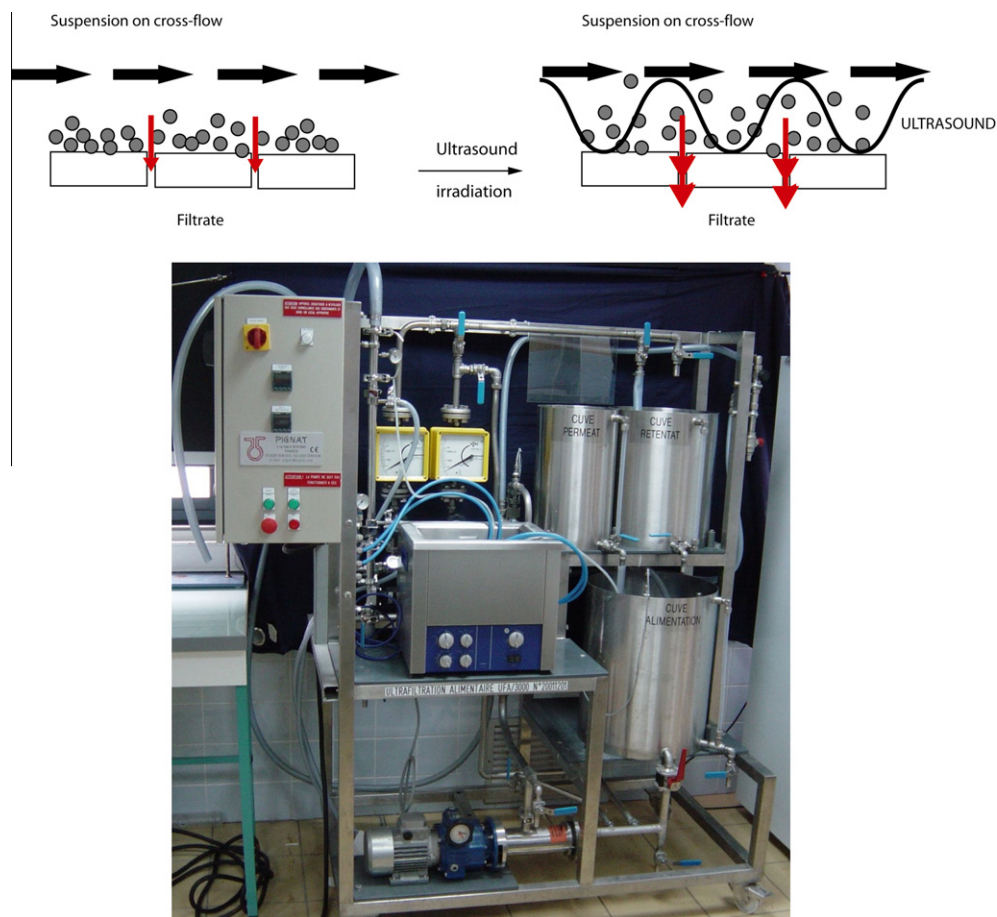


Fig. 1. Enhancement of permeability using ultrasound.

indicated a significant enhancement of flow rate, with ultrasound aiding in preventing blockage of the filter and flow through it by lowering the compressibility of both the initial protein deposit and the growing filter cake.

Additionally, the combination of filter with ultrasounds increases its filter life, as clogging and caking are prevented by continuous cavitation at the filter's surface [17]. All the above described factors are of significance to commercialise the filtration processes and several companies are offering ultrasonic filtration systems as an add-onto existing (vibratory) screens. While, there is still need in its development at laboratory and at commercial level.

2.2. Defoaming

Foam is a dispersion of gas in liquid, with a density approaching that of the gas, i.e., in which the distances between the individual bubbles are very small. Foams find applications in a wide range of industrial processes, including food production and cosmetics. Intensive foaming in the majority of technological processes has the negative consequences expressing in decrease use of useful volume of the technological equipment, infringement of the rules of manufacture and sterility of biotechnological processes, in increase in losses of products and decline of productivity of the equipment, pollution of the environment.

In food manufacturing, foam has historically been controlled by the use of mechanical breakers, lowering packaging container temperatures below the ambient environment, or by the addition of chemical anti-foams [18,19]. Nevertheless, problems remain, for

example: mechanical methods are effective only for coarse foams, heat stresses to rupture bubbles, decrease in surface tension by antifoam agents, and in case of electric current, the mechanism is not entirely clear.

The potential use of sound energy for defoaming has been known for several decades but without real industrial implementation. They were generally based on aerodynamic acoustic sources of various types, including the Hartmann whistle and the siren, but these presented several practical drawbacks, including noise production, high air flow, sterilization of the air flow and high energy consumption. High-intensity ultrasonic waves offer an attractive method of foam breaking since they avoid the need for high air flow, prevent chemical contamination and can be used in a contained environment, i.e. under sterile conditions. This makes it particularly appropriate for implantation in the food and pharmaceutical industries [20].

A system for ultrasonic defoaming has been developed based on a new type of focused ultrasonic generator (Fig. 2). This new system has been successfully applied to control the excess of foam produced in high speed bottling and canning lines of carbonic beverages [21]. The scale-up of such systems to the control of foam has also been achieved (Fig. 2). The focused airborne ultrasonic emitter is mounted on a rotation system that is electronically controlled. The ultrasonic transducer rotates, creating a complex movement and covering a large defoaming area at different rotation speeds. Most of the bubbles break almost instantaneously under the acoustic beam. For the perfect defoaming effect, it is not only important to overcome on the acoustic intensity but also a minimum treatment time is required [22].

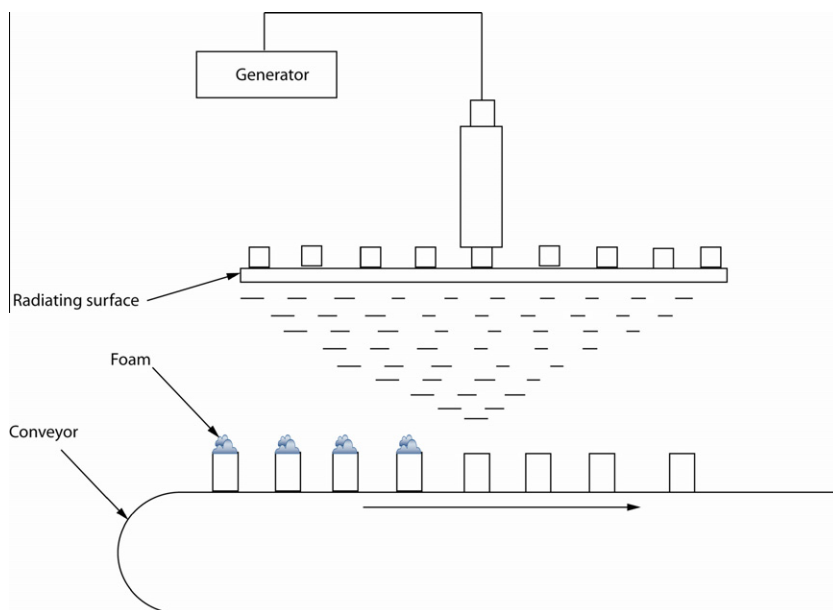


Fig. 2. Ultrasonic transducer for defoaming.

2.3. Degassing/deaeration

A liquid contains gases as a mixed condition, such as dissolved oxygen, carbon dioxide, and nitrogen gas. Two common methods used for degassing are boiling and reducing pressure while ultrasound has an advantage in the small temperature change. Degassing in an ultrasonic field is a highly visible phenomenon when ultrasound, e.g. an ultrasonic cleaning bath, is used with regular tap-water inside. It occurs when the rapid vibration of gas bubbles brought them together by acoustic waves and bubbles grow to a size sufficiently large to allow them to rise up through the liquid, against gravity, until they reach the surface [23,24]. Several acoustic cavitation structures generated in low-frequency ultrasound fields within the range (20–50 kHz) have been investigated and these have been summarized by Mettin [25].

In the food industry, this technique can be used to degas carbonated beverages such as beer (defobbing) before bottling [26]. In the processing of carbonated drinks, the purpose is to displace the air from the liquid surface in order to avoid organoleptic damage of the product by bacteria and oxygen. This process involves coupling a transducer to the outside of the bottle, leading to degassing. Compared with mechanical agitation, the ultrasonic method decreases the number of broken bottles and overflow of the beverage [27]. The application of relatively low-intensity ultrasound during the fermentation of saké, beer and wine resulted in a reduction in the time required by 36–50% [28]. Ultrasonically assisted degassing is particularly rapid in aqueous systems, but the removal of gas is much more difficult in very viscous liquids such as melted chocolate.

2.4. Depolymerization

One of the oldest applications of ultrasound is in the degradation of polymers [29]. The depolymerization process occurs through the effects of cavitation and can involve two possible mechanisms: mechanical degradation of the polymer from collapsed cavitation bubble (Fig. 3) and chemical degradation as a result of the chemical reaction between the polymer and high-energy molecules such as hydroxyl radicals produced from cavitation phenomenon [30].

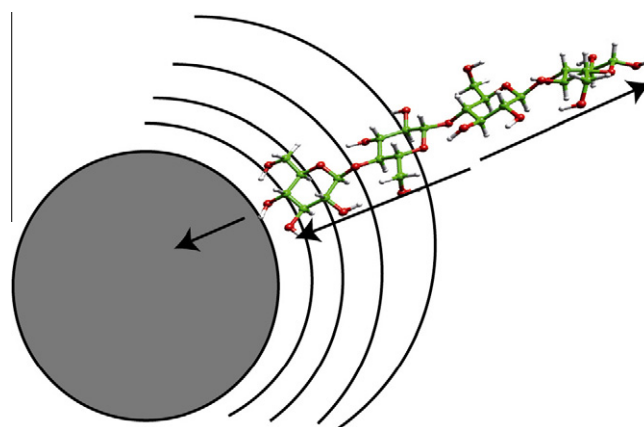


Fig. 3. Representation of depolymerization phenomenon using ultrasound.

In the food industry, an active area is the use of ultrasound to depolymerise starch [31]; this type of research can be traced back to 1933 [32]. Sonication generally provides an efficient depolymerization route in terms of better rates, yields and chemo-, regio- and stereoselectivities and these are generally performed using simple immersion probes operating at 20 kHz. Thus sonication can lead to several improvements in the properties of whey proteins, such as increased solubility and foaming ability [33,34]. The application of low-power ultrasound will generally cause a temporary reduction in the viscosity of polymeric liquids; however, high powers will cause depolymerization and result in a permanent change in their rheology. Such permanent changes are of use in food processing [35,36]. Indeed, the existing ultrasound frequency, ultrasound power, temperature, processing time and other parameters in food processing should be evaluated to not only see their positive effects but also their negative impacts on the food quality and structure [37].

Ultrasonic irradiation offers important potential for the conversion of biomass raw materials such as polymeric carbohydrates to useful lower weight molecules [38]. Recently, a comparative study between ultrasound and some other innovative technologies such

as γ -radiation and microwave heating proved the ultrasonication as the most convenient procedure to depolymerise Xyloglucan [39] and Hyaluronan [40].

2.5. Cooking

In a conventional cooking method, when foods are exposed to elevated temperatures, the outside may be overcooked with the interior insufficiently cooked, and this will lead to a reduction in the quality of the product. Ultrasound has the ability to provide improved heat transfer characteristics, which is a key requirement to avoid such problems, and these have been utilized in cooking [41]. A patent describes a cooking vessel in which ultrasound is applied to a hot oil to provide better and more even overall frying and it is claimed to reduce energy consumption [42].

Although numerous techniques have been employed to cook meat other than conventional heating, e.g. the use of microwaves variations in cooking time, in the end the palatability of the meat together with the sometimes unfavourable energy consumption have proved to be obstacles to the widespread adoption of any single technique. Ultrasound has been compared with convection cooking of beef [43]. Ultrasound cooking resulted in greater cooking speed, moisture retention and energy efficiency, suggesting that it could provide a new, rapid, energy-efficient method that may improve the textural attributes of cooked meat. Ultrasound cooking provides a significantly faster cooking rate and higher post-cooking moisture content and sensory panellists indicated greater myofibrillar tenderness. These last two points can be explained by the fact that the application of high-intensity ultrasound has the potential to increase the water-binding properties of meat [44]. An additional advantage is that ultrasound-cooked muscles have two to five times less cooking losses than those cooked by boiling and convection. This suggests that possible uses of ultrasound in the food processing or food service industries are in the provision of moist precooked or cooked meats for use in prepared meals.

2.6. Demoulding and extrusion

Generally, the industrial cooking of foods leads to adhesion of the products to the cooking vessel or in other operations it must detach from its mould. Ease of removal makes the cleaning and recycling of the container far easier. To remove the cooked product from the mould is difficult because of product/mould adhesion caused by the cooking. To counteract this difficulty in industrial processing of moulded food products, the moulds are fabricated with a surface coating made of a thin layer of silicone or PTFE (polytetrafluoroethylene) or by a coating of white grease. However, it is necessary to replace the mould covering periodically because the shelf-life of the thin lubricating layer is relatively short. Such operations are expensive and, if not 100% successful, i.e. incomplete coverage, the containers will arrive at the cleaning post with product still adhering to them and so disrupt the production line. At present, to solve this problem mechanical methods such as knocking vibration are used to remove the products. An alternative solution to these conventional methods is to release food products by coupling the mould to a source of ultrasound [45]. The device for demoulding industrial food products couples the mould and the ultrasonic source in order to enhance removal of the product contained in the latter by virtue of the high-frequency relative movement between the contact surfaces of the mould and of the product contained in the latter. This technique allows surface coatings to be eliminated and ensures that any residual material in the mould can be cleaned automatically.

A similar property of ultrasound is required to aid extrusion, i.e. the ability to release material from a surface, thus reducing drag.

The energy input is provided by ultrasonic excitation of the metal tubes through which the food is extruded. The ultrasonic source is attached at right-angles to the tube to give it a radial vibration. This process can improve the flow behaviour of sticky or highly viscous materials through the tube by lowering drag resistance and it can also modify product structures [46,47]. In the review of a document, the investment of 120 thousand dollars on Ultrasound-assisted extrusion can give a profit of about 600 thousand dollars per years in comparison to conventional one [48].

2.7. Cutting

The introduction of ultrasound in food cutting has improved the performance of overall food processing. Ultrasonic food cutting equipments provide a new way to cut or slice a variety of food products that streamlines production, minimizes product waste and lowers maintenance costs. Ultrasonic cutting uses a knife-type blade attached through a shaft to an ultrasonic source [49]. The cutting tool itself can be of many shapes and each shape can be considered to be an acoustic horn, part of the whole ultrasonic resonating device.

Cutting with the superimposition of ultrasonic vibration is a direct competitor of technologies such as high-velocity water jet cutting and conventional techniques such as using saws or knives. The low energy requirements for ultrasonic cutting have been presented [50,51]. The ultrasonic cutting characteristics depend on the food type and condition, e.g. frozen or thawed [52]. The most widespread application of ultrasound is in the cutting of fragile foodstuffs. It uses in the particular cases of fragile and heterogeneous products (cakes, pastry and bakery products) (Fig. 4) and fatty (cheeses) or sticky products [53].

Another characteristic of this technique lies in improvements in hygiene since the vibration prevents the adherence of the product on the blade and thus reduces the development of micro-organisms on the surface, i.e. ultrasonic vibrations provide 'auto-cleaning' of the blade. The accuracy and repetitiveness of the cut produce a reduction in losses relative to the cutting (due to cracks, crumbs, etc.) and a better standardization of the weight and dimensions of portions.

2.8. Freezing and crystallization

Freezing and crystallization are linked in that both processes involve initial nucleation followed by crystallization [54]. Sonication is thought to enhance both the nucleation rate and rate of crystal growth in a saturated or supercooled medium by producing a large number of nucleation sites in the medium throughout the ultrasonic exposure. This may be due to cavitation bubbles acting as nuclei for crystal growth and/or by the disruption of seeds or crystals already present within the medium thus increasing the number of nucleation sites.

Cooling and/or freezing as a means of preservation of food has been used for hundreds of years through the use of natural ice or overwinter storage. One very important area related to freezing in the food industry is the formation of ice crystals during the freezing of water present in the food material. The problems related to conventional freezing like non-uniform crystal development, destruction of food material structure and loss in sensory food quality have given rise to use some innovative technologies such as air blast, plate contact, fluidized-bed freezing, immersion freezing, cryogenic freezing, high-pressure freezing and their combinations are the most common methods used in the industry [55,56].

Under the influence of ultrasound, conventional cooling provides much more rapid and even seeding, which leads to a much shorter dwell time [57]. In addition, since there are a greater



Fig. 4. Difference in cutting slices of a bakery product cut with and without ultrasound.

number of seeds, the final size of the ice crystals is smaller and so cell damage is reduced [58]. Accelerated cooling is achieved by improving heat transfer [59,60]. Acoustic cavitation also occurs and acts as nuclei for crystal growth or by the disruption of nuclei already present. In freezing, this phenomenon would lead to fine ice crystals and shortening of the time between the onset of crystallization and the complete formation of ice [61,62], thus reducing damage to cellular structure.

A wide range of foodstuffs have been successfully frozen under the influence of ultrasound [63–65]. In their review, the authors concluded that although commercial development of this technology was not available in 2006, the potential of power ultrasound to aid food freezing is promising, especially for high value food (ingredients) and pharmaceutical products.

Crystallization can often occur in an uncontrolled manner simply due to a slight decrease in temperature or pressure and this can cause severe problems during the manufacturing process [66]. Hence to be able to influence nucleation rate, control the growth and size of the subsequent crystals and prevent fouling of surfaces allows the manufacturer a greater degree of control over the final product [67]. Although most of the literature on sonocrystallization relates to the production of fine chemicals and pharmaceuticals [68], there are clear advantages for the food industry. The technology has been applied to the crystallization of materials such as milk fat [69], triglyceride oils such as a vegetable oil [70] and ice cream [71,72].

It should also be noted that when ultrasound is used to enhance crystallization of any kind, there is an additional benefit from using ultrasound because it helps to prevent encrustation of crystals on the cooling elements. This ensures efficient heat transfer throughout the cooling process.

2.9. Defrosting/thawing

Freezing is widely used method to enhance the shelf life of certain food products. However, its success depends on the optimized thawing conditions [73]. Thawing frozen foodstuffs is intrinsically slow and this can have expensive consequences in industrial food processing and is therefore an inconvenience in both large- and small-scale catering. The process can be made more rapid by generating heat within the food, but when microwave, dielectric or resistive heating methods are employed there are still severe restrictions on the rate at which thawing can be accomplished, due to runaway heating and preferential surface heating. The utilization of acoustic energy to thaw frozen foodstuffs was investigated about 50 years ago; however, the negative aspects of poor penetration, localized heating and high power requirements hindered the development of this method [74]. Recently, work on

the relaxation mechanism showed that more acoustic energy could be absorbed by frozen foods when a frequency in the relaxation frequency range of ice crystals in the food was applied [75]. It was found that the thawing process under the relaxation frequency was faster than that obtained with a thawing process using only conductive heating (Fig. 5).

Experiments showed that blocks of cod required 71% less time by using acoustically assisted water immersion thawing than using water immersion only when 1500 Hz acoustic energy at 60 W was applied. Other work using ultrasound for the thawing of meat and fish indicated that acceptable ultrasonic thawing was achieved at frequencies around 500 kHz, which conformed to a relaxation mechanism [76]. From this, it seems that acoustic thawing is a promising technology in the food industry if appropriate frequencies and acoustic power are chosen. Acoustic thawing can shorten the thawing time, thus reducing drip loss and improving product quality [60].

2.10. Drying

Acoustically assisted drying has been a topic of interest for many years [77,78]. Traditional methods for desiccating or dehydrating food products by a forced stream of hot air are reasonably economical, but the elimination of the interior moisture takes a relatively long time. Moreover, high temperatures can damage

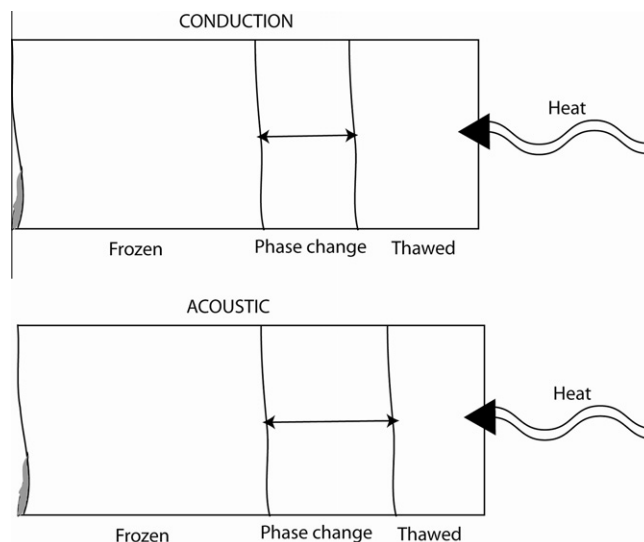


Fig. 5. Phase change difference in conduction and acoustic thawing.

the food, which in certain cases may change the colour, the taste and the nutritional value of the rehydrated product [79].

Alternative methods may eliminate these disadvantages, but some, such as freeze-drying, are expensive and others, such as spray drying, are applicable only to liquids. However, it is known that supplying vibrational energy may stimulate the dehydration and avoid these disadvantages. Diffusion at the boundary between a suspended solid and a liquid is substantially accelerated in an ultrasonic field and heat transfer is increased by approximately 30–60% depending on the intensity of the ultrasound [80]. The mechanism of this process involves a series of rapid and successive compressions and rarefactions in the food material induced by the ultrasound. With each contraction, a very small amount of water is expelled towards the surface of the product, and this water is evaporated by the hot gas stream [81]. A range of products have been examined, e.g. powdered milk [27], vegetables [80] and sugar crystals [82].

Ultrasonically assisted air drying continues to be the focus of considerable attention [83], with new developments now appearing in the design of fluidized bed-type systems [84]. The fluidized reactor is a vertical cylindrical metal tube driven in radial mode at 21.8 kHz with hot air passing through small pieces of carrot cubes and lemon peel cylinders [85]. Ultrasonic osmotic dehydration technology uses lower solution temperatures to obtain higher water loss and solute gain rates [86,87]. Due to the lower temperatures during dehydration and the shorter treatment times, food qualities such as flavour, colour and nutritional value remain unaltered. A hydrodynamic mechanism of mass transfer is observed, significantly increasing the water losses and solute gain. An increase in mass transfer is also instrumental in improving the drying of cheese or meat in salt brine [88].

Ultrasound has also been used as a pretreatment prior to the drying of a range of vegetables [89]. The treatment produced a reduction in subsequent conventional and freeze-drying times and also in rehydration properties.

2.11. Meat tenderization

The quality of meat depends on the aroma, flavour, appearance, tenderness and juiciness. Consumer behavioural has shown that tenderness is most important palatability factor in determining meat quality [90]. The traditional method used for meat tenderization is mechanical pounding, which makes poorer quality meat more palatable. Power ultrasound has also been found to be useful for this process. Ultrasound can act in two ways: by breaking the integrity of muscular cells or by enhancing enzymatic reactions, i.e. via a biochemical effect [27]. A pilot study involving sirloin steak [91] showed that sonicating beef muscle at 2 W cm² for 2 h at 40 kHz produced damage to the perimysal connective tissue, resulting in improved eating texture. Ultrasonic tenderization can be achieved with poultry meat, veal and beef [92].

In the course of investigations into meat sterilization using heat and ultrasound, it was found that a side-effect was tenderization [93]. It proved possible to reduce traditional heat treatment by 50% by using high-energy ultrasound. From these tests, it was concluded that an increased kill effect on micro-organisms could be achieved in various food test systems by using a combination of ultrasound and mild heating, a process known as thermosonication, or with pressure mano-sonication.

Ultrasound has been used in the production of processed meats. In recombined meat products such as beef rolls, the pieces of muscle are held together by a protein gel, formed by the myofibrillar proteins released during processing [44]. Tumbling the meat pieces and adding salt achieve this release. A sticky exudate is formed on the pieces, which binds them together when they are compressed [94]. The binding strength, water holding capacity, product colour

and yields were examined after treatment with either salt tumbling or sonication or both. Samples that received both salt treatment and sonication were superior in all qualities. Hence ultrasound can lead to improved physical properties of meat products, such as water-binding capacity, tenderness and cohesiveness.

2.12. Brining, pickling and marinating

Pickling and marinating are used for a wide variety of vegetables and meat products. Most current salt-brining or pickling-fermentation processes are subject to three main drawbacks: (1) in brining, a very high sodium chloride content is required, which may require a 'desalting' process prior to shipping to reduce the sodium chloride content of the product; (2) there is a potential lack of control in fermentation due to the occurrence of natural outside intrusion of 'wild' fermentation; and (3) any soaking process can lead to enzymatic softening, structural damage and bloating.

All three of these side-effects are detrimental to rapid and efficient food preservation and so alternative technologies are of interest to food producers. Ultrasound allows the pickling time of products to be reduced considerably, particularly those foods with a crunchy texture [95]. It also provides a method for manufacturing a pickle having a low level of sodium chloride compared with the pickles currently on the market. Hence there is no need to 'desalt', repack to reach the desired finished product salt level.

Brining is a two way mass transfer process as the water migrates from the meat to the brine and solute from the brine to the meat. Pork loin slices were immersed in a saturated solution of NaCl at 21 °C for 45 min and different types of agitation of the solution and different levels of ultrasound intensity were applied during brining. It was found that the water and NaCl contents of samples after treatment were higher in sonicated than non-sonicated samples [96]. Moreover, ultrasound reduces the salting time, the formation of a crust and unwanted colouring of raw meat [97]. The process also provides a product which is uniformly salted.

In the cheese industry, the effect of ultrasound on mass transfer during cheese brining has been investigated [98]. Many cheese varieties are salted by immersion in brine. Moreover, the influence of different process conditions, such as the use of agitation, brine concentration, sample: brine ratio and temperature, can be affected by acoustic energy. The rate of water removal and sodium chloride gain increased when ultrasound was applied in comparison with brining performed under static or dynamic conditions, suggesting that ultrasound improves both external and internal mass transfer.

2.13. Sterilization/pasteurization

Conventional thermal pasteurization and sterilization are the most common techniques currently used to inactivate micro-organisms and enzymes in food products. Unfortunately, the intensities of treatment, time and process temperature are also proportional to the amount of nutrient loss, development of undesirable flavours and deterioration of functional properties of food products. Ultrasound provides a method of improving such processes by virtue of the effects of cavitation.

The use of ultrasound in pasteurization continues to be of great interest to the dairy industry. It has proved effective for the destruction of *E. coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes* with no detrimental effect on the total protein or casein content of pasteurized milk [99]. The mechanism of microbial killing is mainly due to the thinning of cell membranes, localized heating and production of free radicals. Investigation on ultrasound effectiveness have also shown the inactivation of enzymes such as pectinmethylesterase, polyphenoloxidases and peroxidases responsible for deterioration of fruit and vegetable juice and

various enzymes pertinent to milk quality [100–103]. In combination with heat, ultrasound can accelerate the rate of sterilization of foods, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat pasteurization include minimization of flavour loss, greater homogeneity of treatment and significant energy savings [104].

2.14. Emulsification/homogenization

Emulsification is an important mean to deliver the hydrophobic bioactive compounds into a range of food products (Fig. 6). Acoustic emulsification offers the following improvements over conventional methods [105]:

- The emulsion produced has particles in the sub-micron range with an extremely narrow particle size distribution.
- The emulsions are more stable.
- Addition of a surfactant to produce and stabilize the emulsion is not necessary.
- The energy needed to produce an emulsion by acoustic waves is less than that needed in conventional methods.

Ultrasonic emulsification is attracting interest for in-line treatment [106]. In the food industry, ultrasonic emulsification is attracting interest for products such as fruit juices, mayonnaise

and tomato ketchup [107], in the homogenization of milk [108] and in aroma encapsulation [109]. It is comparable to microfluidization in terms of generating sub-micron dispersions [110], but there are indications that the emulsification of edible oils might lead to some deterioration in quality [111].

2.15. Miscellaneous effects

With low-power ultrasound, it is possible to accelerate fermentation processes and this has potential for a range of foodstuffs [112]. It is also possible to use ultrasound on foodstuffs to induce premature ageing. Free radicals are produced in water in the presence of the ultrasonic waves because of the temperatures and pressures produced [44]. One of the main applications of ultrasonically aided oxidation is in alcoholic beverages for the ageing of fermented products such as wine and whisky [113]. Indeed, a device has been patented for this purpose using a range of ultrasound between 20 and 80 kHz [114]. Another potential use of ultrasound in winemaking is for the improved dissolution of dyes and the tannins from the initial processing of the fruit.

A patent refers to the use of the ultrasound to reduce the rest period of dough [115]. Indeed, during the mechanical treatment of dough, the yeast is subjected to mechanical stress, which reduces its activity thereafter. The use of ultrasound should make it possible to reduce the stress and the pressures applied to yeast and so reduce the rest period of dough.

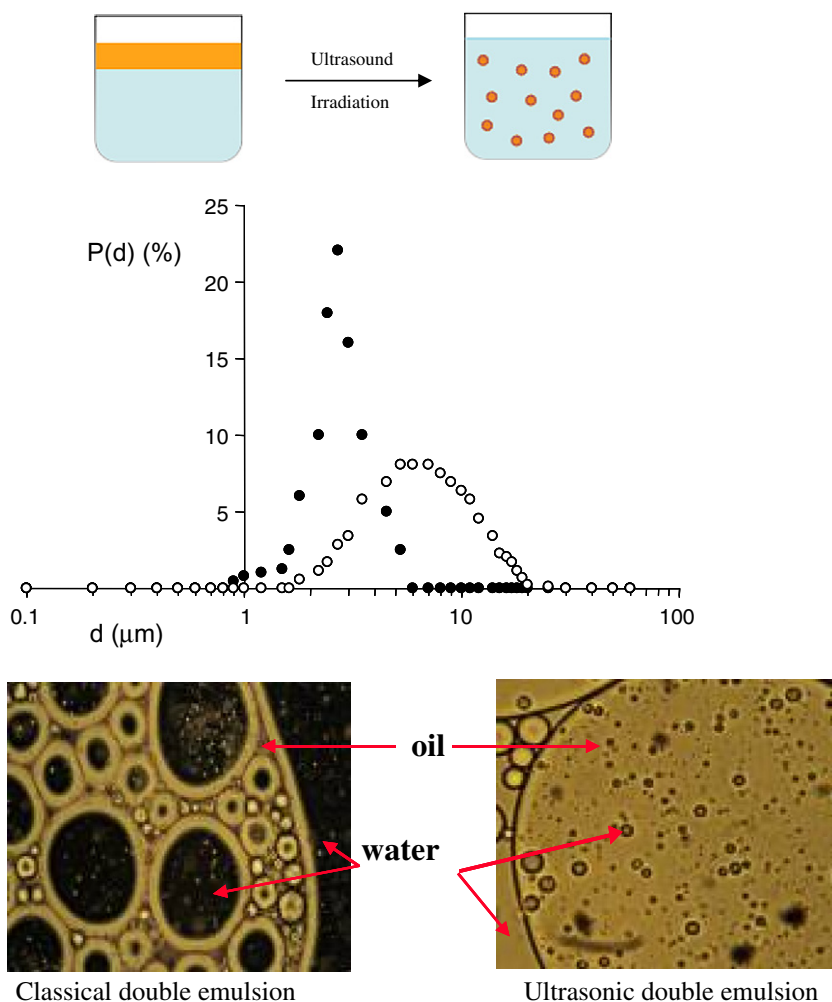


Fig. 6. Phase dispersion of two immiscible solvent and drop size distribution by Power Ultrasound (●) Mechanical Agitation (○).

3. Ultrasound in food preservation

Consumers of the food industry have become more concerned with the hygiene-related aspects of food manufacture. Safety and transparency in production methods have therefore become important terms in the industry. Food processing is a science combining the fundamentals of physics, chemistry, biology and microbiology. Some say that it is a very art to achieve the best in food preservation. Traditionally heat treatment, in occurrence pasteurization and sterilization, was a method of choice having the ability to destroy both micro-organisms and enzymes, the latter being responsible of food deterioration. However its effectiveness is dependent on the treatment temperature and time and this leads to loss of nutrients, development of undesirable flavours, colours and deterioration of the organoleptic properties of food. As a result new non-thermal technologies are receiving great interest. Today's challenge is to combine simultaneously mild thermal preservation techniques with new applications for microbial destruction. The new technologies being developed insure the preservation of food without the use of preservatives, while keeping its nutritional value and organoleptic characteristics (texture, colour, taste) unchanged, with a low consumption of energy, a competitive cost, an environmental friendliness and a high degree of safety.

Ultrasound processing is one of these new methods. While its application in food processing is relatively recent, it has been proved that high-intensity ultrasonic waves can rupture cells and denature enzymes, and that even low-intensity ultrasound is able to modify the metabolism of cells. In combination with heat, ultrasonication can accelerate the rate of sterilization of foods, thus lessening both the duration and intensity of thermal treatment and the resultant damage. The advantages of ultrasound over heat sterilization include: the minimizing of flavour loss, greater homogeneity; and significant energy savings.

3.1. Ultrasound phenomena in food preservation

Micro-organisms and enzymes are the primary factors responsible of food deterioration. Micro-organisms, such as bacteria and spores, need environments rich in nutrients and water to grow and multiply and therefore food is an appropriate medium for them. On the other hand, enzymes present naturally in food, break down the nutrients, for example the break down of fats by lipases or of proteins by proteases. Conventional thermal processing kills vegetative micro-organisms and some spores, and inactivates enzymes. However, the time and temperature of the process are proportional to the amount of nutrient loss, development of undesirable flavours and deterioration of functional properties of food products.

Ultrasound is one of the new preservation techniques that could eliminate microbial activity. High power ultrasound alone is

known to disrupt biological cells. When combined with heat treatment, it can accelerate the rate of sterilization of foods. Therefore it reduces both the duration and intensity of the thermal treatment and the resultant damages. At sufficiently high acoustic power inputs, ultrasound is known to rupture cells [112,116,117]. A cell can be inactivated at an intensity less than that needed to cause disruption. The mechanism of microbial killing is mainly due to the thinning of cell membranes, localized heating and production of free radicals [118].

Acoustic cavitation can be divided into two types, transient and stable [119]. The former occurs when the cavitation bubbles, filled with gas or vapour, undergo irregular oscillations and finally implode. This produces high local temperatures and pressures that would disintegrate biological cells and denature enzymes. The imploding bubble also produces high shear forces and liquid jets in the solvent used that may have sufficient energy to physically damage the cell wall or cell membrane. Stable cavitation, on the other hand, refers to bubbles that oscillate in a regular fashion for many acoustic cycles. The bubbles induce microstreaming in the surrounding liquid which can also induce stress in the microbiological species.

The inactivation effect of ultrasound has also been attributed to the generation of intracellular cavitation and these mechanical shocks can disrupt cellular structural and functional components up to the point of cell lysis (Fig. 7).

Spores appear to be more resistant than vegetative forms while enzymes are reported to be inactivated by ultrasound due to a depolymerization effect. Critical processing factors are the nature of the ultrasonic waves, the exposure time with the micro-organisms, the type of micro-organisms, the volume of food processed, the composition of the food, and the temperature. The effects, however, are not severe enough for a sufficient destruction of micro-organisms when using ultrasound alone [118]. This can be achieved by combining ultrasound with heat or pressure or both. Applications using combination with other preservation methods are:

- *Manosonication*: combination of ultrasound and pressure (MS).
- *Thermosonication*: combination of ultrasound and heat (TS).
- *Manothermosonication*: combination of ultrasound, pressure and heat (MTS).

The combination of heat and ultrasound is much more efficient with respect to treatment time and energy consumption compared with either treatment used individually (Table 2).

3.2. Application

Application of ultrasound in food preservation can be divided into two main categories depending upon its area of utilization:

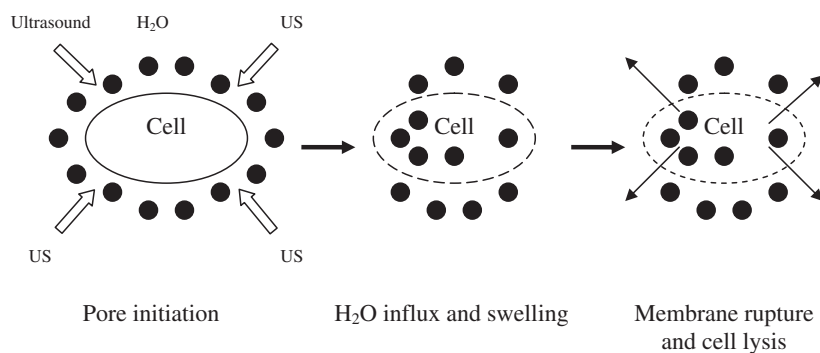


Fig. 7. Mechanism of ultrasound-induced cell damage.

Table 2
Effects of ultrasound in combination with heat and pressure.

Inactivation by/of	Vegetative cells	Spores	Enzymes
Ultrasound alone (US)	+	–	–
US and heat (MT)	+	+	–
US and heat and pressure (MTS)	+	+	+

3.2.1. Directly related to food

3.2.1.1. *Microorganism inactivation*. It has been shown that micro-organisms do not all react in the same way to ultrasound treatment. Factors affecting the effectiveness of microbial inactivation are [120].

- Amplitude of ultrasound waves.
- Exposure or contact time.
- Volume of food processed.
- Composition of food.
- Treatment temperature.

The performance of these above described factors is also affected by type, shape or diameter of the micro-organisms (Table 3) [121]. Larger cells are more sensitive than the small ones. This is probably due to their larger surface area. Gram-positive bacteria are known to be more resistant than gram-negative ones, possibly because of their thicker cell wall which provides them a better protection against ultrasound effects [122]. According to the literature differences in cell sensitivity could also be due to the more tightly adherent layer of peptidoglucans in gram-positive

cells. Concerning the shape of the micro-organisms, cocci are more resistant than bacilli due to the relationship of cell surface and volume. Finally, spores are very hard to destroy compared to vegetative cells which are in phase of growth.

There are many examples of micro-organisms inactivated using ultrasound. Some of these have been studied in culture media and others in food, using ultrasound either combined or alone.

The most frequently studied micro-organisms, not only in the field of power ultrasound, but also among other methods of food preservation are *Saccaromyces cerevisiae* and *Escherichia coli*. The former has been found to be less resistant to ultrasound than other vegetative cells, which is mostly attributed to its larger size. The inactivation of this microorganism has been proven in such food models as water, phosphate buffers, and sabouraud broth [123–127]. The inactivation of *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Listeria monocytogenes* and *E. coli* has been proven in water and phosphate buffers, as well as in foods such as UHT milk [128,129,99]. Ultrasonication in combination with heat was performed to study the inactivation of *Listeria innocua* and mesophilic bacteria in raw whole milk [130]. When applying ultrasound in combination with heat the kill rates were increased when compared to rates of thermal treatment alone and a synergistic rather than an additive effect was observed. Ultrasound produced a good level of inactivation under different treatment conditions and media for *Bacillus* species [131]. The inactivation of *Listeria monocytogenes* by high-power ultrasonic waves (20 kHz) at ambient temperature and pressure has been found to be low with decimal reduction values in 4.3 min. This could be improved however by either an increase in pressure (manosonication) or by increasing

Table 3
Factors affecting the sensitivity of the micro-organisms.


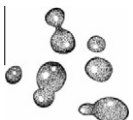




Type of microorganism	Shape	Cell size (µm)	Structure of cell wall
<i>Listeria monocytogenes</i>	Short rods 	0.4–0.5 × 0.5–2	Gram-positive
<i>Saccharomyces cerevisiae</i>	Ellipsoidal shape 	3–15 × 2–8	–
<i>Staphylococcus aureus</i>	Small, irregular coccoid 	0.8–1	Gram-positive
<i>Salmonella typhimurium</i>	Straight rods 	0.7–1.5 × 2–5	Gram-negative
<i>Escherichia coli</i>	Straight rods 	1–1.5 × 2–6	Gram-negative
<i>Bacillus subtilis</i>	Rods with rounded or squared ends 	Small: 0.5 × 1.2 Large: 2.5 × 10	Gram-positive

Table 4

Experiences using ultrasound in food preservation.

Object studied	Suspension medium	Treatment	Frequency (kHz)	Power (W)	Temperature (°C)	Time (min)	Year, Ref.
Total mesophilic aerobes and fungi (yeasts and moulds)	Orange juice	Sonication	500	240	–	15	2007 [156]
<i>Listeria innocua</i>	Milk	Thermosonication + pulse electric field	24	400	55	2.5	2009 [157]
<i>Escherichia coli</i>	Phosphate buffer	Sonication Manosonication Thermosonication Manothermosonication	20	100	40–61 °C/100–500 kPa	0.25–4	2009 [158]
<i>Pichia fermentans</i>	Tomato juice	Sonication	20	0.33–0.81 W/mL	–	2–10	2010 [159]
<i>Salmonella enterica</i> , serovar Enteritidis	Water	Thermosonication	24	400	52–58	2–10	2010 [160]
<i>Alicyclobacillus acidiphilus</i> and <i>A. acidoterrestris</i>	Apple juice	Sonication	25	200–600	–	1–30	2010 [161]
Peroxidase	Watercress	Thermosonication	20	125	40–92.5	0–2	2006 [148]
Lysozyme	Phosphate buffer	Manothermosonication	20	–	60–80 °C/200 kPa	3.5	2006 [162]
Pectinesterase	Lemon	Thermosonication	20	–	40–90	63	2007 [163]
Pectin methylesterase and polygalacturonase	Tomato juice	Thermosonication	20	40	50–75	0–40	2009 [164]
Polyphenol oxidase and peroxidase	Fresh-cut apple	Sonication + ascorbic acid	40	–	–	–	2011 [165]

the power of sonication. Inactivation by manothermal sonication (MTS) proved to be more effective [93,132]. By combining sub-lethal temperatures and higher pressures of 200 kPa the decimal reduction value fell dramatically over 1.5 min to 1.0 min. An advantage of using manosonication instead of heat alone to inactivate bacteria was that recovery of the bacteria was far lower and did not appear to be dependent upon the treatment medium employed. Manosonication at 40 °C and 200 kPa decreased the decimal reduction times of *Streptococcus faecium*, *Listeria monocytogenes*, *Salmonella enteritidis*, and *Aeromonas hydrophila* [93]. Increasing the ultrasonic amplitude reduced these levels even further. Increasing the pressure from 200 to 400 kPa also resulted in a reduction in the decimal reduction rates. Cells grown at 37 °C were twice as heat resistant as those grown at 4 °C. At higher viscosities cavitation is harder to achieve but the final collapse of the bubble is more violent. By employing a calorimetric method of monitoring the power employed directly to the medium they discovered a linear relationship between power entering the medium and decimal reduction times [133]. This suggests that greater rates of kill could be achieved by using higher ultrasonic power that in turn would be dependent upon the frequency and intensity of the ultrasonic power entering the system.

Recently, the use of TS, MS or MTS methods in combination with some novel technologies was found effective in the inactivation of microbes. Investigation was carried out to find out the effect of TS (10 min at 55 °C) in combination with pulsed electric fields (PEF) (40 kV/cm for 150 µs) on the inactivation of *S. aureus* (SST 2.4) in orange juice and then compared with conventional pasteurization (94 °C for 26 s). Using TS/PEF, no significant affect was found on the pH, conductivity, °Brix, juice colour and on the non-enzymatic browning index than thermal treatment [134]. Moreover, the TS/PEF combination has proven the storage life of orange juice without affecting the sensory acceptability by consumers [135]. The combination of steam with ultrasound was found potential to inactivate *Salmonella* and most cost-effective method in comparison to hot water, steam vacuum and lactic acid

treatments [136]. But the combination effect is not always profitable, as no synergistic effect was observed experimentally in combining the ultrasound and non-thermal plasma techniques with aeration [137]. The treatment of ultrasound plays its role not only to inactivate the microbes but also facilitates in the quality improvement of final product [138]. Table 4 groups the some recently studied examples on the microorganisms' inactivation.

3.2.1.2. Spore inactivation. Microbial spores are resistant to extreme conditions such as high temperatures and osmotic pressures, high and low pHs, and mechanical shocks. Those bacterial spores that survive heat treatment may severely restrict the shelf-life of thermally processed foods because of spoilage and poisoning. The endospores of *Bacillus* and *Clostridium* species are very resistant to extreme conditions; for example *Bacillus thermophilus* spores are destroyed by heating only at 100 °C for 4 h.

Bacillus subtilis spores are notoriously difficult to denature. As a result they are often employed to determine the efficiency of various treatment/sterilization processes as, being highly resistant, the success of the process can be weighed clearly against the kill rate of the bacillus spore. Combining the effect of a 20 kHz ultrasonic probe on samples under static pressure indicated that an increase in static pressure resulted in an increased level of spore inactivation [139]. Manosonication treatment at 500 kPa for 12 min inactivated over 99% of the spores. Increasing the amplitude of ultrasonic vibration of the transducer, i.e., the acoustic power entering the system, increased the level of inactivation, for example, a 20 kHz probe at 300 kPa, 12 min sonication at 90 µm amplitude inactivated 75% of the spores. Raising the amplitude to 150 µm resulted in 99.5% spore inactivation. Finally increasing the thermal temperature of the treatment resulted in greater rates of inactivation certainly at 300 kPa compared to thermal treatment alone.

The pathogens *Bacillus cereus* and *Bacillus licheniformis* spores have been found to be resistant to ultrasonic treatment alone [140]. However under MTS treatment chemical are released from

the spores including dipicolinic acid and low molecular weight peptides [141].

The protozoa, *Cryptosporidium parvum*, has become recognised as a cause of the water-borne disease Cryptosporidiosis in humans and many cases have been reported in the news over the last decade. These outbreaks have occurred throughout Europe as well as the USA and are not just associated with the developing countries. The main concern of water suppliers is that conventional treatment methods are inadequate and are not a sufficient barrier in preventing the water-borne transmission of cryptosporidiosis. The most commonly used disinfectant techniques used in water treatment, i.e., chlorination and ultraviolet light is ineffective but ozone seems to be able to destroy some of these oocysts in combination with ultrasound [142].

3.2.1.3. Enzyme inactivation. To prevent denaturation, an enzyme has to keep its native conformation. Hydrophobic interactions, hydrogen bonding, van der Waals interactions, ion pairing, electrostatic forces and steric constraints stabilize the three-dimensional molecular structure of globular proteins.

For stabilisation of some food materials, enzymes must be inactivated or their activity reduced. In fact, the proteolysis caused by some enzymes like proteases can induce some defects of flavour and brown pigments. Enzyme inactivation can be easily achieved by heat treatment. However in some cases the high heat resistance of enzymes may be a problem as heat can negatively modify some food properties such as flavour, colour or nutritional value. This is the driving force for the increased interest in an alternative method of enzyme inactivation: high power ultrasound, i.e. sonic waves above 20 kHz.

The effects of ultrasonic waves on proteins are very complex. Polymeric globular proteins are broken down into subunits and if oxygen is present, the quaternary structure is not recoverable. A partial delipidation of lipoproteins can be obtained and if the ultrasonic irradiation is long enough, proteins can be hydrolysed and polypeptide chains can be broken. The influence of the gas on the intensity of enzyme inactivation has been related to the formation of free radicals by cavitation. Sensitivity to ultrasounds depends on the conditions of the treatment [44] as well as on the nature of the enzyme. Generally, ultrasonication in combination with other treatments is more effective in food enzyme inactivation. In fact, MTS treatment has an increased effectiveness compared with ultrasound alone [132]. MTS treatments inactivate several enzymes at lower temperatures and/or in a shorter time than thermal treatments at the same temperatures. It is possible to quantify the effect of such treatments by calculating the activation energy (E_a) for the process:

$$k = A \cdot \exp(-E_a/RT)$$

The activation energy is obtained from the Arrhenius equation, shown above, by plotting $\log k$ against the inverse of the absolute temperature (T). k is the rate of the reaction, in this case it represents the inactivation. The activation energy is the amount of energy required for the reaction to proceed [119].

Orange juice is usually heated to aid preservation but various thermoresistant enzymes prove to be difficult to denature by thermal treatment alone. Pectinmethylesterase (PME) from oranges is strongly protected by its substrate pectin against thermo-inactivation. However, MTS treatment is unaffected by the presence of that molecule and enzyme inactivation is effective [143]. PME present in oranges was treated by manothermosonication at pressures of 200 kPa in citrate buffer and also in orange juice. At 35.5 °C the inactivation of the enzyme using MTS was estimated to be approximately seven times greater than with thermal treatment alone.

The viscosity of processed tomatoes is one of the most important parameter [144,145] concerning quality and it is highly

dependent on the degree of polymerisation of pectic substances. During the transformation of tomatoes, the viscosity diminishes due to the synergistic activity of endopolygalacturonases (PGs) and pectinmethylesterase (PME). These enzymes depolymerise pectin chains in pulp or serum and cause the decrease in viscosity of the product. Those enzymes are liberated during processing and must be inactivated as quickly as possible. Generally to inactivate pectic enzymes, tomatoes are rapidly heated at temperatures between 82 °C and 104 °C. This step is immediately followed by chopping or crushing [145]. The inactivation of PGs and PME was more efficient when applying MTS than by simple heating. At 37 °C the combination of ultrasound and increased pressure produced higher levels of inactivation compared with those achieved at 62.5 °C with thermal treatment alone [146].

The peroxidase enzyme system present in watercress (*Nasturtium officinale*), is composed of a heat-labile fraction and a heat-resistant fraction. The application of thermosonication was studied to enable less severe thermal treatments obtained from conventional blanching and, therefore, produce a new and healthy frozen product, watercress (*N. officinale*), with minimized colour or flavour changes along its shelf life [147]. The peroxidase and polyphenol oxidase are the principal enzymes involved in the browning process of fresh-cut fruits and vegetables. Browning is important limiting factor in the consumers' acceptance. The study revealed that combined treatment of ultrasound and ascorbic acid had synergistic inhibitory effects on several enzymes related to enzymatic browning [148]. Some other recent publications on enzyme inactivation are presented in Table 4.

3.2.2. Indirectly related to food

One of the major long-established industrial applications of power ultrasound is in surface cleaning and it has proved to be an extremely efficient technology. Ultrasound is particularly useful in surface decontamination where the inrush of fluid that accompanies cavitation collapse near a surface is non-symmetric. The particular advantage of ultrasonic cleaning in this context is that it can reach crevices that are not easily reached by conventional cleaning methods. Objects that can be cleaned range from large crates used for food packaging and transportation to delicate surgical implements such as endoscopes. This was recognised some years ago, see for example a general patent that relates to the use of ultrasound as a method of pasteurisation, sterilization and decontamination of instruments and surfaces used within the medical, surgical dental and food processing industries [149]. The use of ultrasound allows the destruction of a variety of fungi, bacteria and viruses in a much reduced processing time when compared to thermal treatment at similar temperatures. The removal of bacteria from various surfaces is of great importance to the food industry and can be efficiently accomplished with the combined use of sonicated hot water containing biocidal detergents [150]. Typical examples of items requiring repeated regular cleaning are plastic baskets, shackles (the hooks used for hanging poultry on production line) and conveyer belts. Recently, ultrasonic cleaning of a conveyor belt was studied by building a pilot-scale conveyor with an ultrasonic cleaning bath [151]. The purpose was to inactivate the *Listeria monocytogenes* strains using continuous ultrasonic cleaning. The effect of ultrasound with a potassium hydroxide-based cleaning detergent was determined by using the cleaning bath at 45 and 50 °C for 30 s with and without ultrasound. The results indicated that ultrasonic cleaning of a conveyor belt is effective even with short treatment times.

In most countries the use of antibiotics in foods is strictly forbidden. Nevertheless, there have been several reports of the use of ultrasound to improve the effects of antibiotics in surface cleaning, particularly in the removal of biofilms. Thus ultrasound is able to promote the effectiveness of various antibiotics at concentration

that do not, on their own, reduce bacteria viability in biofilms [152]. This synergy reduced the viability by several orders of magnitude for cultures of *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus epidermidis* and *S. aureus*. Measurements of the bactericidal activity of gentamicin against *P. aeruginosa* and *E. coli* demonstrated that simultaneous application of 67 kHz ultrasound enhanced the effectiveness of the antibiotic. As the age of these cultures increased, the bacteria became more resistant to the effect of the antibiotic alone and the application of ultrasound appeared to reverse this resistance. The ultrasonic treatment-enhanced activity was not observed with cultures of gram-positive *S. epidermidis* and *S. aureus*. The synergy was confirmed in studies of biofilms of *E. coli* [153]. The biofilms were up to 300 μm thickness, far greater than those previously examined with *P. aeruginosa*. Treatment with ultrasound alone did not appear to enhance killing rate even after 2 h sonication. Antibiotic alone killed only 82% of the cells within 2 h. A combination of ultrasound and antibiotic killed 99% of the cells within two hours with the best results obtained employing high power densities and low ultrasonic frequencies. Improvement in the killing rate may be due to the ability of ultrasound to introduce the antibiotic further into the biofilm than it would normally have reached on its own.

Sterilization of equipment is often carried out by heating, however, in order to achieve full kill rates high temperatures must be employed which also cause may damage to surrounding materials such as rubbers and plastics. Various chemicals can also be used to sterilise equipment/machinery but these can be difficult to handle and involve health and safety considerations. Glutaraldehyde is used as a chemical steriliser and it is usually employed at room temperature and at a pH greater than 7.4, where it is most efficient. Sierra and Boucher [154] discovered that when employing glutaraldehyde solution in combination with ultrasound the efficiency of the sterilization process is enhanced and neither is it dependent upon the actual pH of the solution employed when used at 70 °C [154]. Ultrasound reduced the time required for glutaraldehyde sterilization at 25 °C from 3 h to just 30 min at pH8. At pH 2.2 and temperatures of 60–65 °C the time required to inactivate the spores was reduced from 10 min to 4 min when employing 20 kHz ultrasound. The authors suggest that a synergistic effect is involved with ultrasound aiding the penetration of glutaraldehyde into the spore where it then acts upon the spore site of inactivation. They also suggest that combining ultrasound with glutaraldehyde at 54 °C is a quick and efficient method of surface sterilization/decontamination when using a liquid phase process.

4. Ultrasound-assisted extraction

Shortcomings of existing extraction technologies, like increase consumption of energy (more than 70% of total process require energy), high rejection of CO₂ and more consumption of harmful chemicals, have forced the food and chemical industries to find new separation “green” techniques which typically use less solvent and energy, such as microwave extraction, supercritical fluid extraction, ultrasound extraction, ultrafiltration, flash distillation, the controlled pressure drop process and subcritical water extraction. Separation under extreme or non-classical conditions is currently a dynamically developing area in applied research and industry.

4.1. UAE in comparison to non-conventional extraction techniques

Using microwaves, extraction and distillation can now be completed in minutes instead of hours with high reproducibility, reducing the consumption of solvent, simplifying manipulation and work-up, giving higher purity of the final product, eliminating

post-treatment of waste water and consuming only a fraction of the energy normally needed for a conventional separation method such as distillation or solvent extraction. Several classes of compounds such as essential oils, aromas, pigments, antioxidants, and other organic compounds have been extracted efficiently from a variety of matrices. The advantages of using microwave energy, which is a non contact heat source, includes: more effective heating, faster energy transfer, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up, increased production, and elimination of process steps [163,164].

Microwave assisted extraction (MAE) has attracted growing interest as it allows the efficient use of microwave energy to extract valuable compounds from solid samples. Pan et al. [165] have found that MAE extraction improves the efficiency of the extraction of polyphenols and caffeine from green tea leaves. However, the moisture content of samples is a defining parameter for the recovery of yield. When using dried samples, the recovery yield drops dramatically. Furthermore, Molins et al. [166] have reported that the use of hexane as the sole solvent in presence of a completely dry sample was not satisfactory. Indeed, the efficiency of MAE is typically low when the solvent lacks a significant dipole moment for microwave energy absorption. The methods are therefore limited in terms of solvents and nature of the solid material.

Supercritical fluid extraction (SFE) is an innovative method with green theme behind its development for the extraction of solid materials using a supercritical fluid [167,168]. Carbon dioxide is the most widely used solvent in SFE because it is non-toxic, non-flammable, cheap, easily eliminated after extraction and endowed with a high solvating capacity for non-polar molecules. Other possible solvents are Freon, ammonia and some organic solvents [169]. In a typical SFE procedure, the supercritical fluid continuously enters the solid matrix where it dissolves the material of interest. The extraction can be achieved with a remarkably high selectivity by adjusting the solvating capacity of the supercritical fluid by changing the pressure or temperature. Major advantages of SFE include pre-concentration effects, cleanliness, safety and simplicity [170]. SFE with CO₂ is a non-conventional technique that can offer very good yields. This technique is suitable for fragrance extraction, giving better yields and good quality essential oil. The drawbacks of SFE are the need of more expensive equipment with the difficulty of extracting polar molecules without adding modifiers to CO₂.

Accelerated solvent extraction (ASE) makes use of the same solvents as traditional extraction methods while operating at elevated temperatures and pressures. ASE, generalized to pressurized fluid extraction, is now well accepted as an alternative extraction technique [171]. The sample is contained in an extraction cartridge heated in a conventional oven and crossed by the extraction solvent. The extraction is performed statically for a short period. When the extraction is complete, compressed gas shifts the solvent from the cartridge to the collecting vessel. ASE allows rapid extraction with small solvent volumes by using high temperatures (up to 200 °C) for increased solvent diffusivity [172,173] and high pressure (up to 20 MPa) to keep the solvent in its liquid state [174]. ASE provides a wide range of applications but the high extraction temperature may lead to degradation of thermolabile compounds. Fisher et al. [175] pointed out that the main drawbacks of ASE are a strong background interference and high detection limit. Moreover, the equipment is expensive.

Ultrasound-assisted extraction is an emerging potential technology that can accelerate heat and mass transfer and has been successively used in extraction field. Ultrasound waves after interaction with subjected plant material alter its physical and chemical properties and their cavitation effect facilitates the release of extractable compounds and enhances the mass transport by disrupting the plant cell walls. UAE is a clean method that avoids

Table 5
Advantages and drawbacks of traditional and recent extraction techniques.

	UAE	MAE	SFE	ASE
Name	Ultrasound-assisted extraction	Microwave assisted extraction	Supercritical fluid extraction	Accelerated solvent extraction
Brief description	Sample is immersed in solvent and submitted to ultrasound using a US probe or US bath	Sample is immersed in solvent and submitted to microwave energy	Sample is placed in a high pressure vessel and crossed continuously by the supercritical fluid	Sample is heated by a conventional oven and crossed by the extraction solvent under pressure
Extraction time	10–60 min	3–30 min	10–60 min	10–20 min
Sample size	1–30 g	1–10 g	1–5 g	1–30 g
Solvent use	50–200 ml	10–40 ml	2–5 ml (solid trap)30–60 ml (liquid trap)	15–60 ml
Investment	Low	Moderate	High	High
Advantages	Easy to use	Rapid Easy to handle Moderate solvent consumption	Rapid Low solvent consumption Concentration of the extract No filtration necessary Possible high selectivity	Rapid No filtration necessary Low solvent consumption
Drawbacks	Large amount of solvent consumption Filtration step required	Extraction solvent must absorb microwave energy Filtration step required	Many parameters to optimize	Possible degradation of thermolabile analytes

the use of large quantity of solvent along with cutting down in the working time. Ultrasounds are successively employed in plant extraction field [176,177]. Ultrasound is well known to have a significant effect on the rate of various processes in the chemical and food industry. Much attention has been given to the application of ultrasound for the extraction of natural products that typically needed hours or days to reach completion with conventional methods. Using ultrasound, full extractions can now be completed in minutes with high reproducibility, reducing the consumption of solvent, simplifying manipulation and work-up, giving higher purity of the final product, eliminating post-treatment of waste water and consuming only a fraction of the fossil energy normally needed for a conventional extraction method such as Soxhlet extraction, maceration or steam distillation. Several classes of food components such as aromas, pigments, antioxidants, and other organic and mineral compounds have been extracted and analyzed efficiently from a variety of matrices (mainly animal tissues, food and plant materials).

Table 5 is clearly representing the advantages of UAE in comparison to these novel extraction methods. Compared to MAE, UAE may eventually be simpler [176] and faster [177]. In addition, UAE is not restricted by the solvent and type of matrix used, or by the moisture content. The use of power ultrasound represents a potentially efficient way of enhancing mass transfer processes. In addition, this is probably the unique practical way to produce agitation in SFE because the use of mechanical stirrers is not possible. Riera et al. [178] examined the effect of ultrasound (20 kHz and 50 W) on the particulate almond oil extraction kinetics using supercritical CO₂. As a consequence of the trials (at 280 bar and 55 °C) at the end of the extraction time (8 h 30 min) the yield of the oil was significantly increased (20%) when SFE was assisted by ultrasound. Alternatively, mass transfer was speeded up to such an extent that yields comparable to those obtained by SFE alone could be achieved in about 30% shorter time when using ultrasound. Also in comparison to ASE, UAE allows an important reduction of extraction time, solvent volume, and sample manipulations [179].

4.2. Extraction mechanisms

Ultrasounds are mechanic waves that necessitate an elastic medium to spread over. The difference between sound and ultrasounds is the frequency of the wave, sound waves are at human earring frequencies (from 16 Hz to 16–20 kHz) while ultrasounds

have frequencies above human earring but below microwaves frequencies (from 20 kHz to 10 MHz). As a sound wave passes through an elastic medium, it induces a longitudinal displacement of particles. In fact, the source of the sound wave acts as a piston on the surface of the medium [180].

This results in a succession of compression and rarefaction phases into the medium. When the piston is in its opened position it induces a compression into the medium and when the piston is in its contracted (pull) position it creates a rarefaction phase. Every medium has a critical molecular distance: below this critical value, the liquid remains intact, but above this distance, the liquid would break down and voids can be generated into the liquid. In the case of ultrasounds, if the rarefaction cycle is strong enough, the distance (d) between contiguous molecules can reach or even exceed the critical molecular distance of the liquid. The voids created into the medium are the cavitation bubbles which are responsive of ultrasonic effect. In fact these cavitation bubbles are able to grow during rarefaction phases and decrease in size during compression cycles. When the size of these bubbles reach a critical point they collapse during a compression cycle and release large amounts of energy. The temperature and the pressure at the moment of collapse have been estimated to be up to 5000 K and 2000 atmospheres in an ultrasonic bath at room temperature. This creates hotspots that are able to accelerate dramatically the chemical reactivity into the medium. When these bubbles collapse onto the surface of a solid material, the high pressure and temperature released generate microjets directed towards the solid surface. These microjets are responsible for the degreasing effect of ultrasounds on metallic surfaces which is widely used for cleaning materials. Another application of microjets in food industry is the extraction of vegetal compounds. As shown in Fig. 8, a cavitation bubble can be generated close to the plant material surface (a), then during a compression cycle, this bubble collapse (b) and a microjet directed toward the plant matrix is created (b and c). The high pressure and temperature involved in this process will destroy the cell walls of the plant matrix and its content can be released into the medium (d). This is a very interesting tool for ingredient extraction from natural products.

4.3. Laboratory and industrial scale reactors

Two different types of ultrasound equipments are commonly used in laboratory (Fig. 9). The first one is the ultrasonic cleaning

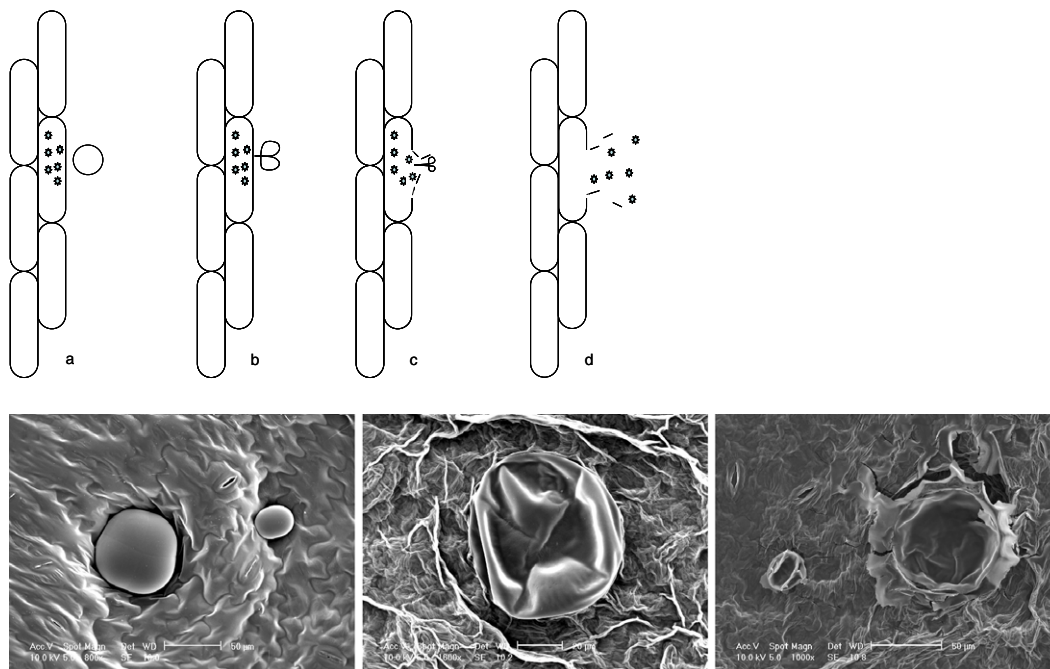


Fig. 8. Cavitation bubble collapse and plant material releasing: example of extraction of essential oil from basil.

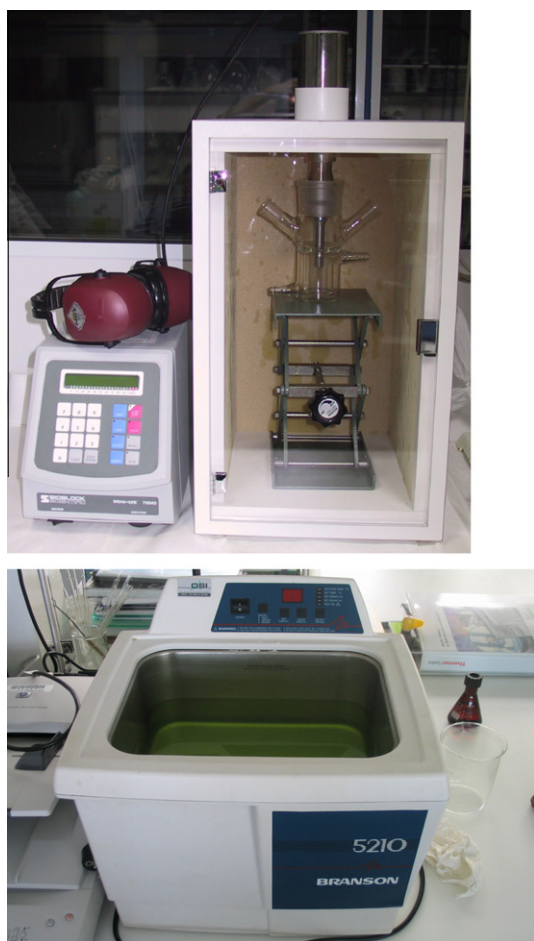


Fig. 9. Commonly used ultrasonic systems (US bath; US probe).

bath which is commonly used for solid dispersion into solvent (ultrasounds will dramatically reduce the size of the solid particles,

which will enhance its solubility), for degassing solutions or even for cleaning small material by immersion of the glassware into the bath. The ultrasonic baths are less used for chemical reactions even if they are easy to handle and economically advantageous because reproducibility of reaction is low. In fact, the delivered intensity is low and is highly attenuated by the water contained in the bath and the walls of the glassware used for the experiment.

The second one, the ultrasonic probe or horn system, is much more powerful because the ultrasonic intensity is delivered on a small surface (only the tip of the probe) compared to the ultrasonic bath. Another change is that the probe is directly immersed into the reaction flask so less attenuation can happen. This system of probe is widely used for sonication of small volumes of sample but special care has to be taken because of the fast rise of the temperature into the sample.

A special sono-extraction reactor (from 0.5 to 3 L) has been developed by REUS (www.etsreus.com, FRANCE) (Fig. 10). The intensity of ultrasounds is about 1 W/cm^2 with a frequency of 25 kHz. In order to keep constant temperature, the reactor is made of a double mantle into which cooling water can circulate. The main advantage of this type of apparatus is that the natural products and the extraction solvent are mixed into a container and the ultrasounds are directly applied to the mixture. To run out industrial trials or to scale-up laboratory experiments, REUS has also developed reactors from 30 to 1000 L (Fig. 11). Pump systems are coupled to the ultrasonic bath in order to fill the ultrasonic bath, to stir the mixture and to empty the system at the end of the experiment.

4.4. Application of UAE in food research

The use of ultrasound can enhance the extraction process by increasing the mass transfer between the solvent and plant material. The collapse of cavitation bubbles leads to better cell disruption through the formation of microjets due to asymmetrical bubble collapse near a solid surface. This allows for improved solvent penetration into the plant body itself and can also break down cell walls [181]. As a consequence, employing ultrasound

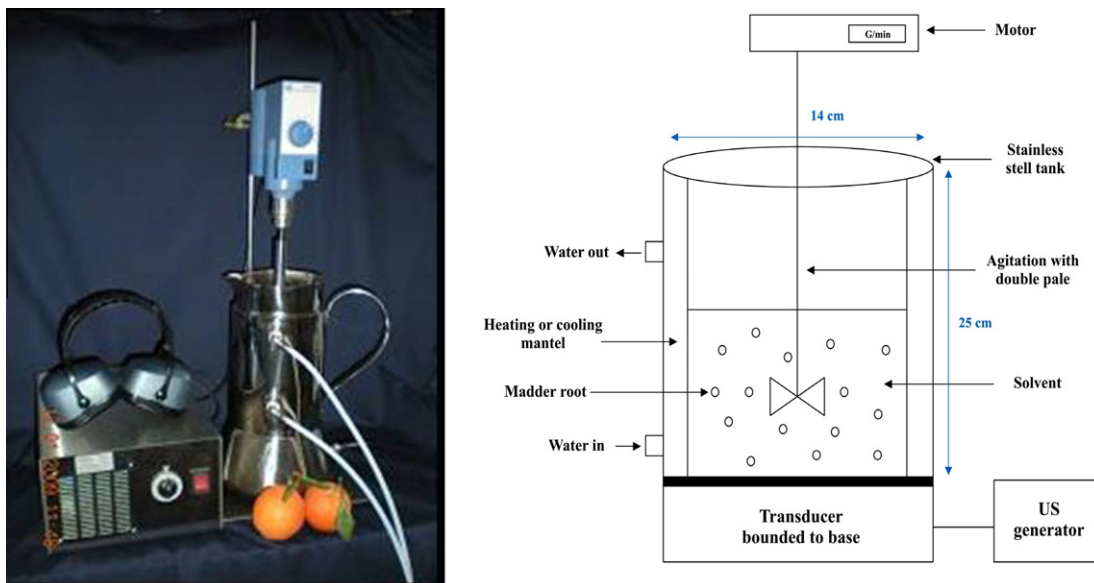


Fig. 10. Laboratory sono-extraction reactor (3 l system).



Fig. 11. Industrial ultrasonic equipments: 50, 500 and 1000 l.

in the use of plant extraction has benefits in increased mass transfer, better solvent penetration, less dependence on solvent used, extraction at lower temperatures, faster extraction rates and greater yields of product [182].

Flavour and fragrances are complex mixtures of volatile compounds usually present in low concentrations. They are present in varieties of aromatic plants, either in the roots, stems, seeds,

leaves, flowers or fruits. The molecules responsive for the flavour of the aromatic plant can be extracted and used in the food industry, medicine or perfumeries. The first aroma extractions are difficult to date precisely. Old testimonies allow concluding that the Hindus controlled fermentation and obtained odorant oils starting from rudimentary distilling apparatuses. Now a day, this distillation process has been improved by its coupling with ultrasound

Table 6
Some examples of UAE.

Matrix	Analyte	Remarks	Ref.
Aged brandies	Aroma Compounds	US bath 20 °C, 3 * 10 min in dichloromethane (50 ml); 37 compounds has been extracted including Linalool (291 µg/L), α-terpineol (248 µg/L), citronellol (397 µg/L)	[183]
Tea	Aroma components	US bath, 40 kHz, 250 W, 3 g/300 ml water, extraction 40 min at 60 °C; enhancement of extraction efficiency of chemical component, volatiles compounds and aroma precursors by US	[184]
Wine	Volatile compounds	US bath, 40 kHz, 25 °C, CH ₂ Cl ₂ , 15 min; 12 compounds extracted with concentrations extended from 0.422 mg/L to 168 mg/L	[185]
Peppermint leaves	Menthol	US bath, 40 kHz at 22 °C, extraction 60 min in hexane; 2% of total product up to 12% of total product at a temperature of 39 °C	[186]
<i>Lavandula angustifolia</i>	Essential oil	US probe, 20 kHz, below 60 °C, 10 g/100 ml 70% EtOH; Increase of 1,8-cineol, camphor and linalyl acetate by 2–3-fold compared to hydrotillation	[188]
Garlic	Essential oil: organo-sulfur compounds	US bath 35 kHz, 25 °C, 100 g/20 ml water, extraction with 50 ml diethylether (or hexane or ethyl acetate) for 30 min; Less thermal degradation with US compared to conventional and microwave assisted distillation	[189]
Citrus Flowers and honey	Linalool	US bath, 25 °C, 5 g/30 ml n-pentane:diethylether (1:2), or 22 ml water + 15 ml solvent, extraction time 10 min; More than 80% of compounds detected are linalool derivatives. Precursors of honey aromas found in flowers	[190]
Vanilla pods	Vanillin	US horn, 22.4 kHz 1 h pulsed mode (5 s.ON/5 s OFF) 1 g/100 ml solvent; 140 ppm vanillin concentration in 1 h vs. 180 ppm in 8 h conventional soxhlet	[191]
Caraway seeds	Carvone and limonene	US bath, 20 kHz, 20–55 °C, n-hexane, 60 min; Carvone (17 mg/g), limonene (16 mg/g). Increased yield by US comparing with the soxhlet extraction	[192]
Greek Safron	Safranal	US Bath, 35 kHz, 25 °C, 5 g/100 ml water/diethyl ether (1:1), 5 * 10 min; Amount of safranal from 40.7–647.7 mg/100 g by US and from 288.1–687.9 mg/100 g by microsimultaneous steam distillation	[193]
Tomatoes	Lycopene	US bath, 40 kHz, 300 W, 1 g/8 ml of ethyl acetate, sonicated for 29 min at 86 °C; 90% total lycopene extracted in 29 min, yield enhance when coupling US with microwaves	[195]
Raspberries	Anthocyanins	US horn, 22 kHz, 650 W, below 40 °C, 60 g/240 ml 1.5 M HCl-95% EtOH (15:85); same yield after 3.3 min (200 s) by US-assisted extraction compared to 53 min with conventional extraction	[197]
Pepper	Capsaicinoids	US Bath, 360 W, 50 °C, 1 g/25 ml methanol for 10 min; capsaicin 448 µmol/kg, dihydrocapsaicin 265 µmol/kg	[198]
Citrus peel	Phenolic compounds	US bath, 60 kHz, 15 °C and 40 °C, 2 g/40 ml 80% methanol for 1 h; higher extraction efficiency by US compared to maceration. Low extraction temperature leads to higher yields	[199]
Coconut shell	Phenolic compounds	US bath, 25 kHz, 150 W, 30 °C, 1.5 g/75 ml ethanol–water (1:1) for 15 min (optimum conditions); 22.44 mg/g of phenolics extracted from coconut shell	[200]
Wheat bran	Phenolic compounds	US bath, 40 kHz, 250 W, 60 °C, 5 g/100 ml of 64% ethanol for 25 min; 3.12 mg gallic acid equivalent per gram of bran	[201]

or even the development of new ultrasound-assisted extraction (UAE) (Table 6). Literature reported the extraction of different aroma components from aged brandies [183], tea [184] and wine [185] by ultrasound baths. UAE allowed better yields and a shorter extraction time compared to conventional methods.

UAE has also been developed for essential oil from aromatic plants such as peppermint leaves [186], artemisia [187] and lavender [188], or from other vegetal matrix such as garlic [189] and citrus flowers [190]. Increased yields of essential oil were found for peppermint leaves (up to 12%) and for artemisia when using UAE, and increase by 2 to 3-fold of the main compounds of lavender essential oil when comparing UAE to conventional distillation. Moreover, UAE not only improved yields but as the method is fast and run at low temperature, the final product usually showed less thermal degradation than traditional methods.

Several studies have been run out on extraction of the main aroma compounds from spices. For example the vanillin was extracted from vanilla pods [191] carvone from caraway seeds [192] and safranal from Greek saffron [193] (Table 6). Yields of vanillin were comparables after one hour by UAE vs. 8 h in conventional extraction. Another publication showed that 80% of the pure vanillin was obtained after only 120 s of ultrasounds (ultrasonic probe) whilst it took 24 h with the conventional method to obtain 100% [194]. For carvone extraction, the UAE method was compared to the Soxhlet. Unwanted fatty materials were extracted by Soxhlet extraction while the extract with UAE was of better quality and richer in carvone than in limonene.

Wide varieties of fruits and vegetables have been studied by UAE because antioxidants are present in different amounts in different varieties of plants and these antioxidants come from different families (Table 6). One of the most common antioxidant is the lycopene extracted from tomatoes [195]. In this particular work, authors not only worked on UAE but also on coupling ultrasounds

with microwaves which gives high lycopene extraction in only 6 min. Herrera et al. [196] showed that similar amounts of phenolic compounds could be extracted from strawberries in 2 min while it took 20 h with conventional method and 3 h using supercritical fluid extraction. Another example is the extraction of anthocyanins from raspberries developed by Chen et al. [197]. A fast UAE of capsaicinoids from pepper as also been set up by Barbero et al. [198] who developed a reproducible ultrasonic extraction method using methanol as solvent. Citrus by-products are not only used for aromatic compounds contained in the essential oils, but phenolic compounds are also extractable from citrus peels [199] by using ultrasound. Other phenolic compounds have been extracted from coconut shell [200] and from wheat bran [201].

Ultrasound has a number of applications for extraction of polyphenols from different plant matrix but the literature search did not yield any reference about earlier reports on the UAE of phenolic compounds from onion by-products just by using water as a solvent. Moving towards green extraction, we have tried to minimise the loss of nutritionally important components from onion by-products by extracting them with ultrasound waves at ambient temperature (25 °C) by using water having pH 7. The objective of this work is to use ultrasound as a novel comparative extraction technique for onion flavonols extraction. We have analyzed the extracts obtained by UAE and conv. method for quantification of flavonols by HPLC, the data revealed the presence of quercetin-3,4'-diglucoside and quercetin 4'-glucoside as predominant form along with possessing higher concentration of quercetin aglycone (Fig. 12) in extracts (unpublished work by Farid chemat et al.).

The application of ultrasound waves resulted with increased yields of these flavonols. Higher concentration of these flavonols in UAE extracts in comparison with their content obtained by traditional extraction process at same conditions reveals clearly the effectiveness of this process in the field of antioxidants extraction.

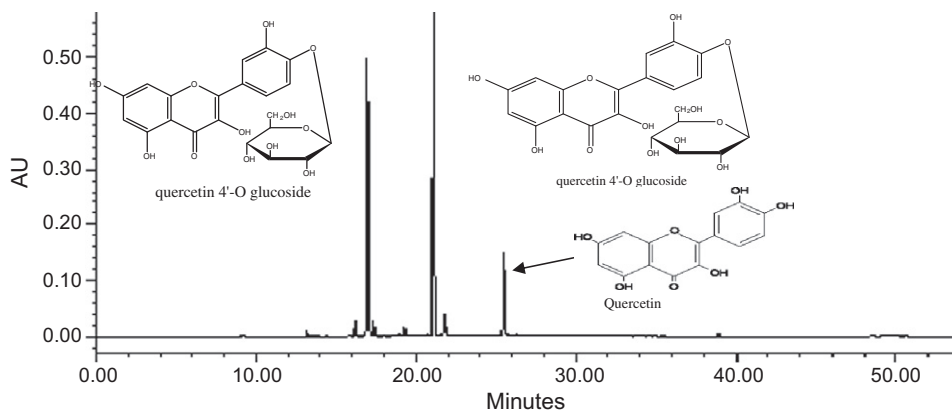


Fig. 12. HPLC chromatogram of ultrasound extracted onion by-products.

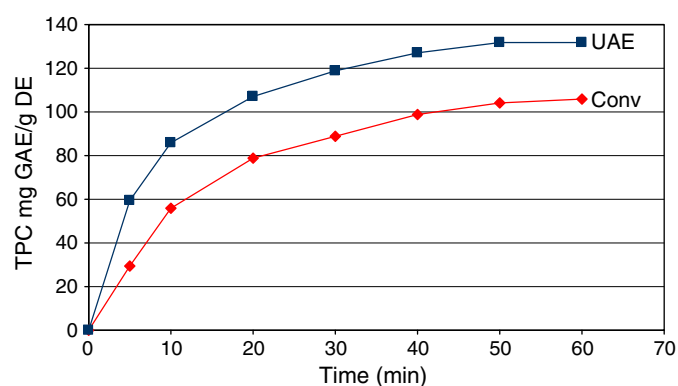


Fig. 13. Comparison of UAE vs. CSE for phenolic contents extraction.

To find the specific impact of ultrasound, extracts (ultrasound and conventional) (Fig. 13) were compared for their total phenolic contents. Ultrasound-assisted extraction lead to a yield of gallic acid equivalents (TPC) (121 ± 3.8 mg GAE/g DW) increased by more than 20% than the yield obtained by conventional maceration method (89.6 ± 2.3 mg GAE/g DW) in 30mins of extraction. The kinetic of extraction was clearly improved, which could be attributed to ultrasonic cavitation while it is the only variable added between both experiments. Chemat et al. showed, by-using electronic microscopy, physical effects of ultrasound on cells when applied on plant material [202].

4.5. Application of UAE in food industry

For the food industry the use of ultrasound assistance is becoming increasingly important. The mains matrix of ultrasound-as-



Fig. 14. Ultrasound extraction platform for onions extracts. Source: with permission www.etsreus.com



Fig. 15. Ultrasound extraction devices for liquid and solid products. Source: with permission www.etsreus.com

sisted extraction is vegetable (seeds and herbs). Compounds extracted will be use as immediately (liquor) or as food and cosmetic additives (essential oil, molecule with special activity). GMC (G. Mariani & C. Spa) is an Italian company specialized in extraction on aromatic herbs using both conventional and innovative method of extraction according the characteristic of herbs (Fig. 14). According to GMC, the use of ultrasound reduce the time of extraction (number of cycle are reduce) [203].

GIOTTI is an Italian company using ultrasound assistance in extraction of food and pharmaceutical additives product and also produce alcoholic drink. Extraction and distillation are reviewed by this company. GIOTTI work with four continuous batch using ultrasound to realizes their extractions (Fig. 15). All tanks are mixing by agitation system on the top and the ultrasound devices are on the side of each tank [204].

Moliserb s.r.l is a company localized in Italy which extracted compound from vegetable for food (food and diary supplements, antioxidatives), cosmetic, fragrance (essential oil, concrete, essence, and absolute) and pharmaceutical. Moliserb works with “soft” temperature extraction condition. Indeed Moliserb using ultrasound-assisted extraction with 23 °C max temperature in order to avoid the degradation of thermolabile compounds [205].

The proposed benefits of UAE for these food industries include, enhancement of overall extraction rate and yield, possibility of use of alternative solvents, use of cheaper raw product sources, enhancing extraction of heat sensitive compounds.

5. HACCP for ultrasound food processing operation

The hazard analysis critical control point (HACCP) system is a process that identifies and assesses the hazards and risks associated with the manufacture, storage and distribution of foods and implement the appropriate controls aiming at the elimination or reduction of these hazards at specific points of the production line. The common terms of HACCP are defined below:

- **Hazard:** Any biological, chemical or physical factor which can lead to an unacceptable risk for consumer safety or product quality.
- **Critical Control Point (CCP):** Any place, personnel, operation or protocol where inadequate control would result in food danger apparition.

A hazard becomes a critical control point (CCP) when an unacceptable risk for the product quality can occur if this step is not monitored. In ultrasound food processing, parameters as temperature and physical properties of the product, time of treatment in ultrasound chamber, frequency and power of the ultrasound, nature of probe are points which need to be monitored. When each CCP is identified, their targets and limits are set. Then, a checking system must be set up to control so that the limits of the CCPs are not exceeded. This system gathers whole control procedures and general hygiene measures to undertake according to the targets. If the checking procedures show that a CCP's limit is exceeded, corrective measures are undertaken in order to meet the requirements and the effectiveness of these actions must be monitored again [206].

For each part of the food process including ultrasound processing, evaluation and classification of hazards are done. In this way, a grade is given to each hazard that has been identified according to its gravity, its risk of occurrence and the ability to detect it. For each hazard, preventive measures are set up with procedures that indicate who will do it, how, when and where. Preventive measures aim primarily at avoiding occurrence of the hazard. For example, a good maintenance plan decreases the risk of metal contamination from US horn (Table 7).

The effectiveness of US in food processing depends on the quality of the US equipment, the parameters used and the initial micro-organism rate of growth in the product. The main causes of contamination by micro-organisms are: (i) poor hygiene of the personnel due to bad work habits (handling contamination) and lack of hygiene formation, (ii) inadequate or non respect of cleaning

Table 7

Possible CCP limits and associated corrective actions in a US food processing operation/system.

Critical control point	Danger	Target	Deviation	Corrective actions
Processing operation	Temperature	Adequate	Does not conform to the requirements	Adjust temperature Reject or reprocess the product
	US frequency	Adequate	Does not conform to the requirements	Adjust frequency Laboratory controls Reprocess the product
	US power	Adequate	Does not conform to the requirements	Adjust the power Laboratory controls Reprocess the product
	Flow rate	Below specifications	Does not conform to the requirements	Adjust flow Laboratory controls Reprocess the product
	Probe spoilage	Absence	Detected	Reject

procedures, (iii) cross-contamination, (iiii) micro-organisms growth abnormally high due to bad storage conditions of the raw materials and/or non respect of requirements by the purveyor. So, to optimize the preservation operation, preventive measures must be set up: (i) controls on raw material supplies and verification of purveyor requirements, (ii) controls of the effectiveness of cleaning; chemical danger must be also controlled in the cleaning in place procedures, (iii) verification of the efficiency of the US system and its parameters.

6. Future trends

Many traditional food processing techniques are reaching their optimum performance while consumers demand's stretch and food and environmental regulations tighten. Research shows ultrasound can play an important role in food technology: processing, preservation and extraction. Although conventional cutting, emulsification and cleaning are often bottlenecks, lack of knowledge keeps industry from implementing ultrasound in their processes. A recent survey and market study of the possible future applications of new process technologies (like microwave, ultrasound) in the food industry has revealed that many companies are reluctant to apply these new technologies. The main reason is poor understanding of these new techniques by food professionals and the reason or weight of tradition.

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