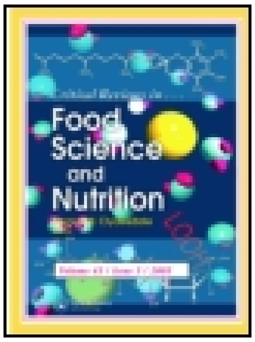

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ABSTRACT

The use of ultrasounds has recently gained significant interest in the food industry mainly due to the new consumers' trend towards functional foods. Offering several advantages, this form of energy can be applied for the improvement of the qualitative characteristics of high-quality foods as well as for assuring the food safety of a vast variety of foodstuffs, minimizing at the same time any negative effects on the sensory characteristics of foods. Furthermore, the non-destructive nature of the technology offers several opportunities in compositional analysis of foods. However, further research is required for the improvement of the related techniques and the reduction of application costs in order to render the technology efficient for industrial use. This review article covers the main applications of ultrasounds as well as the several advantages of the use of the technology in combination with conventional techniques. The effects of ultrasounds on the characteristics, microbial safety and quality of several foods are also detailed.

Keywords: ultrasound, food treatment, drying, freezing, microbial inactivation, ultrasonication

1. Introduction

Ultrasounds are the pressure waves with a frequency of 20kHz or more (Butz and Taucher, 2002). Acoustic waves belong to the category of the mechanical waves and their propagation is performed through a material medium. Usually, their classification is performed using the human audible frequency as a reference. The latter can be placed between the frequencies of 20 Hz and 20 kHz. Lower frequencies are defined as infrasounds while higher frequencies are defined as ultrasounds (Cárcel et al., 2012). Both the physicochemical characteristics and the microstructure of emulsions can be connected to measurable ultrasonic parameters, such as the velocity and attenuation coefficient, by taking into account the scattering of ultrasound by an ensemble of particles (McClements, 1994).

During the last few years consumer's interest on functional foods has been enhanced. This is due to both the supply of nutrients, and their benefits on health or their disease-preventing properties. As a result, it is very important to develop processing methods, which aim at preserving not only the nutrients and sensory characteristics but also the bioactivity of specific constituents (Soria & Villamiel, 2010). The implementation of acoustic energy as a processing aid has been used in industry for over 50 years (Feng & Yang, 2011).

Ultrasound is an emerging technology that can be used for the minimization of processing, quality reservation and assurance of food safety (Knorr et al., 2011). The ultrasound waves present several changes in their characteristics (velocity, attenuation, frequency spectrum) during traveling through a medium (Cárcel et al., 2012) Their frequency range determine their applications in food processing, analysis and quality control. They can be categorized into low

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and high-energy ultrasounds (Piyasena et al., 2003). This technology can also be applied for the quality control of fresh vegetables and fruits, pre- and post-harvest cheese, commercial cooking oils, bread and cereal products, bulk and emulsified fat based food products, food gels, aerated and frozen foods. It has also been used to detect honey adulteration and assess the aggregation state, size and type of proteins (Awas et al., 2012).

As regards food safety, ultrasounds can improve the hygiene standards of food production. Cavitation and related phenomena can loosen impurities and enhance cleaning processes while shortening the application times and limiting the use of chemical substances. However, the singular use of ultrasound is not considered sufficient for effectively inactivating microorganisms to food industry standards (FDA, 2000). Nevertheless, it can be a valuable technology when combined with other preservation processes, such as heat, mild pressure or ozone, since sonication induced cell damage enhances the sensitivity towards other treatments (IFT, 2006).

The versatile application of ultrasound in the field of food technology can lead to improvement of sustainability in food production. Depending on each single application, ultrasound can reduce energy or water needs and limit the use of chemicals and other resources (IUFoST, 2010).

1.1. Low-energy ultrasounds

Low energy (low power, low intensity) ultrasounds are characterised by frequencies of more than 100 kHz at intensities below 1 W/cm^2, and can be used for non-invasive analysis and monitoring of numerous food materials during processing and storage, with the aim of ensuring quality and safety standards. Low power ultrasound has been used to non-destructively evaluate the composition of raw and fermented meat products, fish and poultry (Awad et al., 2012).

Low intensity ultrasounds can be used to provide information regarding several physicochemical properties (Demirdöven & Baysal, 2009). The three factors of high concern for ultrasonic experiments are the ultrasonic velocity, attenuation coefficient and acoustic impedance. These factors are affected by the physical properties of foods such as composition, structure and physical state (McClements, 1995).

1.2. High-energy ultrasounds

High energy (high power, high-intensity) ultrasounds are characterised by intensities higher than 1 W/cm^2 at frequencies between 20 and 500 kHz, which are disruptive and affect the physical, mechanical or chemical/biochemical properties of foods. These effects can find several applications in food processing, preservation and safety (Santos et al., 2009).

This technology has been applied instead of conventional food processing operations to control microstructure and modify the textural properties of fat products (sonocrystallization) as well as for emulsification, defoaming, and modification of the functional characteristics of different food proteins. It can also be used to inactivate or accelerate enzymatic activity, increase shelf life and quality of food products, inactivate microorganisms and facilitate the extraction of several food bioactive ingredients (Awad et al., 2012).

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By applying ultrasound of higher power, the medium can be affected by the acoustic waves generating interesting effects for the food industry. This type of application of ultrasonic technology is known as “power ultrasound” or “high intensity ultrasounds” and aims at inducing changes in products or processes. The frequencies used range between 20-100 kHz (Mason & Lorimer, 2002).

The compression and rarefaction of the medium particles and the consequent collapse of the bubbles is known as cavitation and is the most important effect in high power ultrasonics. The temperature and pressure within these imploding bubbles can reach values of up to 5000 K and 1000 atmospheres, respectively, which in turn generate very high shear energy waves and turbulence in the cavitation zone (Patist & Bates, 2008).

High power ultrasound has very recently been applied for large scale commercial applications, such as emulsification, homogenization, extraction, crystallization, dewatering, low temperature pasteurization, degassing, defoaming, activation and inactivation of enzymes, reduction of particle size and viscosity alteration. Moreover, the technique can be used to alter food structure, softening or disruption and migration as well as protein alterations. By reducing viscosity, the characteristics of the produces can be improved during heat transfers or extrusion processes (Patist & Bates, 2008). While most of the above techniques are still in the process of optimization at laboratory scale, ultrasonic defoaming and sieving have already been established and used at industrial level (IUFoST, 2010).

1.3. Historical evolution of ultrasound

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In 1880 Pierre and Jacques Curie discovered that a plate cut in a particular plate from a Rochelle salt crystal could penetrate an electrical potential under application of mechanical pressure. This phenomenon is defined as piezoelectric effect. The inverse effect takes place when an alternating voltage is applied across a certain plate of the crystal. The alternating voltage changes the linear dimension leading to mechanical vibration. In 1881 crystals were used for the first time for the generation and detection sound waves. Some ceramic materials, such as barium titanate, lead zirconate, and lead titanate can also produce piezoelectricity and are used in ultrasonic transducers, while other techniques that mechanical vibrations, generating ultrasound, include whistles, magnetostrictive methods, and spark discharges in water (Feng & Yang, 2011).

Generally, power ultrasound is considered to be an emerging and promising technology for industrial food processing. The use of ultrasound in processing creates novel and interesting methodologies which are often complementary to classical techniques. There is a wide scope for further research into the use of ultrasound in food processing both from an industrial and academic viewpoint (Dolatowski et al., 2007).

1.4. Benefits of ultrasonication

The development of mild food preservation techniques is of high importance due to the need of the industry to replace the heat-based techniques that can significantly affect the nutritional value of treated foods. One of the most recently applied mild-preservation techniques is ultrasound technology. The benefits of ultrasound applications are among others the protection of qualitative characteristics, such as flavour and odour, visual appearance, nutritional value, as well as the absence of additives. Minimal processing can be used for a vast range of foodstuffs, including foods characterised by a short shelf-life (fresh fruit and vegetables, chilled ingredients, etc.) (Earnshaw, 1998).

The large-scale application of ultrasonication offers numerous advantages including continuous follow-through ultrasound systems, important financial benefits and short payback period. Ultrasound assisted extraction (UAE) is considered to be the most feasible and economically profitable large-scale application of ultrasound in the food industry (Soria & Villamiel, 2010).

Many processes at both semi-industrial and industrial scale have demonstrated that these devices are technologically versatile and effective. As a result, systematic use in large-scale industrial processes signifies an important breakthrough in High-power ultrasonics (HPU) technology (Gallego-Juárez et al., 2010). It is considered that ultrasound significantly affect the rate of numerous processes in the food industry. Using ultrasound, full reproducible food processes can now be performed rapidly, while the technique itself is highly reproducible, limiting the processing cost, simplifying manipulation and work-up, enhancing the purity of the final product, eliminating post-treatment of waste water and requiring significantly less time and energy in comparison to conventional processes. Numerous processes such as freezing, cutting, drying, tempering, bleaching, sterilization, and extraction have been effectively used in the food industry. The benefits of the use of ultrasound for food processing include: more efficient mixing and micro-mixing, faster energy and mass transfer, reduced thermal and concentration gradients, lower temperatures, selective extraction, use of smaller equipment, faster response to process extraction control, faster start-up, increased production, and elimination of process steps (Chemat et al., 2011). Ultrasound techniques are characterised by low cost and simplicity, while, at the same time, they can be very energy efficient and therefore easily became an emerging technology for probing and modifying food products (Awad et al., 2012). In the field of food freezing, the use of power ultrasound is a relatively new subject. However, recent research advances demonstrate its promising potential. The advantages of the application of sound energy are obvious when taking into account the numerous effects that ultrasound generates upon the medium where it transmits. Among these effects, cavitation is probably the most important, since it cannot only lead to the generation of gas bubbles but also to the occurrence of microstreaming. The former can promote ice nucleation while the latter can lead to heat acceleration and mass transfer processes that accompany the freezing process (Zheng & Sun, 2006).

Although further research is necessary for the development of new improved processing equipment that provide less variation in the residence time, the results presented in the study of McClements (1994) demonstrate that high intensity ultrasound implemented with heating in continuous flow can reduce the fat globule size. Use of ultrasound and heat for the treatment of milk can provide important information about the chemical changes of milk proteins (Villamiel & De Long, 2000a). Finally, according to Leadley & Williams (2006), the technique could be effectively applied to a wide range of processes. Current and potential applications include the crystallization of fats and sugars, degassing, foam breaking, extraction of solutes, ultrasonically aid drying, mixing and emulsification, spirit maturation and oxidation processes, cleaning and surface decontamination, cutting, humidifying and fogging, precipitation of airborne powders, stimulating live cells, ultrasonically assisted freezing, ultrasonically aided filtration, enhanced preservation, etc.

1.5. Disadvantages of Ultrasonication

When liquid foods or samples are treated with high frequency ultrasounds, there is a rapid increase of their temperature. This increase can negatively affect certain characteristics of the food. Fat and protein are mostly affected, and these changes have a negative impact on the nutritional value of the food and it's sensory characteristics. With the correct planning of the treatment and the implementation of a cooling stage, the above unwanted results can be avoided. It is generally accepted that ultrasound treatment is not very successful in inactivating microorganisms and enzymes. To achieve this result, higher power ultrasounds should be implemented, along with increased temperature and pressure, which have a synergistic performance in the inactivation (Jambrak et al., 2009).

Free radicals are involved in the catalysis of unwanted reactions that damage proteins, amino acids and fats and can lead to the creation of polymers that could cause subsidence and create uneven texture in the finished product. Although lipid oxidation is considered a deteriorative process responsible for generating off-flavors, specific oxidation products are desirable. Precise sequential reactions that are catalyzed by the enzyme lipoxygenase play an important role in creating a desirable fresh flavor and aroma of cheese and fermented products. (German et al., 1992). Vercet et al (1998) investigated the formation of free radicals after different treatments with ultrasounds combined with temperature and pressure (manotermosonification, MTS). MTS does not affect significantly the nutrient content of the foods. However, it changes the behavior of nonenzymatic browning. According to the study, the increase in temperature resulted in a decrease in the degree of hydroxyl radicals. Temperature effects were studied between 30 and 140 ° C. The amplitude of the ultrasound was changed between the two regimes combining temperature and pressure (70° and 130 °C/200kPa C/500kPa). In both cases, the level of free radicals increased linearly with increasing ultrasound's amplitude. Influence of pressure on the formation of free radicals was determined in the two regimes of the same amplitude at 70 and 130 ° C. At 70 ° C, increase in hydrostatic pressure resulted in an increase of free radicals, while increasing the hydrostatic pressure at 130 ° C did not affect the formation of free radicals.

High power ultrasound is applied in emulsification and milk homogenization, and monitoring possible negative effects like oxidation of fats, inactivation of enzymes and denaturation of proteins is of high importance throughout these processes. Controlled and optimized application of ultrasound requires use of specific ultrasound frequency and optimal treatment time. Ultrasound processing carried out at lower temperatures could prevent any negative side effects on treated materials (Jambrak et al., 2009).

2. Types of ultrasonic waves transducers

The implementation of ultrasound is doable only by using a system that can produce a stable and reliable ultrasonic field from another type of energy, such as electrical. The devices that perform the energy conversion are called transducers. These devices use the energy produced by a power generator to produce mechanical energy in the form of ultrasonic vibrations (Cárcel et al., 2012).

There are two main types of transducer: magnetostrictive and piezoelectric (Soria & Villamiel, 2010). Although the potential use of ultrasonics has been examined for many years, major advances have been made in the last decade taking into account the laboratory-based prototype technology and using it to fully cover operational commercial needs throughout Europe and the USA (Patist & Bates, 2008).

Ultrasonic waves are formed using magnetostrictive or piezoelectric transducers, which convert electrical energy into mechanical oscillations. The latter are transferred into the treatment medium either directly via sonotrodes or indirectly using ultrasonic baths (IUFoST, 2010).

3. Applications of ultrasound

3.1. Microbial inactivation

The effective inhibition of microorganisms is a target that currently affects the decisions of more and more consumers. The reduction of the microbial load to acceptable levels is extremely important in the field of food processing since a single microbial issue could dramatically damage the reputation of any manufacturing company. The minimization of the bacterial population of a product is usually performed by reducing the initial contamination, inactivating any microorganisms present in the product and implementing appropriate measures for the prevention or retardation of subsequent growth of microbial populations which are still active (Sala et al., 1995).

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Although both conventional thermal pasteurization and sterilization can effectively reduce or eliminate the bacterial load of a food, innovative techniques that have reduced impact on the nutritional characteristics and overall food quality are demanded. New preservation techniques have been established that target at eliminating the microbial population while significantly reducing or completely eliminating the use of thermal processing. During the sonication process, longitudinal waves are formed when a sonic wave meets a liquid medium, leading to the formation of regions of alternating compression and expansion. These regions of pressure change cause cavitation to occur, and gas bubbles are formed (Piyasena et al., 2003).

3.1.1. Vegetables and fruits

The aim of the study of José & Vanetti (2012) was the evaluation of the effectiveness of ultrasound treatment, used in combination with commercial sanitizers, in decontaminating minimally processed cherry tomatoes. Pre-selected cherry tomatoes where treated with ultrasound (45 kHz) for 10 min in the presence of 20 and 200 mg/L sodium dichloroisocyanurate, 5% hydrogen peroxide, 10 mg/L chlorine dioxide or 40 mg/L peracetic acid. The limitation of natural microbial contaminants and inoculated Salmonella, adhered to the surface of the tomatoes, were evaluated. It was proved that aerobic mesophilic microorganisms were reduced by 0.7-4.4 log10 cfu/g while molds and yeasts were reduced by 1.1-3.4 log10 cfu/g after use of different sanitizers. The combination of ultrasounds and 40 mg/L peracetic acid significantly reduced the microorganisms naturally present. The adherent *Salmonella* *typhimurium* ACTT 14028 population was reduced by 3.9 log10 cfu/g. It was shown that ultrasounds could potentially be used for sanitizing cherry tomatoes (São José & Vanetti, 2012).

In the study of Sagong et al. (2011) the effectiveness of individual and combination of treatments (ultrasound and organic acids) on limiting the bacterial load of foodborne pathogens on organic fresh lettuce, was examined. Lettuce leaves were inoculated with a mixture of three strains of *Escherichia coli* O157:H7, *S. typhimurium*, and *Listeria monocytogenes* and the following treatments were applied: ultrasound (40 kHz), organic acids (0.3, 0.5, 0.7, 1.0, and 2.0% -malic acid, lactic acid and citric acid) and combined use of ultrasound and organic acids, all for 5 min. For all pathogens, the combination of ultrasound and organic acids further reduced their populations by 0.8 to 1.0 log in comparison to individual treatments, while no quality change (color and texture) on lettuce was observed during a 7 day storage. The maximum reductions in bacterial populations (*E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes* : 2.75, 3.18 and 2.87 log cfu/g, respectively) were achieved by combining ultrasound and 2% organic acid for 5 min. It was proved that the combination of ultrasound and organic acids led to increased bacterial reduction in comparison to individual treatments, with insignificant effects on the quality, and can be used to enhance the microbial safety on organic fresh lettuce (Sagong et al., 2011).

In the study of Seymour et al. (2002) the effects of ultrasound on the microbial decontamination of minimally processed fruits and vegetables were examined. The population of *S. typhimurium* attached to iceberg lettuce was reduced after washing the products with water, chlorinated water, use of ultrasound with water and ultrasound with chlorinated water, by 0.7, 1.7, 1.5 and 2.7 log, respectively. The cleaning action of cavitation acts through removing cells from the surface of the fresh produce, rendering the bacteria less resistant to the sanitizer. For large-scale (40 L) trials, the use of chlorinated water increased significantly the *E. coli* decontamination efficiency. On the other hand, the frequency of ultrasound treatment (25, 32-40, 62-70 kHz) did not significantly affect the decontamination efficiency (P>0.69). The process was characterised by increased capital expenditure and optimization costs, thus making its industrial application non cost-effective. Moreover, the additional reduction of the bacterial population by 1 log, obtained after combining ultrasound and chlorinated water, did not completely eliminate the risk of pathogens on fresh produce (Seymour et al., 2002).

According to Elizaquivel et al. (2012) the efficiency of sanitizers used in fresh-produce industry is commonly assessed using plate counts in selective media. The determination of the effectiveness of a propidium monoazide realtime PCR (PMA-qPCR) technique to monitor the bacterial inactivation degree by ultrasound treatment in fresh-cut vegetable wash water was evaluated. To this aim, lettuce wash water was artificially inoculated with *E. coli* O157:H7 (106 cfu/mL) and treated using continuous ultrasonic irradiation with a power density of 0.280 kW/L. It was proved that the data obtained by PMA-qPCR and plate counts had no statistical differences during the reduction of the bacterial population by 99.996% which corresponds to a reduction level of 4.4 log. Further bacterial inhibition of *E. coli* O157:H7 could not be detected by the PMA-qPCR method due to its limit of detection (20 cfu/mL). Inactivation data obtained by both techniques successfully fitted a linear model, demonstrating that the kinetic parameters did not differ significantly. It was therefore shown that the PMA-qPCR method can be effectively used for the evaluation of the ultrasonic disinfection of vegetable wash water, being capable of distinguishing between live and dead bacteria (Elizaquivel et al., 2012).

A combined thermo-ultrasound (temperature: 50, 55, 60 °C; time: 10, 15, 20 min with the frequency of 40 kHz) and calcium propionate (concentration: 1, 2, 3% w/v) treatment was used for the decontamination of fresh-cut celery from *E. coli* O157:H7 and *S. enterica* serovar typhimurium. The effects of three parameters (temperature, time and calcium propionate concentration) on the bacterial inactivation degree were compared. It was proved that the temperature was of higher importance for bacterial inactivation. No significant differences ($p>0.05$) were observed in the color and shear force resistance of the treated celery. The optimum treatment conditions were 60 °C thermo-ultrasound with 2% calcium propionate for 15 min (*E. coli* O157:H7) and 59 °C thermo-ultrasound with 2% calcium propionate for 17 min (*S. typhimutium*). The use of scanning electron microscopy was used to demonstrate that the membrane of the treated microbial cells was disrupted in each optimal condition. The combination of thermo-ultrasound and calcium propionate significantly enhanced the inactivation (more than 5 log reduction) of *E. coli* O157:H7 and *S. thyphimutium*. The treatment was capable of extending the shelf life of the product without causing any physical quality deterioration (Kwak et al., 2011).

Table 1 presents a variety of methods such as commercial disinfectants, various organic acids and heat, used for reducing the microbial load of vegetables. It can be concluded that the main microorganism of concern found in vegetables is *S. typhimutium*, the population of which can be reduced by 7.3 log cfu/g after 17 minutes of treatment (Kwak et al., 2011).

3.1.2. Beverages

The combination of sonication (50±0.2 W, 20 kHz), and subsequent concentration and storage at high osmotic pressure, was assessed as a method of reducing the levels of Salmonella in different solutions (PBS, sucrose and orange juice) at varying concentrations. The visualization of the effects of the treatment on the cell membranes was performed by following a staining protocol (propidium iodide [PI] and 4',6'-diamidino-2-phenylindole [DAPI]. Sonication alone did not significantly damage the cell membranes while the use of high pressure alone (48h 10.9 MPa affected the membrane permeability of 10% of cells). However, combined use of sonication and high pressure significantly enhanced the degree of membrane integrity loss, leading to dramatic reduction of microbial populations. The use of this combined method for the treatment of contaminated orange juice led to a 5 log10 cfu/ml reduction in the population of Salmonella spp. As a result, it was proved that the innovative method of "Osmosonication"—the synergistic combination of sonication and subsequent storage at high osmotic pressure –can be used to non-thermally decontaminate liquid foods (Wong et al., 2012).

The study of Bevilacqua et al. (2012) examined the use of ultrasound, in combination with either Na–benzoate or citrus extract, for the inhibition of *Fusarium oxysporum* in orange juice. The maximum power attained by the equipment was 130-W frequency, 20 kHz. The validation of the method was performed in orange juice, in combination with either benzoate (0–100 ppm) or citrus extract (0–1,800 ppm), using a mixture design called centroid. It was proved that time as well as and the interaction energy/time/pulse were the most important factors affecting the extension of the shelf life of juice. It was finally reported that the use of benzoate and citrus extract reduced the populations of the microorganism by 5 log cfu/ml for at least 14 days.

The potential use of acoustic energy for ensuring the microbial safety of apple cider was examined. Inactivation tests were performed using *E. coli* K12 at 40 °C, 45 °C, 50 °C, 55 °C, and 60 °C with and without ultrasound, while a validation test (*E. coli* O157:H7 at 60 °C) was also performed. Environmental scanning electron microscopy was used for the observation of the cell morphology of samples treated at 40 °C and 60 °C. The physical quality attributes of the apple cider (pH, titratable acidity, °Brix, turbidity, and color) were evaluated. It was proved that sonication enhanced *E. coli* K12 cell inhibition by 5.3-log, 5.0-log, and 0.1-log cycles at 40 °C, 50 °C, and 60 °C, respectively. Further microbial destruction due to sonication was more pronounced at sublethal temperatures. At 60 °C the rate of death caused by ultrasound did not differ significantly in comparison to the thermal-alone treatment. First order kinetics were used for the description of the inactivation of *E. coli* K12 with heat at 50 °C and 60 °C. For ultrasound treatments, concave upward survival curves were formed, with a shape factor ranging from 0.547 to 0.720 for a Weibull distribution model. Extensive damage was shown for ultrasound-treated *E. coli* K12 cells, including cell perforation. Titratable acidity, pH, and °Brix of the cider were not compromised by the ultrasound treatment, however, small changes in color and turbidity, especially for sonication at 40 °C for 17.7 min, were detected (Ugarte-Romero et al., 2006).

The simultaneous application of heat and ultrasonic waves under pressure (manothermosonication, MTS) was evaluated as a potential method for inhibiting the growth of *Cronobacter sakazakii* in apple juice. At temperatures below 45 °C, the inactivation by ultrasound under pressure did not depend on temperature. At temperatures higher than 64 °C, the lethal effect of ultrasound under pressure was not significant, especially in comparison to the lethality of the heat treatment at the same temperature. Between 45 °C and 64 °C, the lethality of the combined process (MTS) was higher than the lethality obtained by the synergistic effect of independently applied treatments. The maximum synergistic effect (38.2%) was detected at 54 °C. Recovery on selective media indicated that a specific percentage of microorganisms were sublethally injured after MTS treatments. It was also shown that the microorganisms that survived the MTS treatments progressively died during storage under refrigeration (up to 96 h at 4 °C) (Arroyo et al., 2012).

Bauman et al. (2005) performed inactivation experiments with *L. monocytogenes* 10403S, an ultrasound-resistant strain, at both sublethal (20, 30, and 40 °C) and lethal (50, 55, and 60 °C) temperatures in saline solution (pH 7.0), acidified saline solution (pH 3.4), and apple cider (pH 3.4) with and without the use of ultrasound (20 kHz, 457 $mW \cdot ml^{-1}$). The degree of *L. monocytogenes* 10403S survival in apple cider was assessed, and the effects of temperature, ultrasound, pH, and food matrix on the inactivation potential were examined. The use of ultrasound enhanced the degree of inactivation at all temperatures. Higher degrees of lethality were observed as a result of low acidity at lethal temperatures. The reduction of *L. monocytogenes* 10403S survival followed first order kinetics at sublethal temperatures, but at lethal temperatures, a two-section linear model was used to interpret the microbial inactivation. The survival tests of *L. monocytogenes* 10403S in apple cider demonstrated the potential of using a mild treatment in combination with ultrasound for achieving a 5-log reduction in the population *L. monocytogenes.* The existence of *L. monocytogenes* is a common food safety issue in non-pasteurized fruit juices. High-intensity ultrasound in combination with mild heat treatment and natural antimicrobials could alternatively be used to preserve fruit juices. The resistance of *L. monocytogenes* in orange juice to a combination of treatments such as moderate temperature (45

°C), high-intensity ultrasound (600 W, 20 kHz, 95.2-μm wave amplitude), and the addition of different concentrations of vanillin (0, 1.000, 1.500, and 2.000 ppm), citral (0, 75, and 100 ppm), or both was examined in an effort to determine the most effective inactivation treatment. Nonlinear semilogarithmic survival curves were successfully fitted by the Weibull model. The use of vanillin or citral highly enhanced the bactericidal effect of thermosonication and altered the distribution of inactivation times. When the two antimicrobials were applied simultaneously and in combination with ultrasound, narrower frequency shapes, skewed to the right, with low variances and death time means ranging from 1.6 to 2.6 min, were obtained. Orange juices with 1.500 or 1.000 ppm of vanillin and 100 ppm of citral were proved to be of acceptable quality (Ferrante et al., 2007).

In the study of Kiang et al. (2012) the effects of thermosonication and thermal treatment on the viability of *E. coli* O157:H7 and *Salmonella enteritidis* in mango juice were examined at 50 and 60 °C. It was proved that the highest inactivation rate was achieved by thermosonicating the sample at 60° C. Approximately 9 log cycles reduction in *S. enteritidis* was observed after 3 and 7 min with sonication at 60 and 50 °C, respectively. The inactivation degree differed for each microorganism and *S. enteritidis* was proved to be less resistant to thermosonication treatment in comparison to *E. coli* O157:H7. The recovery of *S. enteritidis* was possible for all samples apart from those treated for more than 5 min at 60 °C. Treatment of mango juice samples with sonication at 60 °C reduced the microorganisms population by 5-log cycles after 7 min; however, only 4.4 log cycles reduction was shown when only thermal treatment was used. It was proved that the use of high-intensity ultrasound increased the level of inactivation in comparison to thermal treatment alone. On the other hand, *S Enteritidis* was found in some of the samples after incubating them into universal pre-enrichment broth. Nevertheless, no growth was found after incubation in mango juice (Kiang et al., 2012).

In another experiment, the determination of the efficacy of Dynashock wave power ultrasound was examined as an alternative processing method for ensuring the microbial safety of apple juice. The effects of several implicit, intrinsic and extrinsic properties on the Dynashock wave microbial inactivation potential were also evaluated. The heat penetration profile in Dynashock wave-treated apple juice indicated a temperature increase from 18.07 to 44.03 °C after 30 minutes of treatment. It was reported that the maximum temperatures that allow the growth of enterotoxigenic *E. coli*, *Salmonella spp*., and *L. monocytogenes* are 46, 45–47, and 45 °C, respectively (average 46 °C). Thereof, it was concluded that the microbial inactivation due to temperature increase was minimal. It was also proved that acid adaptation enhanced the resistance of *E. coli* O157:H7 and *Salmonella* spp. but limited that of *L. monocytogenes*. Spoilage yeast mixed inoculum composed of *Debaryomyces hansenii*, *Torulaspora delbrueckii*, *Clavispora lusitaniae*, *Pichia fermentans* and *Saccharomyces cerevisiae* was less susceptible than any of the adapted or non-adapted pathogens. Among the individual acid-adapted *E. coli* O157:H7, the MN-28 isolate presented the highest resistance. It was finally concluded that the Dynashock wave technology, could potentially be used in combination with other antimicrobial hurdles as an alternative to conventional juice processing techniques (Gabriel, 2012).

Char et al. (2010) evaluated the survival rate of single or strain cocktail of *E. coli, Saccharomyces cerevisiae*, and a yeast cocktail in orange (pH 3.5, 9° Brix) and/or apple (pH 3.1, 12° Brix) juices and in 0.1% w/w peptone water treated with high-intensity ultrasound (USc) and/or short-wave ultraviolet radiation (UV-C). USc treatments (20 kHz, 95 µm-wave amplitude) were carried out using a stainless steel continuous flow cell with a 13-mm probe (0.2 L/min, 40 °C). The USc technique reduced the *E. coli* ATCC 35218 population by 2.2 log, while the inactivation followed first order kinetics. A plate count technique was used to monitor the populations of the microorganisms. UV-C efficiency was greatly dependent on media nature. The poor single effect of UV-C light in orange juice was increased by combining it with the effect of USc. Combined treatment provided better results when the techniques were applied simultaneously and not in series of USc−UV-C arrangements.

Figure 1 presents the microbial inactivation of two different Gram-negative microorganisms, which are characterized by highly different inactivation times. The inactivation rates are probably dependent on the type of organism and the treatment applied. **Figure 2** depicts the inactivation route of two different strains of *E. coli* in apple juice. Faster inactivation is observed in the case of the heat treatment under pressure, probably due to the type of processing. The inactivation times are quite close probably due to the fact that the same microorganism is used in both cases. **Table 2** presents the effects of treatment on the microbial populations of two different fruit juices (apple and orange juice). In apple juice, the population *E. coli* were reduced by up to 5,3 log cfu / ml after treating the samples with ultrasound and heat for 17.7 minutes while no changes in pH or ° Brix were observed. However, it is important to mention that slight changes of color and turbidity were detected (Ugarte-Romero et al., 2006). In the case of orange juice and up to 4 log cfu / ml decrease in the population of *L. monocytogenes* was observed after treating the samples with ultrasound for 15 minutes and subsequently exposing them to high osmotic pressure (10.9 MPa) for 48 hours (Wong et al., 2012).

3.1.3. Milk and milk products

A 20 kHz power ultrasound was used to inactivate *Staphylococcus aureus* and *E. coli* in milk containing 4 % milk fat. The planning and performance of the experiments were in accordance with the statistical experimental design. Specifically, a central composite design was employed for the consideration and optimization of three experimental parameters: temperature (20, 40 and 60 °C), amplitude (60, 90 and 120 mm) and treatment time (6, 9 and 12 min). It was proved that Gram-negative bacteria (*E. coli;* D120 mm=2.78 min at 60 °C) presented higher susceptibility to the ultrasonic treatment in comparison to Gram-positive bacteria (*S. aureus*; D120 mm=4.80 at 60 °C). However, it is important to mention that all three factors examined substantially affected the inactivation of both *S. aureus* and *E. coli* in milk treated with ultrasonics. It was also proved that enhanced numbers of microorganisms were inactivated under longer period of treatments, and especially when combined implementation of higher temperature and/or amplitude was used (Herceg et al., 2012).

In the study of Cameron et al. (2009) the potential use of ultrasonication as an alternative to heat pasteurization was examined. Ultrasound led to reduction of spoilage and microbial pathogens to zero or to levels acceptable by South African and British milk legislation, even when initial inoculum loads of 5× higher than permitted were present before treatment. Viable cell counts of *E. coli* were completely eliminated after 10.0 min of ultrasonication. It was also shown that viable counts of *Pseudomonas fluorescens* were limited by up to 100% after 6.0 min. Ultrasonication did not increase the initial levels of protein and lactose of both raw and pasteurized milk. The use of Kjeldahl indicated that ultrasonication did not negatively affect the total protein or casein content of pasteurized milk. However, it was shown that it led to an increase in the fat concentration. The latter can be explained by the larger surface area of the fat globules after ultrasonication, which lead to increased light scattering as observed by the MilkoScan. The use of alkaline phosphatase and lactoperoxidase as potential indicators of an effective ultrasonic treatment was also examined, but it was found that the method was not effective in deactivating these enzymes, which are commonly used by the dairy industry as indicators of effective thermal processes.

In another study the inactivation kinetics of *C. sakazakii* NCTC 08155 and *C. sakazakii* ATCC 11467 and the quantitative determination of the effectiveness of ultrasonic treatments as alternatives to thermal treatment of reconstituted infant milk formula, were examined. The inactivation experiments of *C. sakazakii* inoculated in reconstituted infant formula were carried out at the combined conditions of temperature, i.e., 25 °C, 35 °C, 50 °C and amplitude, i.e., 24.4, 30.5, 42.7, 54.9, 61 μm and the kinetics were interpreted using a range of inactivation models. The relation between specific inactivation rates and applied temperature and amplitude conditions was described using a modified Bigelow-type model. Ultrasound combined with heat treatment effectively reduced the populations of *C. sakazakii*. Specifically, the strains NCTC 08155 and ATCC 11467 were reduced by 9,99 ± 0.49 log cfu / ml at 50 ° C and 61 microns width. (Adekunte et al., 2010).

The inactivation of *Cronobacter sakazakii* using heat and ultrasound treatments under pressure [manosonication (MS) and manothermosonication (MTS)] at several temperatures was examined in citrate–phosphate pH 7.0 buffer and rehydrated powdered milk. The inactivation rate was an exponential function of the treatment time for MS/MTS treatments (35−68 °C; 200

kPa of pressure; 117 µm of amplitude of ultrasonic waves) in both media, and for heat treatments alone when buffer was used as heating media. Survival curves of *C. sakazakii* during thermal processing in milk presented a concave downward profile. Under temperature conditions of up to 50 °C, the lethality of ultrasound under pressure treatments was not dependent on the treatment temperature in both media. At temperatures higher than 64 °C in buffer and 68 °C in milk, the inactivation level obtained using MTS was equal to that of the thermal treatments (at the same temperature). When temperatures between 50 to 64 ºC for buffer and 50 to 68 °C for milk were applied, the lethality of MTS was the result of a synergistic effect, where the total lethal effect was greater than the lethal effect of heat added to that of ultrasound under pressure at room temperature. The maximum synergism was presented at 60 °C in buffer and at 56 °C in milk. A heat treatment of 12 min (60 °C) or 4 min of an ultrasound under pressure at room temperature treatment (35 °C; 200 kPa; 117 µm) was required for the elimination of 99.99% of *C. sakazakii* cells suspended in milk. The same level of *C. sakazakii* inactivation can be reached with 1.8 min of a MTS treatment (60 °C; 200 kPa; 117 µm). Damaged cells were found after heat treatments and after ultrasound- pressure treatments at lethal temperatures. However, no damaged cells were detected when non-lethal temperatures were used (Arroyo et al., 2011a).

Ultrasound was applied to inactivate the populations of *Listeria innocua* and mesophilic bacteria in raw whole milk. Five systems were assessed in an ultrasonic processor (24 kHz, 120 µm, 400 W). Tested amplitudes of ultrasonic waves were 0, 40, 72, 108 and 120 µm, under a temperature of 63 °C for 30 min. Measurements of pH, acidity and color were taken. After the first 10 min and 30 min of treatment, the microbial load of the pasteurized product was reduced by 0.69 log and 5.3 log, respectively. However, use of ultrasound at 60, 90 or 100% combined with increased temperature led to a 5 log-reduction after 10 min. Similar levels of inactivation were found for mesophylic bacterial and Listeria. The heat and the strongest thermo-sonication survival curves were best fitted using a Weibullian model, while an alternative four-parameter model was used for the mildest thermo-sonication treatment (30%). The increase of thermo-sonication intensity led to lower pH (6.64), higher acidity (0.141%) and whiter colour (92.37). According to the same authors, sonication is a new technique capable of inactivating bacteria while significantly reduced treatment times are required. As regards the dairy industry, the inactivation of pathogens such as *L. monocytogenes* can be performed in milk using ultrasound, although inactivation patterns are not in accordance with traditional first order kinetics. Thermo-sonicated milk is characterized by better colour and similar physicochemical properties in comparison to conventional pasteurized milk. The processing time, however, is shorter. From the industrial point of view, thermo-sonication seems to be a viable option for pasteurizing milk, but the use of conventional pasteurization combined with ultrasound presents several advantages. The potential of applying HTST processing in addition to ultrasound is also possible and can lead to even shorter treatment times (Bermúdez-Aguirre et al., 2009).

The viability of *Listeria innocua* in milk was examined after pasteurization (63°C) and thermosonication (63°C, 24 kHz, 129 mW/mL). Heat-treated and thermo-sonicated cells were collected after 10 and 30 min, respectively. Reduction levels of approximately 5-log were observed after 10 min of thermo-sonication, while after 30 min a 6 log reduction was recorded. The bacterial populations were evaluated using common microbiological methods. The specific effects on cell death were assessed through comparison of thermal and thermo-sonication treatments with untreated *Listeria* cells. Environmental scanning electron microscopy (ESEM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used for the evaluation of the effects of cavitation in cells. Two distinct sample preparation methods, hexamethyldisilazane (HMDS) dehydration and freeze-drying (FD), were assessed for SEM. Heat-treated cells presented thinning of cell walls, while the cell walls of thermo-sonicated cells became porous. After 10 min of treatment, some cells presented breakages and fully broke after 30 min. No differences were found between the images of the HMDS and FD techniques. However, it should be mentioned that the FD technique was faster. ESEM provided the highest magnification, allowing the observation of roughness on the cell walls. TEM images indicated clumping of cytoplasm content and disruption of cell membrane (Bermúdez-Aguirre et al., 2011).

In **Figure 3** the inactivation of two Gram-negative microorganisms in fresh milk and milk powder is presented. It is shown that microbial inactivation inactivation can be performed after 6 minutes showing an almost stable reduction rate despite the fact that the initial concentrations are not equal.

Table 3 details the effects of sonication in conjunction with other methods on various forms of milk. It can be seen that the populations of the Gram-positive bacteria did not decrease as much as the populations of the Gram-negative and thus longer treatment times where required. The highest microbial reduction is displayed in the case of reconstituted milk for infants sonicated at 50 ° C for 2.5 minutes. In conclusion, the most effective treatment appears to be the combination of ultrasound and increased temperature (50 ° C) (Adekunte et al., 2010).

3.1.4. Meat products

The meat industry is in need of reliable meat quality information throughout the production process for guaranteeing the production of high-quality meat products. Fast and non-invasive sensors could be used by taking advantage of recent advances in biophysical methods for assessing meat structure. Reliable meat quality information (tenderness, flavour, juiciness, colour) can be provided by assessing the structure of the meat using mechanical (i.e., Warner-Bratzler shear force), optical (colour measurements, fluorescence) electrical probing, electromagnetic waves, NMR, NIR or ultrasonic technology. The data extracted by those measurements are usually applied for the construction of meat structure images that are fused and subsequently processed via multi-image analysis (Damez & Clerjon, 2008). In the work of Corona et al. (2013), the feasibility of applying non-contact ultrasonic techniques (air-coupled and scanning acoustic microscopy, SAM) for the characterization of several dry-cured meat products was evaluated. Air-coupled ultra- sonic measurements were taken on vacuum packaged sliced dry-cured ham, and the data were compared with contact measurements. The average ultrasonic velocity in dry-cured ham was found to be 1846 ± 49 m/s and 1842 ± 42 m/s for air-coupled and contact measurements, respectively. It was suggested that the deviation (1% relative error) between the method was connected to the effect of the heterogeneous structure and composition of dry-cured ham and the transducer focusing. The SAM was applied for the characterization of dry-cured ham and chorizo samples. B-scan images for dry-cured ham and chorizo demonstrated two dominant reflections from the sample, related to reflections in the lean and fatty tissues. It was indicated that contact ultrasonic measurements could be replaced by the air-coupled technique, limiting the measuring time required and the material handling requirements. Also, SAM method allowed the microscopic characterization of dry-cured meat products. In another study, Ozuna et al. (2013) assessed the effect of high intensity ultrasound and NaCl concentration on the brining kinetics (5 ± 1 °C) of pork loin as well as how it can influence the changes in texture and microstructure. The identification of the effect of both factors on NaCl and moisture transfer was performed through kinetics analysis and by taking the diffusion theory into consideration. Both the texture and microstructure of raw and brined meat were analysed with and without the use of ultrasound. It was proved that the brine NaCl concentration not only led to determination of the final NaCl content in meat samples, but also effectively determined the direction of water transport. The NaCl and moisture effective diffusivities were enhanced. The final NaCl and moisture content and the use of the ultrasound led to changes in instrumentally measured meat texture. The latter was confirmed via microstructural observations.

In the study of Siró et al. (2009), immersion of pork loins (*Longissimus dorsi*) in NaCl brine (40 g L^{-1}) and treatment at 5 °C was carried out using one of the following treatments: (1) static brining, (2) vacuum tumbling, or (3) ultrasonic brining at low-frequency (20 kHz) and low-intensity (2–4 W cm^{-2}). The effect of ultrasonic and tumbling assisted curing technology on porcine tissue microstructure, protein denaturation, water-binding capacity (WBC), water-holding capacity (WHC), sodium chloride diffusion coefficient (D) and on meat textural profile (TPA) were evaluated. It was proved that both ultrasonic treatment and tumbling improved the microstructure of the meat tissue. Water-holding capacity and texture were improved in samples treated with ultrasonic in comparison to tumbled and static brined samples. Nevertheless, the above positive effects were significantly affected by the ultrasonic intensity. Higher intensities and/or longer treatment times led to protein denaturation. The constant diffusion coefficient model effectively described the NaCl diffusion kinetics during brining. Significant enhancement of the diffusion of salt was observed in ultrasonically treated samples in comparison to samples brined under static conditions, while the diffusion coefficient exponentially increased with enhanced ultrasonic intensity.

The evaluation of 3 commercially available ultrasound instruments was performed by Fortin et al. (2004) using pigs representative of the Canadian pig population: CVT-2, UltraFom 300 and AutoFom. Sampling was stratified by fat thickness. The original data set (n = 236) was divided randomly into a calibration set (n = 194 [85 barrows and 109 gilts]) for calibrating the instruments and a validation set (n = 72[32 barrows and 40 gilts]) in order to validate the calibration models. As regards salable meat yield, RMSE values for the calibration models were: HGP2, 1.56; CVT-2, 1.57; UltraFom, 1.70; and AutoFom, 1.68. For lean (kg) in butt, picnic, loin and ham, and weight (kg) of the skinless, trimmed belly, RMSE for the calibration models was almost the same for all four instruments(0.19–0.21, 0.21–0.23, 0.31–0.37, 0.35–0.40 and 0.35–0.36 kg, respectively). Validation results for the prediction of salable meat yield indicated that no significant improvement in precision and/or accuracy occurred by using the UltraFom 300 or AutoFom, over the baseline reflectance probe HGP2. On the other hand, a significant improvement was seen when the CVT-2 was used. However, validation results for the prediction of kg of lean in the primals demonstrated no obvious advantage for the three commercially available ultrasound instruments over the baseline reflectance probe HGP2. After calibrating and validating the instruments, it was proved that, in terms of precision and accuracy of predicting salable meat yield and/or kg of lean in the primals, ameliorations over the baseline reflectance probe HGP2 negligible; particularly as regards the UltraFom 300 or AutoFom.

Tenderness is one of the most significant qualitative characteristics of meat and the use of high power ultrasound towards the disruption of muscle structure could be effectively applied for the reduction of both myofibrillar and collagenous toughness. The experiment was performed using *Longissimus lumborum et thoracis* and *Semitendinosus* muscles from 3 to 4 year old steers. The treatment of the raw beef samples (60 × 40 × 20 mm) were performed using high power ultrasound (24 kHz, 12 W/cm^2) for up to 240 s, and aged for up to 8.5 days before assessing the pH, drip loss, cook losses Warner–Bratzler shear (WBS), compression hardness, and colour. Ultrasound treatment caused a significant reduction in WBS force and hardness, while increasing the pH. Ageing highly reduced hardness and WBS force, but no significant interaction between ultrasound treatment and ageing time was found. Ultrasound treatment had no effects on the colour parameters (L*a*b*, chroma and hue) but the ageing time highly increased the lightness, chroma and hue. The drip loss, was not significantly affected by ultrasound, however the treatment caused reduction of the cook and total loss. During ageing, cook loss and total losses were significantly enhanced. It was therefore shown that high power ultrasound could reduce objective texture measurements of beef without affecting the other quality factors investigated (Jayasooriya et al., 2007). Also, The use of ultrasonics as an analytical technique for the estimation of the composition of a fermented meat-based product (sobrassada) was evaluated by Simal et al. (2003). Measurements of moisture, fat and protein contents and ultrasonic velocity at 4, 8, 12 and 25 °C were taken in samples using different formulae. For the purpose of this study, it was considered that a meat-based product is constituted of fat, water and protein + others. A semiempirical equation, was used to relate the ultrasonic velocity with the composition and the ultrasonic velocities of fat, water and protein. The ultrasonic velocity temperature dependence was used for determining fat, moisture and protein + others contents. The explained variance was found to be 98.0% for protein + others, 97.6% for fat and 95.6% for moisture. The data extracted demonstrated the feasibility of using ultrasonic velocity measurement for rapidly and non-destructively assessing the composition of a meat-based product. Similarly, the application of ultrasonic velocity measurements for determining the composition of dry fermented sausages was evaluated by Benedito et al. (2001). Preparation of ground lean and fatty tissues mixtures was performed in an effort to cover a vast range of fat (2±90 wt.%), moisture (7±76 wt.%), and protein (2±21 wt.%) contents. According to the authors "the ultrasonic velocity in fat decreased on average 5.6 ms^{-1} per °C increase in temperature, due to the negative temperature coefficient for fat and the fat melting, which is observed in (DSC) differential scanning calorimetry analysis". The ultrasonic velocity temperature dependence was used for the determination of the fat, moisture and protein+others content, through measurement of the ultrasonic velocity in the mixtures at 4 and 25 °C and by employing a semi-empirical equation. The explained variance was 99.6%, 98.7% and 85.4% for fat, moisture and protein+others, respectively. It was proved that ultrasonic velocity measurement can be effectively used for evaluating the composition of meat products in a rapid and non-destructive way. Finally, in another study, Llull et al. (2002) examined how the textural properties of a meat-based product (sobrassada de Mallorca) and ultrasonic velocity are related. Measurements of textural properties (at 4 °C) and ultrasonic velocity (at 4, 8, 12 and 25 °C) were taken. A mathematical relation between the ultrasonic velocity and the textural characteristics, such as hardness and compression work (CW) was found. It was proved that the use of ultrasonic measurements, can be applied to non-destructively estimate the hardness and CW. Furthermore, by measuring the ultrasonic velocity at different temperatures it was shown that velocity was decreasing by increasing the temperature. Therefore, this non-destructive technique could be used for effectively assessing the moisture and fat contents of the meat-based product.

3.1.4. Fermented Food Products

The optimization and modeling of an ultrasound-assisted extraction process of phenolics (such as anthocyanins from wine lees) were performed. An ultrasound bath system with the frequency of 40 kHz was applied and the acoustic energy density during extraction was found at 48 W/L. The effects of extraction time, extraction temperature, solvent-to-solid ratio and the solvent composition on the extraction yields of total phenolics and total anthocyanins were all taken into consideration. The simulation and optimization of the extraction process was performed using artificial neural network (ANN) and genetic algorithm (GA). The constructed ANN models accurately predicted the extraction yields of total phenolics and total anthocyanins according to the statistical analysis. The optimization of the input space of the ANN models was performed using GA, with the aim of maximizing the extraction yields. Under the optimal conditions, the experimental yields of total phenolics and total anthocyanins were 58.76 and 6.69 mg/g, respectively, in agreement with the predicted results. Moreover, extraction of higher amounts of total phenolics and total anthocyanins was achieved using ultrasound at the optimal conditions compared to using conventional maceration. Furthermore, the stability of phenolics in the liquid extracts obtained from ultrasound-assisted extraction during storage was assessed.

After storing the product for 30 days, the total phenolic contents in extracts stored at 4 °C and 20 °C decreased by 12.5% and 12.1%, respectively. Additionally the stability of anthocyanins was higher at 4 °C while tartaric esters and flavonols were more stable at 20 °C. Finally, it was concluded that the loss of phenolics during storage was not excessively high (Tao et al., 2013). According to Vila et al. (1999), wine volatile compounds were rapidly extracted using a technique based on ultrasonics and a mix of n-pentane–diethylether (1:2) as solvent. Factorial designs were applied for optimizing the sonication process. Parameters such as sample volume, extraction time and solvent volume were taken into account. The suitable conditions for the ultrasound extraction of aroma compounds of wine were found using a statistical approach. It was proved that the use of lower sample volume (from 1025 ml to 100 ml) and solvent volume (from 60 to 50 ml) significantly improved the extraction efficiency.

In another study, a technique relied on ultrasound-assisted emulsification–microextraction (USAEME) was used for determining the Brett character responsible compounds (4-ethylguaiacol (EG), 4- ethylphenol (EP), 4-vinylguaiacol (VG) and 4-vinylphenol (VP)) in wines. After the selection of the extraction solvent, the influence on the extraction efficiency of ratio between extraction solvent and sample volumes, temperature, ionic strength and time was evaluated by employing experimental design methodology. The optimum conditions found for USAEME of 5 mL of wine were determined at 160 μL of chlorobenzene at 60 °C during 5 min without salt addition. Afterwards, the analytical performance of the optimised USAEME procedure was assessed. The methods revealed that correlation coefficients were over 0.984 for all compounds studied. Satisfactory repeatability (below 10%) and inter-day repeatability (below 11%) were achieved by using different concentration, while the detection limits of the technique were either similar or lower than the limits observed in previous studies. It is probably the first study that uses USAEME for determining the Brett character responsible compounds in wine (Pizarro et al., 2011). Also, Pizarro et al. (2012), applied an ultrasound-assisted emulsification microextraction combined with solidification of floating organic drop method (USAEME–SFOD) to accurately determine haloanisoles and volatile phenols in wines. The effects of several factors that affected the procedure the procedure (type and volume of extraction solvent, temperature, time and ionic strength) were considered with the aim of optimizing the efficiency of the process. Subsequently, the linearity, detection and quantification limits, precision, recoveries and applicability to real samples were all evaluated and excellent method performance results were obtained. Furthermore, comparison of USAEME–SFOD with other liquid–liquid microextraction methods [such as dispersive liquid–liquid microextraction (DLLME) and ultrasound-assisted emulsification microextraction (USAEME)] was performed. It was revealed that the USAEME–SFOD method is suitable for simultaneously detecting cork taint and Brett character responsible compounds in wines.

Moreover, in the study of Cabredo-Pinillos et al. (2006), a liquid–liquid extraction method was employed for the extraction of volatile compounds from wine using dichloromethane and ultrasounds. The method can be used to simultaneously extract different samples and is highly reproducible. After the preliminary tests, numerous parameters (sample volume, solvent volume and extraction time) were optimised using a factorial design in order to find the most relevant variables. The detection limits ranged from 0.0238 mg L^{-1} for 1-pentanol to 0.261 mg L^{-1} for octanoic acid. the calibration of the extraction yields was performed and the results ranged from 9.16 to 1.2%.

In the bakery industry, a Biot–Allard model was applied for characterising the porous structure of bread crumb. The model described the wave propagation in porous materials and is usually applied for evaluating the sound absorbing properties of soft poro- elastic foams. A fine grain bread (FGB) with small cells and a coarse grain bread (CGB) with larger gas cells were subjected to analysis using both image analysis (IA) and ultrasonic techniques. IA-extracted results for mean cell area, cell to total area ratio and number of cells/cm^2 were 0.25 and 0.47, 0.32 and 0.41, and 112.8 and 81.6, respectively for FGB and CGB. The ultrasonic method employed non- contact air coupled transducers at ultrasonic frequencies. The measurement of the phase velocity and attenuation of ultrasonic waves was performed in transmission and reflection experiments. The results of these experiments indicated that there were indeed structural differences between the two different bread types and highlighted the usefulness of the Biot–Allard model for describing wave propagation in bread crumb for a wide frequency range. Moreover, the use of non-contact ultrasound was used for the estimation of flow resistivity, open porosity, a measure for the size of the intersections in the crumb cell walls, and tortuosity (Lagrain et al., 2006). Additionally, in the study of Elmehdi et al. (2003), ultrasonic techniques were employed in order to examine how the mechanical properties of bread crumb are affected by changes in the size, concentration and shape of the crumb cells. Since the determination of the structural integrity of the bread crumb, is connected to the gas cell, these effects are essential for the generation of methods towards the prediction of loaf quality, and are therefore highly important to food and cereal scientists. The preparation of freeze-dried bread crumb samples was performed using red spring wheat flour. The density (r) of crumb ranged from 100 to 300 kg/m^3. Both the ultrasonic velocity and the amplitude of 54 kHz longitudinal waves increased with r.

The signal amplitude increased linearly with density. The investigation of the effects of anisotropy in the cell structure of bread crumb was performed by uniaxially compressing freshly baked samples, thereby transforming the cells from spherical to ellipsoidal. Ultrasonic measurements were taken in the directions parallel and perpendicular to the applied stress. Compressed and non-compressed samples were characterised by opposite density dependence of the velocity, while the velocity decreased by increasing compression, more prominently along the direction parallel to the stress. Signal amplitude increased slightly. It was proved that the mechanical properties of the compressed and non-compressed samples differ. The sensitivity of ultrasonic waves to changes in the size and shape of crumb cells indicates that they could potentially be used as a tool for the characterization of the mechanical and structural properties of bread crumb, and therefore for the measurement of bread quality factors.

3.1.5. Fish and seafood

Ultrasound-assisted extraction of As, Se, Ni and V from fish and shellfish was used as a fast and reliable sample pre-treatment method to accurately determine the four elements by electrothermal atomic absorption spectrometry with Zeeman (As, Se) or Deuterium (Ni, V) background correction. A multivariate optimization approach was applied for investigating the effect of variables affecting the extraction process. Under suitable conditions, quantitative extractions occurred from a 10 mass sample (particle size <100 lm) suspended in 1.5 mL of acidic extractant (0.5 or 3% v/v HNO_3) and treated with high intensity ultrasound (50% amplitude; 3 min). The validation of the method was successfully performed against NRCC-

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DORM-2 dogfish muscle, NRCC-DOLT-2 dogfish liver, NRCC-TORT-2 lobster hepatopancreas, NIST-SRM 1566b oyster tissue and BCR 627 tuna fish. The samples subjected to analysis derived from hake (*Merluccius merluccius*), sole (*Solea solea*), clam (*Venerupis rhomboides*), prawn (*Panaeus kerathurus*), cuttlefish (*Sepia officinalis*), shrimp (*Palaemon elegans*), razor shell (*Ensis ensis*), cockle (*Cardium edule*), Mussel (*Mytilus galloprovincialis*), edible crab (*Cancer pagurus*), meagrin (*Lepidorhombus whiffiagonis*). The concentration ranges (lg/g, dry weight) for the elements examined were: As (12.6–190), Se (0.73–2.34), Ni (2.94–46) and V (0.82–5.14). The detection limits in dry tissue were as follows: 0.6, 0.3, 0.2 and 0.4 lg/g for As, Se, Ni and V, respectively (Lavilla et al., 2008). In another study, an ultrasound-assisted solid–liquid extraction technique, using diluted mixed acid solution was employed for determining cadmium, copper and zinc in fish and mussel samples. The influence of several factors such as nitric acid concentration, hydrochloric acid concentration, hydrogen peroxide concentration, leaching solution volume, and sonication time were taken into account. A 30-min sonication, 56 °C operating temperature and 6 mL of 1:1:1 HNO_3(4 M):HCl(4 M):H_2O_2(0.5 M) were applied for 0.5 g of dried sample. The determination of cadmium and copper was performed using graphite furnace atomic absorption spectrometry, while the determination of zinc was performed using flame atomic absorption spectrometry. The data extracted were compared with the results obtained by microwave-assisted digestion. Ratio (%) of metal amount obtained from leaching technique to amount obtained from digestion for cadmium, copper and zinc ranged from 92% to 114% for fish and from 88% to 103% for mussel samples. The accuracy of the technique was assessed through analysis of a dogfish muscle certified reference material (DORM-2). Recoveries were obtained in the order of 80.9 ± 0.3 and 87.2 ± 0.6%

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(Manutsewee et al., 2007).

Finally, in the study of Neves et al. (2009), determination of calcium, magnesium, manganese and zinc in fish feed samples was carried out by applying ultrasound in the analyte extraction process. The quantification was subsequently performed using flame atomic absorption spectrometry (FAAS). Specifically, 0.10 mol/L of HCl as extraction solution were used and the optimal conditions of extraction were: 100 mg of sample mass; sample granulometry of less than 60 lm; sonication time of three cycles of 10 s and sonication power of 102 W. The technique was used for studying the digestibility of these nutrients in samples of fish feed used for feeding Nile tilapia juveniles. The results obtained were congruent with those obtained from the mineralization of fish feed samples in the metal nutrient extraction process.

3.1.6. Simulated food models

The examination of cell damages as a result of high-intensity ultrasound treatment using classical plate count technique and flow cytometry were examined in cells of *E. coli* (Gram-negative bacteria) and *Lactobacillus rhamnosus* (Gram-positive bacteria). The use of plate counts indicated that *E. coli* (D-value 8.3 min) was far less resistant to ultrasound intensity applied (20 kHz, 17.6 W). The dye precursor carboxyfluorescein diacetate (cFDA) could easily diffuse across the cytoplasmic membrane of intact cells of Gram-positive bacteria *L. rhamnosus*, resulting in its intracellular enzymatic conversion and emission of green fluorescence. On the other hand, the outer membrane of the *E. coli* (characteristic of all Gram-negative bacteria) restricted the penetration of cFDA. In this experiment, ultrasound did not significantly affect the cytoplasmic membrane even though plate counts demonstrated a population reduction. It was suspected that ultrasound induced cell death, which is not necessarily connected to membrane damage. The limited use of bacteriocins, which aim at destabilizing the cytoplasmic membrane but are inhibited by the outer membrane, can now be expanded due to supportive use of ultrasounds, which physically disrupt the outer membrane (Ananta et al., 2005).

The heat resistance of the bacterial spores can be reduced by combining ultrasonic and heat treatments. To study this fact, ultrasonic treatment (20 kHz, 120 W, 12 °C, 30 min) was applied on *Bacillus stearothermophilus* spores in aqueous medium, and the substances released were examined. Calcium, dipicolinic acid and a glycopeptide of 7 kDa molecular weight were found in the treated medium. Fatty acids, acyl glycerols and glycolipids (but no phospholipids) were also detected. It was suggested that the decreased heat resistance resulted from the release of low molecular weight compounds of the spore protoplast, which consequently modified its hydration state (Patil et al., 2009).

Arroyo et al. (2011b) examined the resistance of *Cronobacter sakazakii* to ultrasonic waves under applied pressure (manosonication, MS). The DMS value (decimal reduction time value) of *C. sakazakii* under standard conditions (35 °C, 117 µm, 200 kPa, citrate–phosphate buffer pH 7.0) was 0.41 min, which was higher compared to that of *Yersinia enterocolitica* (DMS=0.19 min) and lower in comparison to those of *Salmonella enterica serovar Enteritidis* (DMS=0.61 min), *L. monocytogenes* (DMS=0.86 min), and *Enterococcus faecium* (DMS=1.2 min). The different strains examined (ATCC 29544, NCTC 8155, 9238, and 9529), the growth temperature (10, 20, 30, and 37 °C), and pH of the treatment media (4.0, 5.0, 6.0, and 7.0) had no significant effects on *C. sakazakii* MS resistance. On the other hand, the beginning of the stationary growth phase, the decrease of water activity of the treatment media (0.98, 0.96, and 0.94), and treatment in foods (apple and orange juices, chicken and vegetable soups, and rehydrated powdered milk) led to DSM change of up to a 1.6-, 3.9-, and 2.5-fold, respectively. It is important to mention that the amplitude of ultrasonic waves and the DMS values were exponentially related, whereas static pressure and DMS values were better related using a quadratic equation. The transfer of energy into the medium critically affected the lethality of the ultrasonic waves under all combinations of pressure (0, 50, 100, 200 and 300 kPa) and amplitude (34, 62, 90, 117 and 145 μm) used. The DMS values and the power input were exponentially related. Specifically, by increasing the power by 134W, a ten-fold increase of the inactivation rate was observed for all treatment mediums. The MS treatments did not cause injuries to the MS cytoplasmic membranes or oxidative damages to *C. sakazakii*, but it was proved that damage to the outer membrane preceded microbial death (Arroyo et al., 2011b).

A continuous flow ultrasonic treatment apparatus was employed for the application of high-intensity ultrasound, aiming at inactivating bacteria of high significance for the dairy industry. A comparative study was performed using a conventional tubular heat exchanger and similar process conditions. Ultrasound was used to inactivate *Pseudomonas fluorescens* and *Streptococcus thermophilus* cells after inoculation into Trypticase Soy Broth (TSB). It was shown that the Gram-negative bacteria were characterised by lower resistance in comparison to Gram-positive bacteria. Although the ultrasounds are less effective at increased temperatures, an additive effect between heat and ultrasound was detected. The latter was detected through computer simulations, which compared ultrasounds with conventional heating. The results obtained for milk confirmed that continuous-flow ultrasonic treatment could be effectively used for milk processing offering numerous advantages such as increased energy efficiency (Villamiel & De Jong, 2000b).

In another study, the effects of amplitude of ultrasonic waves, static pressure and temperature on the inactivation rate of *L. monocytogenes* were studied. Additionally, the effects of growth temperature, pH and composition of treatment medium, as well as the use of NaCl in the recovery medium of the bacterium were studied and compared with its heat resistance. The inactivation degree of *L. monocytogenes* under application of high power ultrasonic waves (20 kHz, 117 mm) at room temperature and pressure was quite low (D =4.3 min). The UW increase of relative pressure (MS) to 200 kPa limited D value to 1.5 min while by increasing MS to 400 kPa, D values of 1.0 min were observed. An exponential increase of inactivation rate was observed under application of MS with ultrasonic waves ranging from 62 to 150 mm. Amplitude increase of 100 mm decreased the MS resistance by approximately six times. Under application of MS, the inactivation rate was not initially affected by temperature. However at temperatures higher than 508 °C, the lethality effect of this combined process (MTS) was significantly enhanced. Inactivation by MTS included the combined effects of heat and ultrasound under increased pressure conditions. Cells grown at 378 °C were twice as heat resistant as those grown at 48 °C. The decimal reduction time values (MS: 117 mm, 200 kPa, 40 °C) did not differ between the two suspensions. While the inactivation rate in buffer of pH 4 under application of heat was two- and five-fold higher than that of buffer with pH 7 and skimmed milk, respectively, the D values presented under these conditions differed by less than 60%. The use of sucrose (57%) in the medium enhanced both D_{61} (from 0.22 to 5.7 min) and D (from 1.5 to 3.1min). The

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use of 3% sodium chloride to the MS recovery medium caused D_{60} to decrease without modifying the DMS values. (Pagán et al., 1999).

Broda (2007) examined the survival degree of 'blown pack-causing *C. estertheticum* spores after four treatments: thermal processing, ultrasound followed by thermal processing, use of peroxyacetic acid (POAA)-based sanitizer followed by thermal processing and POAA sanitizer followed by thermal processing in 20% animal fat. No cells survived after treatment with sanitizer alone for 5 min at room temperature. The use of heat alone or ultrasound followed by thermal processing did not completely inactivate the spores treated under any treatment combinations other than 240 s at 100 °C. The data obtained from this experiment provide a basic background for the development of methods for controlling 'blown pack'-causing clostridia on dressed carcasses and meat plant environments (Broda, 2007). **Table 4** shows the differences in microbial inactivation between several food models. It is obvious that the highest reduction of *Pseudomonas fluorescens* (4.2±0.8 cfu/ml) occurs in TSB medium as a result of the use of ultrasound for 1.705 minutes (Villamiel & De Jong, 2000b).

4. Use of Ultrasound in food drying processes

According to Schössler et al. (2012) a contact ultrasound system was developed and evaluated for use in combination with freeze-drying processes for treating vegetables. Red bell pepper cubes were freeze-dried on a stainless steel screen, which acted as the sound transmitter. The effects of ultrasound attenuated and absorbed in the product and the relevant process factors were assessed. The use of continuous ultrasound at an excitation amplitude of 6.7 µm led to

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instant sample heating under reduced ambient pressure and a loss of freeze-drying conditions. By reducing the excitation amplitude to 4.9 μm and the net sonication time to 10%, through application of an intermittent treatment with an interval of 10 s ultrasound and 90 s recovery phase, freeze-drying at enhanced sublimation rates was allowed while no sample heating was observed during freeze-drying for 7 h. As drying proceeds reduced amount of sublimation energy is demanded while partial transformation of sound energy into heat is observed. This heat can be used for accelerating moisture removal during secondary drying. The use of ultrasound limited the treatment time required for obtaining moisture levels of 10% d.b. The ultrasound treatment had no effects on the qualitative characteristics (bulk density, color, ascorbic acid content, and rehydration characteristics) of the product. Although freeze-drying is a highly important method for producing high quality food products, its application is frequently limited due to its high-energy requirements for sublimation and vacuum generation, which render it very cost intensive. Current technologies developed for improving the drying rates (heated plates, infrared radiation, microwave application) can degrade the quality of the food. This study aimed at developing a contact ultrasound treatment, close to industrial freeze-drying, where the product is dried on trays. Ultrasound takes advantage of the positive effects of heating due to attenuation and adsorption as well as the mechanical effects of pressure waves, improving drying rates and can therefore be used to shorten drying times and reduce the related processing costs (Schössler et al., 2012).

Atmospheric freeze-drying (AFD) is a dehydration-based technique capable of producing high-quality end products for food, pharmaceutical, and biological industries. Both evaporation and sublimation that occurs under the applied conditions are generally low. Airborne ultrasound can be applied to enhance drying rates. The examination of the influence of the drying temperature, drying time, and ultrasonic power during freeze-drying in the presence of an airborne ultrasonic field was performed. Accelerated effective diffusion of up to 14.8% was detected for atmospheric freeze-drying with a fluid bed. The higher drying rate in ultrasonic-assisted atmospheric freeze-drying is due to the enhanced mass transfer rate at the solid-gas interface, as a result of a reduced boundary layer due to a higher turbulent interface. As a result, high intensity airborne ultrasound applied in combination with drying systems can effectively accelerate drying, reduce cost and enhance the qualitative characteristics of the foods (Bantle & Eikevik, 2011).

In the study of Sabarez et al. (2012), ultrasound was used to assist in convective food drying. The application of ultrasound is relied on transmitting ultrasonic energy as combined airborne contacts and through several solid contacts between the ultrasound element and the product tray. A computer-based ultrasonic drying setup was used to continuously record the process variables in real time and enable simulation of dehydration under controlled conditions and drying parameters. The drying setup was used to dry apple slices, and the effects of ultrasound combined with conventional hot air drying on drying kinetics and product quality, were examined. It was demonstrated that ultrasound can simultaneously be used for the acceleration of the processing time (i.e., reduced energy consumption and enhanced production throughout) in conventional hot air drying with no effects on the qualitative characteristics of the product. It was suggested that the potential of ultrasound to enhance the air-drying process largely depends on the process variables employed. Specifically, the effectiveness of ultrasound on improving the efficiency of the convective drying process is optimized under low temperature conditions and high ultrasonic power. The results of this experiment could potentially be taken into account when the effective dehydration of heat-sensitive products is needed or when shorter drying times are demanded to receive products with improved functional and nutritional properties. **Table 5** presents the effects of ultrasounds during drying and freezing of foods in connection with their organoleptic characteristics and the processing method used. It is shown that the apple slices required the minimum treatment, mainly due to their small surfaces.

5. Use of ultrasound in food freezing processes

Freezing is defined as the process of ice crystallization from supercooled water. Ice morphological characteristics (e.g., the size and shape of crystals) is a very significant parameter that affects the quality of frozen foods as well as several freeze-related processes such as freeze concentration and freeze drying. These characteristics can significantly affect the sensorial characteristics of foods consumed in the frozen state. For example, the perceived texture of ice cream is partly attributed to a large number of small ice crystals (<50 μm in size). Furthermore, the shape of ice crystals is a significant factor since smooth and rounded crystals slide past one another easily, rendering ice cream smooth while crystals with jagged edges and rough surfaces (as in popsicles) do not flow evenly during consumption. Ice morphology, developed during the freezing stage, is also of high importance in freeze-drying since it highly affects the time of sublimation (Petzold & Aguilera, 2009). Li & Sun (2002) evaluated the combined use of immersion freezing and ultrasound for the treatment of potatoes. The effect of ultrasound on freezing rate was affected by ultrasound power, exposure time and freezing phase. By increasing the ultrasound power or the exposure time, stronger sonication was observed. Nevertheless, both the ultrasound power and the exposure time should always be selected by taking into account the thermal effect of ultrasound. In this experiment, great improvement of freezing rate was achieved through application of 15.85 W ultrasound power for 2 min (P < 0:05). Application of ultrasound to the phase change period during the freezing process highly enhanced the freezing rate (P < 0:05) (Li & Sun, 2002).

The use of ultrasound in the field of food freezing, and specifically for the shortening of freezing time of ice cream, was evaluated. Ultrasound of 20 kHz was used and it was demonstrated that application of this technology can effectively shorten the freezing time required. Evaluation of the qualitative characteristics of the treated product revealed reduced crystal size and prevention of incrustation of freezing surface (Mortazavi & Tabatabaie, 2008). In another study the use of ultrasound-assisted immersion freezing was evaluated as potential technique for treating apples. Because of the mechanically anisotropic nature of the apple parenchyma, the effects of the application of ultrasound on radial or tangential orientated samples were also evaluated. Apple cylinders were immersed in an ultrasonic bath system, operating at 40 kHz. The experiments were performed using power of 131.3 W (0.23 W/cm^2), and the application of ultrasound was performed intermittently for different times and temperatures below and close to the initial freezing point. It was indicated that ultrasound application at 0 °C or −1 °C for 120 s in total, with 30 s intervals, led to significant improvement of the freezing rate in comparison to immersion freezing without ultrasound. Results of the effects of ultrasound waves, used for the treatment of radial or tangential cut samples sonicated for 120 s from −1 °C and/or 0 °C, showed that at the power level studied the treatments of the ultrasonic radial and tangential irradiated samples did not differ significantly, while the freezing rates increased and found to be quite different ($P<0.05$) in comparison to the control treatment. Some evidence was also found for the ability of ultrasound to cause primary nucleation (Delgado et al., 2009).

6. Use of ultrasound in quality control processes

Quality control is becoming more and more important in the fish and seafood industry and the fat content is one of the most important parameters measured since it highly affects the quality of these products. The measurement of the lipid content can be performed through extraction of lipids from a homogenised sample using solvents. However, the destructive nature of this method render it appropriate for use in a representative number of samples only. As a result, such measurements cannot always give results as accurate as those required for labeling purposes (Shannon et al., 2004).

The ultrasonic velocity and attenuation coefficient of samples were measured using a Frequency Scanning Ultrasonic Pulse Echo Reflectometer or FSUPER. The lipid, protein, moisture and ash concentrations of cod fillets were assessed using officially recognised techniques. The ultrasonic velocity of the fillets varied between 1575 and 1595 m s^{-1}, decreasing linearly with increasing moisture content ($r^2 > 0.8$ for 26 samples). No systematic relationship was found between the attenuation coefficient and moisture content. The study demonstrated the potential of using ultrasonic velocity measurements for the rapid and nondestructive determination of the moisture content of cod fillets (Ghaedian et al., 1997).

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Commercial catfish farming is one of the most profitable industrial fields in North America; over 250 million kg of catfish were processed in 1998. Processed catfish are usually sold as dressed carcasses (head, viscera, and skin removed) or boneless filleted fishes. As a result, enhanced meat yield (carcass or fillet) results in more retail product per unit weight of live fish produced and increases the profits of the industry. Selective breeding has been extensively used to improve carcass yield in other livestock species, and could similarly be used in catfish products. However, one of the main disadvantages of the technique is that direct measurement of carcass traits can only be performed by killing the animal and removing it from the pool of potential breeders. Development of accurate, quick, and nondestructive methods to predict meat yield in live fish can lead to improvement of selection efficiency and accelerate genetic improvement of catfish meat yield. An initial trial with 30 channel catfish showed significant correlations among weight-adjusted residuals for muscle area measured from transverse ultrasound images and transverse sections at five locations along the trunk musculature ($r = 0.30$ to 0.70). A single ultrasound measurement was used to explain 40 to 50% and 16 to 23% of the variation in meat yield traits of females and males, respectively. The best three-variable model using ultrasound and body shape traits could explain 48 to 56% and 31 to 38% of the variation in meat yield traits in females and males, respectively. It was proved that ultrasound could be effectively used for the prediction of meat yield in live fish, but prediction accuracy needs to be improved (Bosworth et al., 2001).

Meng et al. (2003), examined the potential use of ultrasounds and nano-zinc oxide (ZnO) coating both combined and individually in preserving the qualitative characteristics of fresh-cut kiwifruit. The preparation of the nano-ZnO coating solution was performed by mixing the ZnO nanoparticles in premixed chitosan–acetic acid solution. The fresh-cut kiwifruit were dipped in NaClO solution (50 μL L^{-1} sodium, control), and treated with ultrasound (40 KHz, 350 W, 10 min), or coated with nano-ZnO solution. The combination of the two methods was also evaluated. All samples were stored at 4 °C for 10 days. Different quality factors such as production of carbon dioxide and ethylene, mass loss, and flesh firmness were evaluated. It was proved that the combined use of the two treatments (40 KHz with 1.2 g L^{-1} nano-ZnO coating) led to production of less ethylene (1.86 μL kg^{-1} h^{-1}) and carbon dioxide (10.01 mg kg^{-1} h^{-1}), water loss (0.46 %), and texture (7.87 N). It was therefore proved that the combination of ultrasound treatment and nano-ZnO coating can be effectively use for extending the shelf-life of fresh-cut kiwifruit.

Shannon et al. (2004) examined the white muscle content of salmon samples and evaluated the results of velocity measurements on a group of samples characterised by different fat contents. Since, the fat content in the muscle of an individual animal can vary along the muscle by as much as 16%, the velocity can be highly affected. Myosepta are the most important intramuscular fat depositories in salmon, containing up to 40% of the fat. Thus, the amount of myosepta in the ultrasound path can result in significant velocity variations through the sample. To ensure that the myosepta pattern was as similar as possible in all measurements, the sample was positioned in the rig allowing the acoustic wave to travel from the edge of the sample closest to the dermis, to the inside edge of the sample closest to the spine. It was demonstrated that it was possible to group the velocities into “high fat”, “medium fat” and “low fat” categories but the accurate prediction of the fat content was not possible due to the inhomogeneity in the structure of the muscle.

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Brøndum et al. (1998) used the Autofom ultrasound system with the aim of grading pork carcasses. The system consisted of 16 ultrasound transducers positioned in a frame. The measurement of the carcass was performed automatically at 3,200 positions to a depth of approximately 12 cm with a depth resolution of .19 mm. A three-dimensional ultrasound image was produced, which was subsequently processed to reduce noise, detect orientation and extract 127 features that gave a description of the composition of the carcass. The image features were used in a multivariate regression model, employed for on-line predictions. The mean percentage was predicted with an accuracy of 1.58 to 1.95%, while the fat thickness could also be determined.

7. Conclusions

Although the application of ultrasound in the food industry is well known, numerous innovative and well-promising applications have been presented during the last few years in an effort to improve both the technology and the bioactivity of the processed foods. Ultrasonics not only represents a rapid, efficient and reliable alternative for the improvement of the qualitative characteristics of food, but it can also be used for the development of new products with unique functionalities (Soria & Villamiel, 2010). In the field of microbial inactivation, although enhanced level of ultrasound amplitude can inhibit the survival of acid adapted non-toxigenic strain of *E. coli* O157:H7, it is still of high importance to examine the higher D-values observed. Future studies should investigate the mechanism of resistance of acid-adapted cells to ultrasound treatment. For fruit juice processing, parameters such as juice type, presence of pulp and

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viscosity can significantly affect the determination of the inactivation rate obtained and the treatment time required for the achievement of the desired microbial inactivation (Patil et al., 2009).

In conclusion, the interest of the food industry has recently turned into ultrasound technology and its promising potential in processing and preservation of foods. It has been proved that due to the nature of this technology, it can be used for the improvement of the qualitative characteristics and safety of high-quality foods. However, although a huge variety of applications and potential uses have been proposed, extensive research is still demanded in order to further develop the industrial applicability of the method and fully investigate the effects of ultrasounds on the characteristics of foods.

8. Abbreviations

US	Ultrasound
HPU	High-power ultrasonics
MTS	Manothermosonication
TS	Thermosonication
AFD	Atmospheric freeze drying
MS	Manosonication
FD	Freeze-drying
UAE	Ultrasound assisted extraction

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Table 1. Use of different ultrasound treatments for the inactivation of microorganisms and vegetables and effects of the treatments on sensory characteristics.

Food	Equipment	Processing conditions	Combination of methods	Effect on microorganisms	Effects on sensory characteristics	References
Cherry tomatoes	Ultrasonic Cleaner Model 08849-00	45 kHz , 10 min	Ultrasound & commercial sanitizers (20 and 200 mg/L sodium dichloroisocyanurate, 5% hydrogen peroxide, 10 mg/L chlorine dioxide or 40 mg/L peracetic acid)	*Salmonella typhimurium* - 3.9 log N/No	-	São José & Vanetti, 2012
Organic fresh lettuce	Lab Companion ultrasound tank UC-05	40 kHz, 5 min	Ultrasound & 2% Organic acids (0.3, 0.5, 0.7, 1.0, and 2.0% malic acid, lactic acid, and citric acid)	*Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes* - 1 log cfu/g	No significant color and texture change (Minolta CR300 colorimeter)	Sagong et al., 2011

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Fresh iceberg lettuce	Kerry Ultrasonics tank KS450	32–40 kHz, 10 min	Ultrasound & Chlorine (0–250 mg/ L)	*Salmonella typhimurium*	No significant color change (Sensory)	Seymour et al., 2002
				- 3 log cfu/g		
Fresh pepper	Kerry Ultrasonics tank KS451	32-40 kHz, 10 min	Ultrasound	*Salmonella typhimurium*		
				- 1.5 log cfu/g		
Fresh-cut Iceberg lettuce	Branson Sonifier S-450A	0.280 kW/L, 30min, 20 kHz	Ultrasound (COD 500 ppm)	*Escherichia coli O157:H7*	-	Elizaquível et al., 2012
				- 2.5 log cfu/ml		

Fresh-cut celery	JAC-4020(P), KODO	40 KHz, 60 °C, 15 min	Ultrasound, Heat & 2% calcium propionate	*Escherichia coli O157:H7*	No statistically significant changes were observed in the color or resistance against shear force	Kwak et al., 2011
				- 7.02±0.15 log cfu/ml		
Fresh-cut celery		40 KHz, 59°C, 17 min	Ultrasound, Heat & 2% calcium propionate	*Salmonella Typhimurium*		
				- 7.30±0.12 log cfu/ml		

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Table 2. Effects of different ultrasound treatments on the microbial populations, physicochemical and organoleptic characteristics of apple cider and orange juice.

Food	Equipment	Processing conditions	Combination of methods	Effect on microorganisms	Effect on physicochemical parameters	Effects on sensory characteristics	References
Apple Cider	VC-750 ultrasound generator	20kH, 17.7 min 40°C	Ultrasound & Heat	*Escherichia coli* - 5.3 log N/No	No change to pH and ° Brix	Minor color changes and turbidity of the samples	Ugarte-Romero et al., 2006
Apple Cider	450 W Branson Digital Sonifier® ultrasonic generator	54°C, 117 mm, 0,75 min 200 kPa	Ultrasound & Heat under pressure	*Cronobacter sakazakii* - 4.5 log N/No	-	-	Arroyo et al., 2012
Apple Cider	Ultrasonic processor 750-W	20 kHz, 5 min 60ºC	Ultrasound & Heat	*Listeria monocytogenes* - 4.8 log cfu/ml	-	-	Bauman et al., 2005
Orange Juice	Model Rotovisco RV12, thermostatically bath	20 kHz, 15 min, 600 W, 95.2 μm, 45ºC	Ultrasound, Heat and Natural Antimicrobials	*Listeria monocytogenes* - 4 log N/No	-	Pleasant flavor for consumers	Ferrante et al., 2007

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Orange Juice	Cole-Parmer 750-Watt, 115 VAC, 50/60 Hz	20 kHz, 20 min 50±0.2 W	Ultrasound & storage at high osmotic pressure	*Salmonella*	-	-	Wong et al., 2012
Apple juice	Honda Electronics Co, W-338	Dynashock waves (28,45,100 kHz), 30 min	Ultrasound	*Escherichia coli O157:H7*	-	-	Gabriel, 2012
				- 1,2 log cfu/ml			
				Salmonella spp.			
				- 1,2 log cfu/ml			
				Listeria monocytogenes			
				- 1,8 log cfu/ml			
Apple juice	Sonic & Material Vibra cell VCX 601	21 kHz, 95 µm, 20 min	Ultrasound	*E. coli ATCC 35218*	-	-	Char et al., 2011
				- 2 log cfu/ml			
Orange juice				*E. coli ATCC 35218*			

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				- 1 log cfu/ml			
Orange juice				*Salmonella cerevisiae KE162*			
				- 1 log cfu/ml			
Mango juice	Ultrasonic Elma cleaning bath Model TI-H-10	7min, 60°C, 25 kHz, 200 W	Ultrasound and Heat	*Escherichia coli O157:H7*	-	-	Kiang et al., 2012
				- 5 log cfu/ml			
		7min, 50°C, 25 kHz, 200 W	Ultrasound and Heat	*Salmonella Enteritidis*			
				- 9 log cfu/ml			

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Table 3. Use ultrasounds for inactivation of microorganisms in different types of milk and effects on the physicochemical parameters and organoleptic characteristics of the products.

Food	Equipment	Processing conditions	Combination of methods	Effect on microorganisms	Effect on physicochemical /organoleptic characteristics	References
Milk 4%	Misonix Sonicators S-4000	20 kHz, 2.78 min	Ultrasound & Heat 60°C	*Escherichia coli* - 1 log cfu/g	-	Herceg et al., 2012
		20 kHz, 4,8 min, 120 µm	Ultrasound & Heat 60°C	*Staphylococcus aureus* - 1 log cfu/g	-	Herceg et al., 2012
Raw milk (pasteurisation)	Vibra-Cell High Intensity Ultrasonic Processor VCX 750	20 kHz, 10 min, 750 W	Ultrasound	*Escherichia coli* - 5.34 log cfu/g	No reduction in protein or lactose	Cameron et al., 2009
		20 kHz, 6 min, 750 W		*Pseudomonas fluorescens* - 5.64 log cfu/g	No reduction in protein or lactose	Cameron et al., 2009

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		20 kHz, 10 min, 750 W		*Listeria monocytogenes* - 2.07 log cfu/g	No reduction in protein or lactose	Cameron et al., 2009
Raw whole milk	Hielscher UP400S	24 kHz, 10 min, 120 µm, 400 W, 90%		*Listeria innocua* - 5 log N/No	Slightly lower pH, acidity increase, Whiter color samples	Bermúdez-Aguirre et al., 2009
Reconstituted powdered infant formula	VC 1500, Sonics and Materials	20kHz, 10min, 61 µm, 1500W	Ultrasound & Heat 50°C	*Cronobacter sakazakii* - 9.99 ±0.49 log cfu/ml	-	Adekunte et al., 2010
Rehydrated powdered milk	451 W Branson Digital Sonifier ultrasonic generator	117 mm, 30 min	Ultrasound & Heat 56°C under pressure 200 kPa	*Cronobacter sakazakii* - 3.5 log N/No	-	Arroyo et al., 2011a
Raw and whole cow's milk	UP400S (Hielscher, Inc., Ringwood,NJ)	63±0.5°C, 24kHz, 129mW/mL, 400 W, 120 mm, 10min	Ultrasound & Heat	*Listeria Innocua* - 5 log cfu/mL		Bermúdez-Aguirre et al., 2011

Table 4. Use of ultrasound for the inactivation of different microorganisms in simulated food models

Food	Equipment	Processing conditions	Combinations of methods	Effect to microorganisms	References
Phosphate buffer, pH = 7	Sonopuls HD 2070 homogenizer	20 kHz, 20 min 17.6W	Ultrasound	*Escherichia coli*	Ananta et al., 2005
				- 2.6 log N/No	
		20 kHz, 22 min 17.6W		*Lactobacillus rhamnosus*	
				- 1.2 log N/No	
TSB (Tryptone Soya Broth)	450 Soni®er II ultrasonic cell disruptor	20 kHz, 20 min, 12.7 mm, flow of 11ml/min		*Pseudomonas fluorescens*	Villamiel & De Jong, 2000
				- 4.2±0.8 log cfu/ml	

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		20 kHz, 20 min, 12.7 mm, flow of 11ml/min		*Streptococcus thermophilus* - 2.7 ±0.1 log cfu/ml	
TSAYE (Tryptone Soya Broth with 0.6% of yeast extract)	Branson Sonifier ultrasound generators	20 kHz, 1 min 400 kPa, 117µm	Ultrasound & Heat under pressure	*Listeria monocytogenes* - 4 log cfu/ml	Pagan et al., 1999
TSAYE-BS (Tryptone Soya Broth with 0.6% of yeast extract and 0.3% (w/v) of bile salts)	451 W Branson Digital Sonifier ultrasonic generator	35°C, 117 mm, 1,1 min 200 kPa	Ultrasound under pressure	*Cronobacter sakazakii* - 3.4 log N/No	Arroyo et al., 2011b
Model orange juice, pH = 3	VC750 ultrasound generator	20 kHz, 15 min 37.5µm	Ultrasound	*Escherichia coli* - 2.7 log cfu/g	Patil et al., 2009

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Model apple juice		20 kHz, 15 min 0.4µm, 5.3 min		*Escherichia coli*	Patil et al., 2009
				- 3 log cfu/ml	
Phosphate buffer saline (PBS)	Sonic and Material Vibra cell VC600	20 kHz, 0.012 W cm^{-3}, 15 min		*Escherichia coli*	Joyce et al., 2011
				-1.3 Log cfu/ml	
		21 kHz, 0.012 W cm^{-3}, 15 min		*Klebsiella pneumonia*	
				- 2.3 Log cfu/ml	
Peptone water		20 kHz, 95 µm, 20 min		*E. coli ATCC 35218*	Char et al., 2010
				- 2.2 log cfu/ml	

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Table 5. Use of ultrasounds in drying and freezing of foods and effects on sensory characteristics.

Food	Equipment	Method	Processing conditions	Effects on sensory characteristics	References
Cubes of red pepper	Ultrasound processor UIP1000 with two sonotrodes BS2d34	Freeze-drying	4.9 µm, 7 hours, 10 s ultrasound and 90 s recovery phase	No effect on product quality	Schössler et al., 2012
Peas	Sonotronic, DN 20/2000	Freeze-drying	6ºC, 24 hours	No change in color	Bantle & Eikevik, 2011
Apple slices	Transmitter model H290 connected to a microprocessor controller model 2604	Ultrasonic-Assisted Convective Drying	90W, 3.5 min	Minor differences in texture, not statistically significant $P < 0.05$	Sabarez et al., 2012
Potatoes	Bath system CQBF-1025	Immersion freezing	15.85W, 2 min	-	Li & Sun, 2002

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Ice cream	Model Dr Hielscher	Freezing Process	20KHz, 20 min	Better texture because of downsizing the crystals	Mortazavi & Tabatabaie, 2008
Apple	Ultrasonic bath system Elma MC 300 LC	Immersion freezing	40 kHz, 131.3 W, 0.23 W/cm2, 272.6s	-	Delgado et al., 2009

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Legends

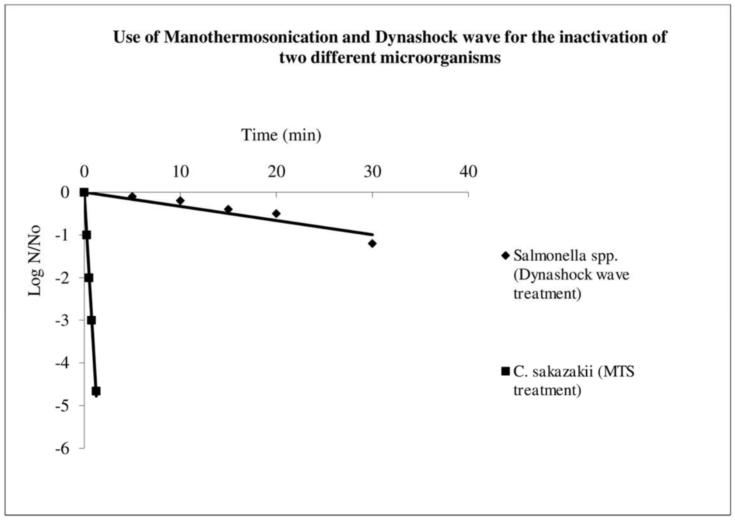

Figure 1. Inactivation of *C. sakazakii* and *Salmonella spp* in apple cider through Manothermosonication (54 °C, 117 mm, 200 kPa) and Dynashock wave treatment, respectively. (Arroyo et al., 2012; Gabriel, 2012).

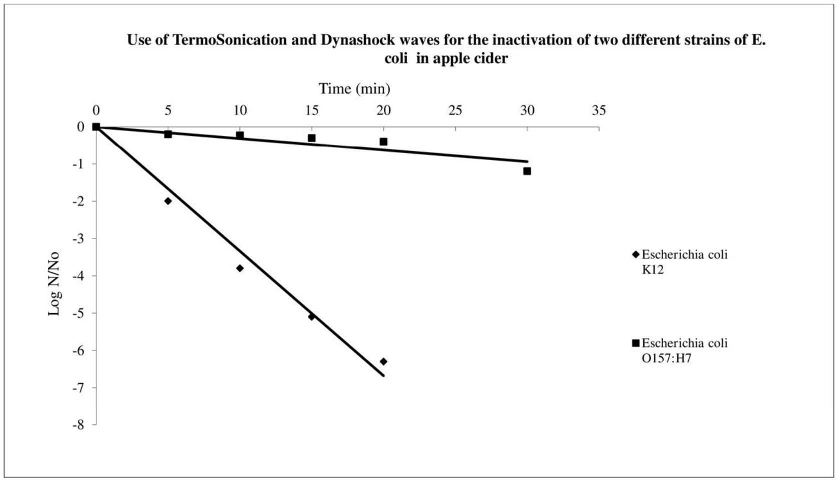

Figure 2. Use of TermoSonication (50 °C) and Dynashock wave treatment in apple cider for the inactivation of *Escherichia coli* K12 and *Escherichia* coli O157:H7, respectively (Ugarte-Romero et al., 2006; Gabriel, 2012).

