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Review

Pasteurization of Foods with Ultrasound: The Present and the Future

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Abstract: In the last two decades, much research has been carried out using ultrasound as an alternative for pasteurization. Cavitation, the main effect of ultrasound, can disrupt and perforate cell membranes, generate free radicals, and produce sonoluminescence. Ultrasound in combination with additional hurdles such as temperature, pressure, or antimicrobials can achieve a 5-log reduction. Pathogens, spoilage microorganisms, yeast, and molds have been successfully inactivated by this novel technology. Currently, ultrasound is investigated as an option to reduce the content of aflatoxins during pasteurization. Ultrasound can inactivate those enzymes related to the stability of pasteurized food products, extending the shelf-life of the products. New uses of sonication are surging; for example, ultrasound has been studied as an option for pasteurizing plant-based foods. An important area of research is ultrasound's effect on food's bioactive compounds. Results exhibit an increase in the concentration of phenolics, carotenoids, anthocyanins, and other nutrients after the use of ultrasound because of an extractive effect. Finally, an area of concern in the early ages of ultrasound has been studied, food quality. In most cases, sonicated products have similar quality parameters to raw products. Lastly, there are some areas of opportunity in ultrasound's future, such as the equipment improvement, regulation, and toxicology of sonicated products.

Keywords: sonication; pasteurization; bioactive compounds; quality; enzyme inactivation; microbial inactivation

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1. Introduction

Ultrasound is a novel technology that has been under active research in the last two decades. Although the first research was focused on microbial inactivation using only model systems, the positive results encouraged scientists to study the effect of ultrasound in real and complex food systems. The main effect of ultrasound in microbial cells is because of cavitation. This physical phenomenon occurs when sound waves enter contact with a liquid medium [1,2]. The passing of sound waves through the liquid generates tiny bubbles or cavities. During the formation of bubbles, there are micro implosions and explosions inside the medium. These explosions increase temperature and pressure, damaging the cellular membrane [3,4]. However, the intelligent combination of processing conditions can use these physical effects to promote the microbial growth of some beneficial microorganisms without damaging cellular structures. This specific feature of ultrasound can accelerate fermentation processes [3]. Ultrasound has also been studied for enzyme inactivation with promising results in stabilizing pasteurized products. Cavitation can modify the protein structure and affect enzyme functionality [5]. The combination of ultrasound and mild thermal treatment often achieves the required 5-log reduction as pasteurization standard with minimal effects on the quality product. Furthermore, the study of the sonicated products has shown the cavitation effect in breaking the cells of vegetable tissue and the release of some bioactive compounds [6–8], making these nutrients more available for quantification and absorption in the digestive system. The positive effects of ultrasound for pasteurizing liquid foods have been widely reported and documented, not only in terms of microbial inactivation but also in the generation of specific characteristics in the sonicated product [9].

This review presents an update on the current research on low-frequency or power ultrasound focused on pasteurized products. The different microorganisms and enzymes studied under this novel technology are discussed. Furthermore, a comprehensive list of bioactive compounds in sonicated products is presented and discussed. The overall quality of several sonicated products is included. At the end of the manuscript, some of the current research needs of ultrasound are discussed to evaluate its potential future as a novel technology in food processing.

2. The Science behind Ultrasound

Ultrasound has a very diverse range of applications in different scientific areas. In the case of food processing and preservation, ultrasound can have physical and chemical effects on the material when it is treated in the range of 16 to 100 kHz, known as low frequency, high intensity, or power ultrasound. The power intensity for low-frequency ultrasound goes from 10 to 1000 W/cm^2 [10]. Most of the research has been conducted with devices operating from 18 to 30 kHz. Opposite that, high-frequency ultrasound works in frequencies higher than 1 MHz, and it is utilized more for nondestructive uses such as quality examination of products. The power intensity for high-frequency ultrasound is <1 W/cm^2 [10]. There is a range of specific applications for sonochemistry reactions, from 300 to 600 kHz. In this range of frequencies, the production of free radicals is promoted to the maximum [11]. For example, the generation of $OH^\bullet$ radicals is higher at 358 kHz than at higher frequencies [12]. The production of free radicals has an antimicrobial effect during processing, acting directly on the DNA of the microorganism [13]. Power ultrasound is the one that has been used for pasteurization. Then, the term ultrasound will be used in this manuscript to refer to low frequency, high-intensity, or power ultrasound.

The primary mechanism of ultrasound is based on acoustic cavitation, the generation of thousands of bubbles or cavities when the sound waves travel through a liquid medium. Cavitation can have a different energy source, like heat, pressure, or shear [14]. The term cavitation in this manuscript will refer to acoustic cavitation. The physical effects of cavitation are the formation of shockwaves and turbulence inside a liquid [1]. The bubbles from cavitation will create micro-storms and micro-currents with critical temperature and pressure increases. Although cavitation is the primarily visible phenomenon during sonication, there are additional effects during microbial inactivation. The rise in temperature and pressure, the generation of free radicals, recombining of these radicals with other molecules, and the breakdown of macromolecules are associated with the antimicrobial effect [2]. All factors will affect the microbial cells and vegetable and animal tissue differently.

Cavitation can be classified as stable and transient. In Figure 1, there is a comparison between both types of cavitation. During stable cavitation, bubbles grow and dissolve in the liquid. There is no collapse of bubbles. However, small eddies are formed in the medium that has a rubbing effect on microbial cells. This effect can create pores in the cell membrane. During transient cavitation, bubbles grow and collapse violently. There is the generation of sonoluminescence. In this cavitation, there are drastic changes in temperature and pressure, reaching extreme values (i.e., 5500 °C, 50 MPa) and creating zones known as "hot spots." Free radicals are formed from the sonolysis of water (i.e., OH^- and H^+) and the breakdown of other macromolecules [13]. The bubble collapse will release potential energy stored in the bubbles; this collapse occurs in picoseconds [14]. Transient cavitation is responsible for the damage to cell membranes, creating not only pores but also disrupting and breaking down cellular structures.

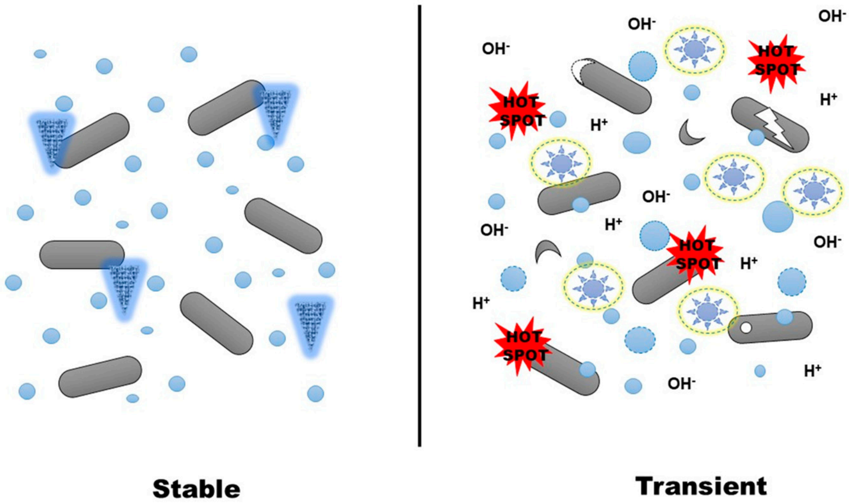

Figure 1. Comparison between stable and transient cavitation during ultrasound processing. In "stable cavitation," bubbles grow and dissolve, and micro eddies are formed. In "transient cavitation," bubbles grow and collapse violently, there is sonoluminescence, generation of hot spots and free radicals (OH^-, H^+), and breakdown of macromolecules.

The additional antimicrobial effect of temperature, pressure, luminescence, and free radicals are part of ultrasound's inactivation mechanism. The size of bubbles during cavitation is affected by the ultrasound frequency. As the frequency decreases, the bubble size increases, promoting more violent cavitation. Hence, low frequency or power ultrasound is used for microbial inactivation. At higher ultrasound frequencies, the bubble size decreases, and cavitation is less violent [13,14].

At higher temperatures, the vapor pressure also increases, affecting the surface tension and viscosity of the liquid, leading to a higher generation of bubbles [14]. If the temperature is not controlled during processing, the heat generated can influence the product's microbial inactivation and nutritional and overall quality. The generated heat during sonication depends on several variables. Some of them are intrinsic to the ultrasound generator itself, such as the power, the frequency, and the way to apply the sonication (constant or pulsed). Others are related to the process, such as the time, the product and its thermal properties, and any additional thermal treatment applied together, the volume of the treatment chamber. The type of microorganism also influences the inactivation. Larger cells are more susceptible to cavitation. Gram-positive bacteria are more resistant to sonication because of their thicker and tight cell wall. Coccoid cells are more resistant than rod-shaped ones [4,13].

Acoustic energy is often used to measure the level of ultrasound applied during processing [15]. However, the conversion of electrical into acoustic energy is not always complete. Some energy is lost by thermal, chemical, sound, and other effects [16]. The acoustic power (AC) has been widely used in ultrasound and is measured based on a calorimetric method [16,17]. Equation (1) allows the measurement of AC:

$$AC = mC_p\left(\frac{dT}{dt}\right) \tag{1}$$

where m is the mass of the product to be treated (kg), Cp (J/kg °C) is the specific heat of the product, and dT/dt (°C/s) is the change of temperature over time. The power density (PD, W/mL) is a measurement of the AC per unit of volume [16,17] and can be estimated using Equation (2):

$$PD = \frac{Acoustic\ power}{Sample\ volume} = \frac{AC}{Volume} \tag{2}$$

Both parameters should be reported while working with ultrasound to compare treatments and reduce replication of experiments or lack of information.

3. Microbial Inactivation

Pasteurization is commonly a thermal process that requires holding the product at a specific temperature for a certain period. Pasteurization aims to inactivate at least 5-log reductions or 99.999% of the pathogen of concern in the product [18,19]. This thermal process also reduces the enzymatic activity and spoilage microorganisms in the food providing stability during storage. Pasteurized products are kept under refrigerated conditions to support the slow growth of microorganisms. However, pasteurization is a strong thermal process that might affect the nutritional quality of some products (i.e., denaturation of vitamins or proteins) and affect the sensory quality of food (i.e., changes in color, texture, taste). Then, ultrasound has been evaluated as an alternative to pasteurization, using sound waves and the effects of cavitation to inactivate microorganisms and enzymes and preserve the quality of the food.

Ultrasound can be applied alone in the product (i.e., juice, milk, or liquid food) or combination with heat and/or pressure. The combination of ultrasound and heat (thermo-sonication), ultrasound and pressure (mano-sonication), ultrasound, heat, and pressure (mano-thermo-sonication) has been evaluated in different products [20–22]. From the microbial point of view, ultrasound by itself is not strong enough to inactivate microorganisms at the pasteurization level, and it has been widely reported [2,23–26]. Though cavitation can produce cell permeabilization, known as "sonoporation," this is a temporary and reversible increase in the permeability of the cell membrane allowing the transport of nutrients [3,27]. Hence, the combination of heat and/or pressure enhances the process, making the microorganisms more vulnerable to cavitation.

In terms of microbial inactivation, many foodborne pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* have been studied under ultrasound with positive results [2,28–32]. Furthermore, several studies deal with spoilage microorganisms such as yeast and molds [33–36]. A few studies have also been conducted on spore-formers microorganisms [37–39]. The acoustic cavitation is responsible for the cellular wall's breakdown and further damage to the cell membrane. Stable cavitation promotes the erosion of the cell wall because of the formation of eddies. Transient cavitation is more aggressive in the cells creating orifices, breaking the cell walls, and releasing cytoplasmatic content [40]. Because ultrasound generates heat by itself, the cells also have thermal effects, such as the weakening of the cell wall and thermo-coagulation of proteins, leading to cellular death [41].

The most recent ultrasound reports for pasteurization are focused mainly on the microbial inactivation of aerobic microorganisms and yeast and molds in some novel fruit and vegetable juices and dairy products, as presented in Table 1. This table shows the need to use ultrasound plus temperature (at least 60 °C) to inactivate the microorganisms successfully.

Table 1. Microbial inactivation in food products using ultrasound.

Product	Microorganism	Processing Conditions	Log Reduction	Reference
FRUITS AND VEGETABLES JUICES				
Amora (*Spondius pinnata*) juice	Aerobic microorganisms and yeast and molds	44 kHz, 500 W, 40 °C, 60 min	>1 and >1, respectively	[21]
Cloudy apple juice	Aerobic microorganisms and yeast and molds	1125 W, 70 °C, 12 min	>3.5 and >3.5, respectively	[42]
Chinese bayberry juice	*Bacillus subtilis*	20 kHz, 400 W, 63 °C, 20 min	>5	[43]
Mandarin juice	Aerobic microorganisms	19 kHz, 750 W, 50 °C, 36 min	1.3	[44]
Pear juice	Aerobic microorganisms and yeast and molds	20 kHz, 750 W, 65 °C, 10 min	~4 and ~3.5, respectively	[45]

Table 1. *Cont.*

Product	Microorganism	Processing Conditions	Log Reduction	Reference
Hog plum juice	Aerobic microorganisms and yeast and molds	40 kHz, 400 W, 60 °C, 30 min	>5 and >4, respectively	[46]
Spinach juice	Aerobic microorganisms and yeast and molds	30 kHz, 600 W, 60 °C, 20 min	>4 and >3, respectively	[47]
Tomato-based beverage	Aerobic microorganisms and yeast and molds	37 kHz, 240 V, 15 min	~1 for each kind of microorganism	[48]
PLANT-BASED MILK				
Almond milk	Aerobic microorganisms and yeast and molds	40 kHz, 600 W, 60 °C, 40 min	>5 and >4, respectively	[49]
Hazelnut milk	Aerobic microorganisms and yeast	62.37 W/cm^2, 75 °C, 15 min	>5 and >3, respectively	[50]
DAIRY AND POULTRY				
Butter	Aerobic microorganisms, yeast and molds, and *B. cereus*	20 kHz, 200 W, 40.76 W/cm^2, 85 °C, 15 min	>6, >2, >1, respectively	[51]
Chocolate milk beverage	Aerobic microorganisms	19 kHz, 400 W, 3 kJ/cm^3	>3.5	[52]
Liquid whole egg	*S. typhimurium*	968 W/cm^2, 35 °C, 20 min (with or without lysozyme)	4.2 and 3.3, respectively	[53]
Camel milk	Aerobic microorganisms, *Staphylococcus* spp.	210 W, 20 kHz, 24 °C, 10 min	> 4 for both	[54]
Goat whole milk	Aerobic microorganisms, coliforms, *lactobacillus*, yeast and molds	20 kHz, 90 °C (ending temperature), 10 min	>6, >6, >5, >4	[55]
Goat whole milk	*Brucella melitensis, S. Typhimurium, E. coli,* and *L. monocytogenes*	20 kHz, 90 °C (ending temperature), 10 min	> 6	[55]
Skim milk	*Anoxybacillus flavithermus* and *B. coagulans*	20 kHz, 5000 W, 10 min	~0.5 and ~1.2, respectively	[56]
Whole milk −3.50%	Aerobic and psychrotrophic microorganisms	24 kHz, 200 W, 15–25 °C, 16 min	~3 and ~3.5, respectively	[57]
Whole milk	Aerobic microorganisms and enterobacteria	20 kHz, 1500 W, 45 °C, 15 min	~2 and ~3.7, respectively	[58]
Whole milk	Aerobic mesophilic heterotrophic	19 kHz, 475 W, 85 °C, 6 min 9 s	3.9	[59]
ALCOHOLIC BEVERAGES				
Beer (0% alcohol content)	*Saccharomyces cerevisiae*	24 kHz, 161.6 W, 70 °C, 30 s, continuous and batch treatment	0.2 and 2.4, respectively	[33]
Beer (4.8% alcohol treatment)	*S. cerevisiae*	24 kHz, 161.6 W, 70 °C, 30 s	1 and 2.7, respectively	[33]

Milk processing with ultrasound continues to be a topic of interest. Recently, several critical reviews about the use of ultrasound for milk pasteurization have been published [15,23,60,61]. New milk sources, like goat, camel milk, or even the latest plant-based milk, are in the scope of ultrasound research. Ultrasound has also been tested against thermoduric spore-formers microorganisms in dairy products. It is well known that *Bacillus* spp. is one of the main problems during milk storage, shortening the shelf-life [62] considerably. However, also, *Bacillus* spp. is one of milk's most heat-resistant species. Khanal et al. [56] studied the inactivation of spore-formers vegetative cells in milk using ultrasound (5000 W, 20 kHz, 80% amplitude, 10 min). The results showed an excellent inactivation of *B. coagulans* (4.53) and *A. flavothermus* (4.26); when thermal pasteurization (63 °C, 30 min) was followed by ultrasound, both microorganisms were fully inactivated. Using ultrasound as a unique technology to pasteurize milk is not feasible without alternative hurdles like heat or pressure. However, ultrasound can modify some functional properties of milk and dairy products [23]. It can also promote the growth of microorganisms, speed up fermentation processes in dairy products, like cheese or yogurt, or increase yield [63–65]. However, the use of ultrasound for milk and dairy products needs to be optimized in processing conditions to deliver those benefits that ultrasound can offer and minimize some drawbacks related to cavitation.

In the inactivation of yeast and molds with ultrasound, aflatoxins became an emerging research topic. Aflatoxins are some of the most toxic compounds for human beings. These toxins are produced by fungi, mainly *Aspergillus flavus* and *A. parasiticus*. Although indirectly through feeding, the presence of aflatoxin M_1 (AFM_1) has been reported worldwide in milk [58,66,67] and dairy products [68,69]. If the cattle consume animal feed contaminated with mycotoxins, the milk will contain mycotoxins. AFM_1 is produced by the liver excretion of AFB_1 in milk. Aflatoxin B_1 (AFB_1) has potent toxic, mutagenic, immunotoxic, teratogenic, and carcinogenic effects, affecting the liver and kidneys [69]. Thermal pasteurization can decrease the concentration of aflatoxins in milk [69]. Other technologies have been tested recently as an alternative to reduce the levels of AFM_1 in milk, such as ultraviolet light, high voltage atmospheric cold plasma, and ultrasound [58,66,67]. Milk processed under pasteurization conditions using ultrasound (20 kHz, 95% of amplitude, 10 min) showed the lowest levels of AFM_1 after the first day of storage (0.15 pg AFM_1 E/mL), reducing the concentration considerably compared to untreated milk [67]. In several countries, there are specific limits on AFM_1; in the United States, the Maximum Residue Level (MRL) for AFM_1 is 0.5 µg/L [58].

Few promising studies have been conducted with parasites and ultrasound [70,71]. Although the studies are focused more on wastewater and effluents, the effect of ultrasound on the oocyst viability can be easily transferred to those food products in which the presence of parasites became a safety problem, such as herbs, salads, and fresh produce. The Centers for Disease Control and Prevention [72] list *Cyclospora* as the main parasite of concern in recent foodborne outbreaks. Hence, the importance of ultrasound research for parasite inactivation in food safety. Abeledo-Lameiro et al. [70] studied the effect of ultrasound (20 kHz, 53.1, 70.8, and 88.5 W/cm^2) on the oocyst viability of *Cryptosporidium parvum*, a protozoan parasite of concern in water. Their findings listed the reduction of oocyst viability from 98.57% to only 4.16% in distilled water after 10 min of treatment.

Finally, an area of concern in ultrasound is related to sublethally injured cells and how some microorganisms can recover from storage as viable cells. Microorganisms can use homeostasis mechanisms (i.e., metabolic and chemical reactions) to repair injuries and recover from stress. This is a critical point that could lead to a food safety risk. Several studies show that hurdle technology and sonication can reduce the number of sublethally injured cells [25,28,73]. Sublethal injured cells have been observed in several novel technologies such as pulsed electric fields [74,75], ohmic heating [76], radio frequency [77], high-pressure carbon dioxide [78], high pressure, and cold plasma [73], among others.

4. Enzyme Inactivation

During pasteurization, some enzymes should decrease or stop their activity from providing the product with stability during storage. Depending on the product, some necessary enzymes can be evaluated during pasteurization. For example, the relevant enzymes for milk pasteurization are alkaline phosphatase (ALP), lactoperoxidase (LP), lipase, and plasmin (alkaline protease). ALP indicates an adequate pasteurization process, while LP frequently checks for overprocessing [79]. Meanwhile, in fruit and vegetable products, the enzymes of interest are pectin methylesterase (PME), polyphenol oxidase (PPO), peroxidase (POD), lipoxygenase (LOX), and polygalacturonase (PG), among others. These enzymes affect the stability of the product in terms of cloudiness, color (discoloration, browning), viscosity, flavor, and nutritional value [80].

Like other novel technologies, ultrasound can increase the enzymatic activity when the proteins unfold because of the sonication effect. During this process, there is mass transfer and better contact between enzyme and substrate, boosting enzymatic activity [41]. However, if the sonication is combined with pressure and/or temperature, the proteins (enzymes) can be denaturized, and any enzymatic activity can be stopped [60]. Enzymes are denatured with ultrasound because of the effect of cavitation in hydrogen bonds and van der Waals forces between the polypeptide chains, but also when the free radicals produced during cavitation react with the amino acids of the enzyme modifying its functionality [5,81].

Some examples of enzyme inactivation in pasteurized products are presented in Table 2. Like microbial inactivation, ultrasound is not enough to achieve important enzyme inactivation [82]. Scudino et al. [59] studied the inactivation of two target enzymes in milk pasteurization, ALP and LP. After 6 min of processing with ultrasound and thermal treatment (85 °C), both enzymes were fully inactivated. Both enzymes are often correlated with microbial inactivation during pasteurization; the complete inactivation of these enzymes is a good indicator of an effective pasteurization process. Usually, the inactivation of ALP is generally used as an indicator of the efficacy of the pasteurization treatment. In a different study, ALP was fully inactivated when ultrasound was applied with heat (20 kHz, 120 µm, 150 W, 75.5 °C, 102.3 s) regardless of the type of milk. However, LP was only partially inactivated when using the cited conditions, remaining with 30% activity in whole milk and 47% in skim milk [82].

Table 2. Enzyme inactivation in selected food products by ultrasound.

Product	Enzyme	Processing Conditions	Inactivation	Reference
FRUIT AND VEGETABLE PRODUCTS				
Araçá-boi pulp	Polyphenol oxidase (PPO)	19 kHz, 400 W, 88.9 °C, 8.75 min	1.19 U/min	[83]
Goldenberry puree	Peroxidase (POD)	20 kHz, 1000 W, 90% amplitude, 65 °C, 10 min	92%	[84]
Mango juice	Polyphenol oxidase (PPO), Peroxidase (POD), Pectinmethylesterase (PME)	20 kHz, 600 W, pulsed (5 s), 10 min, plus UV (20 W, 254 nm)	100% for all	[85]
Pear juice	Peroxidase (POD), Polyphenol oxidase (PPO), Pectinmethylesterase (PME)	20 kHz, 750 W, 65 °C, 10 min	95.70%, 98.09% and 96.75%, respectively	[45]
Spinach juice	Peroxidase (POD) and Polyphenol oxidase (PPO)	30 kHz, 600 W, 60 °C, 20 min	0.66 and 0.016 Abs/min, respectively	[47]
PLANT-BASED MILK				
Almond milk	Peroxidase (POD), Lipoxygenase (LOX)	40 kHz, 600 W, 60 °C, 40 min	93.65% and 94.88%, respectively	[49]
DAIRY				
Skim milk	Alkaline Phosphatase	20 kHz, 5000 W, 10 min	101 mU/L	[56]
Whole milk	Alkaline Phosphatase and Lactoperoxidase	19 kHz, 475 W, 85 °C, 6 min 9 s	100% for both	[59]

The case of pear juice in Table 2 shows three of the target enzymes to inactivate during pasteurization, Peroxidase (POD), Polyphenol Oxidase (PPO), and Pectin methylesterase (PME), almost inactivated (>95%) after 10 min of treatment. However, it is essential to highlight that ultrasound was applied together with thermal treatment (65 °C) [45]. Additional examples of enzyme inactivation with ultrasound include polygalacturonase in tomato juice and paste; PPO in apple juice and puree, strawberry puree, pineapple juice, and carrot juice; and POD in apple juice, carrot juice, watercress leaves, guava cubes, carrot slices, and green beans [81].

5. Bioactive Compounds

One of the latest and growing trends in the food industry is processing plant-based foods. These are alternative foods to standard animal products (i.e., meat, chicken, eggs, dairy, fish, seafood) but processed only from vegetable sources. The plant-based foods market showed a 3-year growth of about 54%. The sales went from 4.8 billion dollars in 2018 to 7.4 billion in 2021. Plant-based milk has been one of the main areas with strong and constant growth because of the several health benefits and innovative products. Some plant-based milk sources are sesame seeds, oats, flax, hemp seeds, upcycled barley, pea protein, cassava root, potato starch, mushrooms, bananas, cashews, coconut, macadamia nuts, pili, pistachio, pecans, walnuts, and hazelnuts [86].

Plant-based foods are often considered over-processed because of the number of processing steps and list of several ingredients required to provide the product with similar characteristics in terms of flavor, color, and texture as the animal product. Ultrasound has also been tested on plant-based food products, not only for pasteurization purposes but also to study the effect of processing on the bioactive compounds and overall quality. Almond milk, currently one of the commercial plant-based options, was tested under sonication. A comprehensive study showed that ultrasound is an excellent technology for inactivating aerobic microorganisms, yeast, molds, and enzymes. Most of the quality parameters and bioactive compounds exhibited minor variations. The cloudiness in the sonicated almond milk was increased because of the breakdown of suspended particles during the cavitation. Some of these particles in almond milk are pectin, lipids, cellulose, proteins, and hemicellulose [49]. In another plant-based product, hazelnut milk increased the total phenolic content when processed at 62.37 W/cm^2, 75 °C for 15 min. However, the soluble protein decreased by about 1%, and there was also a slight decrease in the free radical scavenging activity [50]. Ultrasound promoted better stability in hazelnut milk, showing an improvement in the stability index because of cavitation's effect in reducing the components' particle size [87]. Additional plant-based milk studied under ultrasound is peanut, coconut, soy, rice, and oat milk. Besides successful microbial inactivation, these products showed stability after processing [88].

Table 3 contains a compilation of different products treated with ultrasound and the changes in bioactive compounds. It is evident in most of the examples that ultrasound influenced the concentration of some of these. First, the total phenolic content increased in most of the reported products. Products such as amora juice, blueberry juice, and spinach juice are among the ones with a significant increase in phenolic content (139%). Wu et al. [89] reported an increase in the total phenols as the intensity of the sonication treatment was increased too. Authors mention the disruption of the vegetable tissue due to the cavitation and the formation of small cavities increasing the mass transfer as responsible for a higher concentration of phenols. Total flavonoid content was also increased in some of the products listed in Table 3. Again, amora juice and blueberry juice showed the highest concentration after sonication. Nayak et al. [21] mentioned the release of phenolic and flavonoids from pectic and cellulose components from the cell wall and the addition of hydroxyl molecules to the aromatic ring of these compounds as the cause of an increase in the quantification. The antioxidant activity of the sonicated products presented in Table 3 was increased almost for all the items listed. That fact can be attributed because the general increase of the phenolic content in the sonicated products attributed to the previously mentioned reasons. In kiwifruit juice, there was a significant increase in total phenolics (108.65%), flavonoids (105.56%), and antioxidant capacity (65.67%) after the sonication process (25 kHz, 400 W, 16 min) [90]. Under the same processing conditions, strawberry juice also increased total phenolics, flavonoids, and antioxidant capacity [91].

Table 3. Effect of ultrasound on bioactive compounds in food products.

Product	Bioactive	Processing Conditions	Results			Reference
FRUIT AND VEGETABLE PRODUCTS						
Amora (*Spondius pinnata*) juice	Citric acid (CA) Total Phenolic Content (TP) Total Flavonoid Content (TF) Antioxidant activity (AA) Vitamin C (VC)	44 kHz, 500 W, 40 °C, 60 min	 CA (%) TP (mg GAE/mL) TF (mgCE/mL) AA (%DPPH inhibition) VC (mg/100 mL)	Ctrl. 2.62 130 0.05 58 50	Proc. 2.29 310.45 0.16 84.65 35.39	[21]
Apple juice (cloudy)	Total Polyphenol Content (TP) Antioxidant activity (AA)	1125 W, 70 °C, 12 min Pasteurized: 85 °C, 10 min	 TP (µg/mL) AA (%DPPH inhibition)	Ctrl. 437.52 14.17	Proc. 515.12 25.73	[42]
Araçá-boi pulp	Vitamin C (VC), Total Phenolic Content (TP), Total Flavonoids (TF), Antioxidant Activity (AA)	19 kHz, 400 W, 88.9 °C, 8.75 min	 VC (mg/100 mL) TP (mg GAE/g) TF (mg CE/g) AA (µM TE/g)	Ctrl. 9.33 1.15 0.06 1.21	Proc. 13.67 1.33 0.09 1.91	[83]
Chinese bayberry juice	Anthocyanin content (AC)	20 kHz, 400 W, 63 °C, 20 min	 AC (mg/100 mL)	Ctrl. ~28.5	Proc. ~27	[43]
Blueberry juice	Total Phenolic Content (TP) Total Flavonoid Content (TF) Total anthocyanin content (TAC) Antioxidant activity (AA) Hydroxyl activity (HA)	20 kHz, 600 W, 60 °C, 15 min	 TP (GAE mg/mL) TF (RE mg/mL) TAC (Cyc mg/100 mL) AA (% DPPH inhibition) HA (% OH inhibition)	Ctrl. 3.56 0.58 1.7 41.8 45.68	Proc. 8.52 2.04 3.3 65.22 51.13	[89]
Goldenberry puree	Carotenoids (C)	20 kHz, 1000 W, 90% amplitude, 65 °C, 10 min	 (C) µg/100 g	Ctrl. 3800	Proc. 4000	[84]
Grapefruit juice	Ascorbic acid (AA), Total Phenolic Content (TP), Total Flavonoids (TF), Antioxidant Capacity (AC)	28 kHz, 70% power set, 20 °C, 90 min	 AA (mg/100 mL) TP (GAE µg/g) TF (CE µg/g) AC (Ascorbic acid equivalent µg/g)	Ctrl. 27.83 757.96 462.27 276.72	Proc. 35.75 826.27 603.18 308.89	[8]
Cape gooseberry juice	Vitamin C (VC), Total Phenolic Content (TP), β-carotene, α-carotene, β-cryotxanthin, lycopene, Retinol Activity Equivalent (RAE)	42 kHz, 240 W, 30 °C, 40 min	 VC (mg/100 mL) TP (GAE µg/g) β-carotene (mg/L) α-carotene (mg/L) β-cryotxanthin (mg/L) Lycopene (mg/L) RAE (µg/L)	Ctrl. 17.17 801.45 3.26 2.13 4.01 3.36 1.46	Proc. 3.75 918.1 6.2 4.18 7.72 5.84 3.18	[92]
Mandarin juice	Glucose, Vitamin C (VC), Total Phenolic Content (TP), Total Carotenoids (C)	19 kHz, 750 W, 50 °C, 36 min	 Glucose (g/100 mL) VC (mg/100 mL)	Ctrl. 2.93 26.82	Proc. 2.85 24.49	[44]

Table 3. *Cont.*

Product	Bioactive	Processing Conditions	Results			Reference
			TP (mg/100 mL) C (mg/100 mL)	43.78 1.22	32.07 1.25	
Orange juice	Vitamin C (VC)	20 kHz, 1500 W, 25 °C, 0.88 W/mL	 VC (mg/100 mL)	Ctrl. 46.62	Proc. 44.21	[93]
Pear juice	Vitamin C (VC), Total Phenolic Content (TP), Total Flavonoid Content (TF), Antioxidant Capacity (AC)	20 kHz, 750 W, 65 °C, 10 min	 VC (mg/100 mL) TP (GAE µg/g) TF (CE µg/g) AC (Ascorbic acid equivalent µg/g)	Ctrl. 4 346 217.81 237.7	Proc. 3.65 325.74 200.19 206.62	[45]
Hog plum juice	Vitamin C (VC), Total Phenolic Content (TP), Total Carotenoids (C), Antioxidant Capacity (AC)	40 kHz, 400 W, 60 °C, 30 min	 VC (mg/100 mL) TP (mg GAE/mL) C (µg/100 mL) AC (%)	Ctrl. 24.1 8.01 95.15 38.72	Proc. 22.66 7.61 77.56 43.33	[46]
Spinach juice	Total Phenolic Content (TP), Chlorophyl a + b (CL), Carotenoids (C), Anthocyanins (A)	30 kHz, 600 W, 60 °C, 20 min	 TP (GAE µg/g) CL (µg/mL) C (µg/mL) A (µg/mL)	Ctrl. 753.87 51.04 3.56 37.32	Proc. 902.15 61.43 4.22 43.65	[47]
Tomato-based beverage	Vitamin C (VC) Chlorogenic acid (CA), Gallic acid (GA)	37 kHz, 240 V, 15 min	 VC (mg/L) CA (mg/L) GA (mg/L)	Ctrl. 3.85 4.8 5.5	Proc. 3.58 4.6 6.3	[48]
PLANT-BASED MILK						
Almond milk	Total Phenolic Content (TP) Total Flavonoid Content (TF) Total Flavonols (TS) Condensed tannins (CT)	40 kHz, 600 W, 60 °C, 40 min	 TP (GAE µg/g) TF (CE µg/g) TS (CE µg/g) CT (CE µg/g)	Ctrl. 702.2 412.3 8.9 191.3	Proc. 692.9 388.6 8.5 159.2	[49]
Hazelnut milk	Total Phenolic Content (TP) Soluble protein (SP) Free radical scavenging activity (DPPH)	62.37 W/cm^2, 75 °C, 15 min	 TP (GAE µg/g) SP (%) DPPH (µmol TE/g)	Ctrl. 162.78 4.09 65	Proc. ~170 3.1 ~60	[50]
DAIRY						
Chocolate milk beverage	Antioxidant Activity (AA) ACE inhibitory activity	19 kHz, 400 W, 3 kJ/cm^3 HTST: 72 °C, 15 s	 AA (%) ACE inhibitory activity (%)	Ctrl. 76.7 70.7	Proc. 87.2 82.4	[52]
Whole milk	Total Phenolic Content (TP), Antioxidant Activity (AA)	20 kHz, 1500 W, 45 °C, 15 min	 TP (mg GAE/L) AA (µmol TE/L)	Ctrl. 45 1500	Proc. 57 5500	[58]

Ctrl., Control; Proc., Processed; CE: Catechin equivalents; GAE: Gallic acid equivalents; ACE: Angiotensin-converting enzyme; HTST: High-Temperature Short Time pasteurization.

Some natural pigments found in food, such as anthocyanins, carotenoids, and chlorophyll, have also been quantified after sonication. The results for some of the products containing these pigments are presented in Table 3. In general terms, the anthocyanin content of the listed products was increased after the ultrasound treatment. Blueberry juice and spinach juice had a significant increase in anthocyanins. Wu et al. [89] showed that as the ultrasound intensity gets stronger, the concentration of anthocyanins is higher. These authors mention the disruption of cell walls because of the cavitation releasing the anthocyanins into the medium. They compared this effect to using ultrasound to extract pigments with higher yields than conventional technologies. Besides, ultrasound positively affects the inactivation of peroxidase and polyphenol oxidase enzymes (as shown in Table 2). Both enzymes can naturally lead to the degradation of anthocyanins.

In a Polish beverage (*Berberis amurensis* juice), the main anthocyanin, peonidin-3-O-glucoside, showed an increase of 20% in the concentration after being processed with ultrasound (20 kHz, 140 W, 70% of amplitude, 10 min) boosting the antioxidant content of the product [94]. Carotenoids were also increased in goldenberry juice, cape gooseberry juice, mandarin juice, and spinach juice after sonication, as shown in Table 3. As part of the carotenoids, lycopene was also increased in the cape gooseberry juice. In a different study, β-carotene showed a significant increase in sweet potato juice after sonication. The concentration of this pigment went from 246.3 μg/g to 298.9 μg/g after 8 min of treatment (26 kHz, 0.66 W/cm^2) and exhibited a high bioaccessibility (76.6%) compared to raw juice (10.3%) [95]. Like anthocyanins, carotenoids are extracted from the cell walls because of the effect of cavitation, but they are protected from enzymatic activity because of the enzyme inactivation by ultrasound. However, a threshold value of acoustic energy can damage these pigments [47,92].

Opposite to the previous bioactive compounds, the concentration of organic acids such as ascorbic acid (vitamin C) and citric acid was decreased in almost all the products presented in Table 3 after sonication. It is well known and documented the heat sensitivity of ascorbic acid [96,97], and this is one of the reasons for evaluating alternative technologies for pasteurization, mainly for citric juices, which have a high content of vitamin C. Ascorbic acid is a very unstable molecule and can be degraded because of the effect of pH, light, temperature, oxygen, and metallic catalysts [97]. During sonication, there is constant contact with oxygen, and temperature increases, mainly in the “hot spots” and the deposition of metallic particles from the sonotrodes. These factors can decrease ascorbic acid in the evaluated products after sonication. Nayak et al. [21] attributed the decrease of vitamin C in amora juice because of the oxidation process during sonication and the generation of free radicals interacting with the ascorbic acid. Similar findings were reported by Ordóñez-Santos et al. [92] during the sonication of cape gooseberry juice. If the processing is conducted under atmospheric conditions, oxygen will be part of the process leading to the oxidation and decrease of the ascorbic acid.

In other products, ultrasound (42 kHz) was tested in a different type of honey and its bioactive compounds, such as total phenols, antioxidant capacity, and flavonoids, showed a significant increase after processing for a few minutes (5–15 min). Additional studies in sonicated honey exhibited increased antibacterial activity, mainly for *Salmonella typhimurium* [98]. Camel and bovine milk were successfully treated with ultrasound (20 kHz, 105–210 W, 5–10 min), reducing total plate count and Staphylococcal counts with no viable cells after the strongest treatments. The study also investigated the effect of sonication on the protein of both kinds of milk, showing no degradation in this macromolecule. Additional analyses were conducted on the antioxidant properties of camel and bovine milk, and the results showed the retention or enhancement of these properties [54].

Ultrasound offers advantages during processing in protecting some bioactive compounds essential for human health. In most of the studies, the concentration of these compounds has been boosted to significant levels. However, pasteurization requires the inactivation of pathogens and enzymes and the protection of some of these nutritive compounds. Then, there is a need to find the best processing conditions for each product to meet all these needs and provide the consumer with a product with good sensory properties.

6. Quality

Ultrasound is one of the newest technologies that offer the possibility to have novel ingredients for product development. Some changes in quality parameters have been reported in some products and can be used for product development. In the case of milk, ultrasound can affect some quality characteristics of the product. For example, the color parameters L^*, a^*, and b^* can differ from raw milk if the product contains fat. The main effect of cavitation in milk is perfect homogenization. During this process, the fat globules suffer a reduction in size and combine with other molecules present in the milk. The luminosity of milk, L^* is increased as a result [58,59]. This fact can be used for dairy product development for those products that require a whiter color and perfect homogenization (i.e., yogurt). However, in skim milk, where there are no fat globules, cavitation acts between the lactose and the milk proteins, likely promoting a Maillard reaction and decreasing the luminosity (L^*) [63]. The b^* parameter tends to move to the yellowish region when whole milk is processed with ultrasound, likely because of the presence of β-carotene in the fat.

On the contrary, plant-based milk decreased the L^* parameter after sonication. The main reason is the increase in the cloudiness of these products. Some of the particles suspended in these plant-based milk are breakdown because of the cavitation, increasing the turbidity of the product and decreasing the luminosity. Examples of almond and hazelnut milk are shown in Table 4. In fruit and vegetable juices, color parameters such as L^* remained unchanged or slightly higher. In this case, the cavitation breaks down the vegetable tissue and increases the product's luminosity.

The Browning Index (BI) is slightly increased in sonicated products, as shown in Table 4. Amora juice, hog plum juice, and almond milk showed a higher value of BI. These minor changes in BI can be attributed to the Maillard reaction triggered by ultrasound. However, this chemical reaction does not occur when the processing temperature is below 50 °C [46]. Two of the three products with higher BI listed in Table 4 were thermo-sonicated at 60 °C. Additional variations were observed in other color parameters such as chroma, hue, and yellow index for the products listed in Table 4. The most significant change was observed for the blueberry juice, where the chroma was from 4.86 to 0.98, the hue was from 22.88 to −28.61, and the yellow index changed from −12.26 to −2.61. The authors of this research discussed these changes in the color parameters of sonicated blueberry juice based on the production of free radicals. During this process, hydroxylation of the aromatic ring of phenols occurs as previously described. This chemical change affects the juice's color, increasing the product's red component and changing chroma and hue values [89].

Regarding the pH of the sonicated products, most values remained close to the raw product. Minor variations were observed in those products that exhibited a longer processing time. The only significant change in pH presented in Table 4 was for the liquid whole egg. Bi et al. [53] explain this fact based on the decomposition of carbonic acid in the egg white during sonication. Because of the release of CO_2 into the medium, there is an increase in the pH of the product.

Some rheological properties have also been reported for sonicated products. For almond milk, the viscosity was increased after sonication, as shown in Table 4. For butter, the firmness and the work of shear were also increased for the sonicated product. Chocolate milk beverage showed an increase in the apparent viscosity and flow behavior index after treatment but decreased in the consistency index. These changes in rheological properties can be explained based on the emulsifying effect of the ultrasound. For dairy products, ultrasound acts on the fat globules, breaking the fat globule membrane and reducing the size of the globules. This size reduction confers an emulsifying effect on the product achieving an almost perfect homogenization after processing and during storage.

Table 4. Quality changes in food products after ultrasound processing.

Product	Quality attribute	Processing Conditions	Results			Reference
FRUIT AND VEGETABLE PRODUCTS						
Amora (*Spondius pinnata*) juice	pH, Browning index (BI), Cloud value (CV)	44 kHz, 500 W, 40 °C, 60 min		Ctrl.	Proc.	[21]
			pH	3.6	3.65	
			BI	0.7	0.77	
			CV	0.35	0.19	
Cloudy apple juice	*L**, Brix, Water soluble pectin (WSP), Cloud stability (CS)	1125 W, 70 °C, 12 min Pasteurized: 85 °C, 10 min		Ctrl.	Proc.	[42]
			*L**	29.92	34.13	
			Brix	13.37	13.33	
			WSP (mg/mL)	135.47	352.01	
			CS (%)	39	41	
Araçá-boi pulp	pH, Brix, *L**, Chroma (C), Hue (h), Yellow Index (YI)	19 kHz, 400 W, 88.9 °C, 8.75 min		Ctrl.	Proc.	[83]
			pH	3.2	3.15	
			Brix	5	5.8	
			*L**	49.37	52.17	
			C	43.53	43.35	
			h	82.29	82.44	
			YI	124.85	117.69	
Chinese bayberry juice	pH, Brix, *L**	20 kHz, 400 W, 63 °C, 20 min		Ctrl.	Proc.	[43]
			pH	3.08	3.07	
			Brix	10.4	10.27	
			*L**	20.49	20.15	
Blueberry juice	*L**, Chroma (C), Hue (H), Yellow Index (YI)	20 kHz, 600 W, 60 °C, 15 min		Ctrl.	Proc.	[89]
			*L**	21.19	25.65	
			C	4.86	0.98	
			H	22.88	−28.61	
			YI	−12.26	−2.61	
Goldenberry puree	Chroma (C)	20 kHz, 1000 W, 90% amplitude, 65 °C, 10 min		Ctrl.	Proc.	[84]
			C	47.5	54	
Cape gooseberry juice	pH, Brix, *L**, Chroma (C), Hue (h), Yellow Index (YI)	42 kHz, 240 W, 30 °C, 40 min		Ctrl.	Proc.	[92]
			pH	3.98	4.02	
			Brix	6.82	6.7	
			*L**	37.73	37.08	
			C	20.55	17.47	
			H	83.14	86.46	
			YI	77.82	67.15	
Mandarin juice	pH, Brix, *L**	19 kHz, 750 W, 50 °C, 36 min		Ctrl.	Proc.	[44]
			pH	3.43	3.45	
			Brix	13.31	13.43	
			*L**	43.88	45.8	
Pear juice	pH, Brix	20 kHz, 750 W, 65 °C, 10 min		Ctrl.	Proc.	[45]
			pH	12.77	12.63	
			Brix	4.82	4.81	
Hog plum juice	pH, Brix, *L**, Cloudiness, Browning Index (BI)	40 kHz, 400 W, 60 °C, 30 min		Ctrl.	Proc.	[46]
			pH	2.35	2.34	
			Brix	5.58	5.13	
			*L**	49.48	47.34	
			Cloudiness	23.5	29.3	
			BI	0.09	0.13	
Spinach juice	*L* *, *a**, Chroma (C), Hue (h), Cloud stability (CS)	30 kHz, 600 W, 60 °C, 20 min		Ctrl.	Proc.	[47]
			*L**	49.24	52.32	
			*a**	−5.38	−6.05	
			C	7.62	8.64	
			h	45.11	46.17	
			CS (%)	43	55	
Sugarcane juice	*L**, *a**, *b**	20 kHz, 750 W, 80 °C, 36 min		Ctrl.	Proc.	[99]
			*L**	31.57	31.9	
			*a**	0.67	0.29	
			*b**	1.08	1.73	
Tomato-based beverage	Brix, pH, *a**	37 kHz, 240 V, 15 min		Ctrl.	Proc.	[48]
			Brix	9.9	9.8	
			pH	4.4	4.4	
			*a**	0.43	0.64	

Table 4. *Cont.*

Product	Quality attribute	Processing Conditions	Results			Reference
PLANT-BASED MILK						
Almond milk	pH, Brix, viscosity, browning index (BI), *L**, cloudiness	40 kHz, 600 W, 60 °C, 40 min		Ctrl.	Proc.	[49]
			pH	6.12	6.11	
			Brix	4.48	4.27	
			Viscosity (cP)	3.55	4.2	
			BI	0.173	0.18	
			*L**	64.38	63.26	
			Cloudiness	6.5	9	
Hazelnut milk	pH, *L**, Syneresis	62.37 W/cm^2, 75 °C, 15 min		Ctrl.	Proc.	[50]
			pH	6.51	6.42	
			*L**	75.16	73.91	
			Syneresis (%)	63.39	60.92	
DAIRY AND POULTRY						
Butter	Moisture content (MC), a_w, pH, firmness (F), and work of shear (WS)	20 kHz, 200 W, 40.76 W/cm^2, 85 °C, 15 min		Ctrl.	Proc.	[51]
			MC (%)	15.8	13.71	
			a_w	0.968	0.958	
			pH	6.48	6.25	
			F (N)	129.1	210.6	
			WS (Ns)	176.29	217.36	
Chocolate milk beverage	Apparent viscosity (μ) Consistency index (k) Flow behavior index (n)	3 kJ/cm^3 HTST: 72 °C, 15 s		Ctrl.	Proc.	[52]
			μ	25	26	
			k	183	120	
			n	0.55	0.64	
Liquid whole egg	pH, *L**, Protein Solubility (PS), Foaming Capacity (FC), Foaming Stability (FS), Gelation temperature (Tg)	968 W/cm^2, 35 °C, 20 min		Ctrl.	Proc.	[53]
			pH	7.79	8.52	
			*L**	61.57	73.11	
			PS	73.07	64.38	
			FC	52.81	33.36	
			FS	27.09	23.35	
			Tg	64	75	
Skim milk	pH, *L**	20 kHz, 50,00 W, 10 min		Ctrl.	Proc.	[56]
			pH	6.8	6.8	
			*L**	75.61	72.33	
Whole milk	pH, specific gravity (SG), Solids-non-fat (SNF), *L**, Chroma (C), Hue (h)	20 kHz, 1500 W, 45 °C, 15 min		Ctrl.	Proc.	[58]
			pH	6.73	6.71	
			SG (g/mL)	1.032	1.032	
			SNF	8.69	8.64	
			*L**	63.02	64.04	
			C	6.5	6.75	
			h	−57.07	−57.95	
Whole milk	*L**, Chroma (C), Hue (h)	19 kHz, 475 W, 85 °C, 6 min 9 s		Ctrl.	Proc.	[59]
			*L**	74	80.7	
			C	2.89	3.05	
			h	179	141	

Ctrl., Control; Proc., Processed; Control: Raw product.

In recent years, one of the most explored categories in ultrasound research is related to the sensory evaluation of products. In the early stages of the technology, products were not thoroughly evaluated on sensory attributes because of the lack of information regarding toxicological aspects. Besides, former ultrasound research showed some drawbacks in sonicated products' sensory attributes and considered the future of ultrasound in food processing. For example, even though whole milk was whiter and with a perfect emulsion after ultrasound processing, there were some red flags in the aroma and chemical composition of this dairy product after processing. The aroma of milk after sonication was often reported as "rubbery" or "burnt," and the deposition of metallic particles from sonotrodes was often cited as one of the main areas for equipment optimization.

Milk processing with ultrasound has been widely studied in recent years [1,15,23,60,61]. The sensory evaluation of sonicated milk used in cheese-making and yogurt production has been documented. The generation of off-flavors in milk because of the effect of cavitation has now been analyzed in detail. These aromas reported as "rubbery," "burnt," "plastic," or "metallic" have also been identified in cheese and yogurt processed with sonicated milk. In sonicated yogurt, GC-MS analysis showed an increase in the concentration of ketones, aldehydes, hydrocarbons, and dimethylsulphide. Meanwhile, in cheese, the sensory evaluation showed good scores for the product even though there was an increase in the bitterness of the product made from sonicated milk (300 W, 30 kHz, 12 °C). This fact is attributed to the combination between some volatile compounds, free fatty acids, and the ripening process of the cheese [1].

Furthermore, chemical analyses of sonicated milk showed the presence of free radicals and some non-desirable compounds in milk, such as benzene, toluene, and other hydrocarbons generated likely from pyrolytic reactions during cavitation [100]. These authors reduced the intensity of the "rubbery" off-flavor of the sonicated milk while reducing the power of the treatment. The addition of CO_2 to milk has also been studied as an alternative to reduce the production of off-flavors during sonication [1]. Recent research shows more specific and analytical experimentation on flavor compounds of products after sonication [44,101–103]. For example, Mu et al. [101] studied the effect of ultrasound (20 kHz, 400 W, 0–9 min) on the flavor components of soybean milk. They comprehensively evaluated the sonicated product using sensory panelists, an electronic nose, an electronic tongue, and gas chromatography-mass spectrometry (GC-MS). The untreated soybean milk exhibited some off flavors described as grease oxidative, metallic, and grassy. However, ultrasound reduced them during the first 7 min of treatment. After this threshold value, the off flavors were increased. The electronic nose and tongue could distinguish between sonicated and unsonicated milk products. The GC-MS analysis showed a decrease in aldehydes, furans, ketones, and alcohols in the sonicated samples. The threshold value between off flavors can be explained due to the generation of free radicals and oxidative processes, enzyme inactivation, and changes in the protein conformation.

In recent studies, the sensory evaluation of hog plum juice showed positive results. Several ultrasound treatments were conducted at 40 kHz, 400 W, with temperatures from 40 °C to 60 °C, and the processing time was from 5 to 30 min. The sensory evaluation of the juice included taste, color, flavor, mouthfeel, raw juice's overall acceptability, thermal pasteurization (90 °C, 60 s), and the different sonication treatments. Untrained panelists ($n = 50$) used a 9-point Hedonic scale, showing the preference for those juices treated with ultrasound in all the sensory characteristics evaluated, followed by the raw juice, and leaving the thermal pasteurized juice in the last place [46]. Apple juice was also assessed in sensory properties by a trained panel ($n = 20$) using the same 9-point Hedonic scale. In this case, the evaluation showed that appearance, odor, cloudiness, and the general acceptability of sonicate juice were preferred over thermal pasteurized juice (85 °C, 10 min). The only trait that was not showing positive feedback was the taste of the sonicated apple juice [42]. Amora juice was also evaluated in sensory attributes and compared to raw and thermal pasteurized juice. Twenty-five untrained panelists assessed with a 9-point Hedonic scale the taste, odor, and overall acceptability. Results showed excellent results for all the juices (>8.5) and no difference in taste or smell after sonication and up to 30 days of refrigerated storage between thermal or sonicated products [21].

These results represent a milestone in ultrasound research. Now, the study of ultrasound is more comprehensive and is not only limited to microbial inactivation. Besides, scientists are playing with the processing conditions to find the best combination between ultrasound and other parameters, such as temperature, that ensures microbial inactivation and delivers a high-quality product.

7. Following Stages of Ultrasound Research

Ultrasound has the potential to be used in several food applications, and one of them is pasteurization. However, several research needs still need to be addressed to be used in commercial settings for microbial inactivation. These needs are discussed in this section, from equipment to product toxicity, as well as regulatory and harmonization of units.

7.1. Equipment

It has been widely documented that ultrasound technology's main challenge is delivering the same treatment intensity when the equipment is scaled up to industry settings. Several factors must be considered, such as the volume of the treatment chamber and the system's operation mode (batch or continuous), among others. However, one of the main drawbacks, as of today, is the sonotrode. The acoustic energy during sonication is delivered in the treatment medium by a sonotrode or horn tip. Most of the research in ultrasound for food processing and preservation has been conducted using devices with an attached sonotrode. These tips are commonly made of titanium and its alloys. However, the intense cavitation also affects the surface of the sonotrodes.

The implosions and explosions during sonication can remove metallic particles from the sonotrode surface. It has been observed and widely reported that the erosion or pitting of sonotrodes during processing [11,104,105]. This is a significant concern in food processing because of the deposition of metal particles in the product. A few studies have been conducted about the toxicity of these metallic particulates in food after processing [105]. The findings reported that these particulates do not represent a toxicological concern for human health.

Furthermore, a few attempts have been made to reduce this problem, and some solutions include covering the sonotrodes in epoxy or using inert materials such as quartz or pyrex [12,106]. These efforts have reduced the number of metallic particles eroded from the horn tips. However, this change is also reducing the intensity of the treatment and hence the microbial inactivation. Some promising materials in constructing improved sonotrodes include niobium and its alloys [107]. However, the re-design of sonotrodes to reduce the leaching of metallic particles into the medium is a research need that must be addressed while optimizing the pasteurization process by ultrasound soon. In this stage, the construction of sonotrodes needs to be made from strong materials that can provide the same intensity of treatment as titanium horn tips but also reduces the erosion of the sonotrode surface and are made of materials considered safe to be in contact with food.

7.2. Harmonization of Processing Conditions

Today, thousands of publications are dealing with ultrasound for food pasteurization. The number of microorganisms, enzymes, products, processing conditions, and quality analysis is vast. Most of the publications display encouraging results in microbial and enzyme inactivation but also in preserving the quality of the product. However, in many of the publications, some processing conditions are lacking. As a new area of research, some parameters that need to be reported are sometimes missing from the experimental details. Until today there is no general rule of what needs to be reported while working with ultrasound, making it challenging to compare experiments [23,38].

It is also known that each country has unique standards regarding food safety and regulations, but also, the meaning of some words differs from country to country. A Global Harmonization Initiative started a few years ago in different areas of food science. A specific working group is devoted to novel food preservation technologies. Even though various regulations worldwide can affect the development of novel technologies, this working group will provide, exchange and recap knowledge with the scientific community to align to international regulations and ensure the technology can be validated and approved in the future [108]. The development of previous emerging technologies and currently commercial applications, such as high hydrostatic pressure or microwave, set the pathway to be followed. It has been a joint effort between academia, industry, and government, with many years of research behind it. Ultrasound pasteurization is not the newest emerging technology; it has at least a few decades of research. At this point, it is necessary to explore the next stages of research and the pathway to follow for further validation and approval.

7.3. Toxicity

Even though ultrasound offers several advantages in the pasteurization area, indeed, ultrasound still has some current challenges. Because of the cavitation effect, some macromolecules break down, not only water, releasing free radicals. These radicals can combine with other compounds in the product and generate new ones. Hence, these new compounds must be studied and quantified to ensure the product does not contain toxic substances.

Recently, ultrasound has been tested for pesticide degradation in some food products. The comprehensive study of the intermediate and after-sonication chemical composition has been targeted in several experiments [108,109]. In specific research to degrade the pesticide parathion methyl in milk, scientists discovered a reduction of up to 97% of the pesticide concentration in milk, affected by the intensity of the treatment and the initial concentration of the chemical. However, they also found three transformation products, P_1, P_2, and P_3, likely from oxidation during sonication; these products presented higher toxicity than the original pesticide [109]. Opposite to these results, Yuan et al. [110] studied the degradation of an organophosphate insecticide chlorpyrifos in milk using sonication and ultraviolet. The researchers found that both technologies could degrade the chemical up to 97% because of the electrophilic attack on the P=S bond, converting it into a P=O bond, besides other chemical reactions, reducing the toxicity of the chlorpyrifos.

Although the experiments mentioned above were conducted to evaluate pesticide degradation, processing conditions are similar to the research aimed at pasteurization. Also, the presence of pesticides is familiar in many food commodities. Then, the generation of these compounds is likely probable to find in pasteurized products by ultrasound. More research is needed in the area of toxicology, not only to find the concentration of these toxic compounds in different products but also to detect the processing conditions that decrease the formation of these chemicals while keeping the microbial safety and quality of the product.

8. Conclusions

Ultrasound is a novel food processing technology that can pasteurize liquid foods. However, an ultrasound must be used with other preservation factors (i.e., heat, pressure, antimicrobials) to achieve pasteurization standards. Microbial inactivation is possible with ultrasound as it can be widely documented in the last twenty years. Recent ultrasound research has focused on evaluating bioactive compounds and the overall quality of the products after sonication. Thermolabile vitamins can be affected during sonication because of oxidative reactions. Sensory evaluation of sonicated products represents an opportunity to continue the research in ultrasound, mainly to improve the taste and odor of foods after treatment. Further research needs to be conducted with equipment manufacturers to evaluate resistant materials for sonotrodes and the scale-up of the technology. Toxicological studies of sonicated products and the regulatory aspects of this novel technology represent two milestones to explore and meet in the coming years.

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